Microbial Response to Urbanisation and Earthquake Damage in New Zealand Streams

By

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Supervised by: Dr Kevin Simon

Abstract

Microbial organisms play a pivotal role in stream nutrient cycling by taking up nutrients from organic and inorganic sources; this supports higher trophic levels and prevents downstream nutrient export. Nutrient loading to urban streams is likely to alter the identity and magnitude of limiting nutrients, this is important to understand if we are to manage nutrient pollution effectively by placing controls on the limiting nutrient. The aim of this study was to determine how urbanisation influences microbial nutrient limitation in New Zealand streams. Nutrient limitation was assessed in Auckland and Christchurch over Spring and Summer using three commonly used methodologies: 1) *in situ* organic and inorganic nutrient diffusing substrates (NDS), 2) sediment extracellular enzyme activity (EEA), and 3) water chemistry ratios. Nutrient diffusers experimentally tested nutrient limitation, and nutrient limitation was inferred from ratios of EEA and water chemistry. Biofilms demonstrated a clear switch in the identity of limiting nutrients from nitrogen (N) limited in native sites to phosphorus (P) limited in urban and agricultural sites, with urban sites demonstrating N-saturation at 30% land-use. Demonstrating rough agreement with the Redfield ratio, organic biofilms demonstrated a switch in nutrient limitation from N limited at <19N:1P to P limited at >15N:1P. Earthquake damage also produced noticeable effects including N and P suppression on biofilms and water column N-concentrations up to seven-fold lower. Limitation predicted by sediment EEA suggested a predominance of N limitation and did not align with limitation inferred from water chemistry which suggested P limitation; however enzyme activity did vary with urbanisation impact suggesting this could be a promising bio-assessment tool. Sediment EEA was also not coherent when compared to limitation from organic biofilms; but EEA on organic biofilms from nutrient diffusers was more accurate. Results demonstrate the complexity of nutrient limitation between stream compartments as microbial organisms are reliant on the water column for N and P to different degrees. Additionally, nutrient limitation according to nutrient diffusers was often co-limited or secondarily limited, suggesting that controls need to be placed on both N and P. Future

NDS studies should incorporate organic substrates as these may be a valuable tool for consistently gauging microbial response to land-use change.

Keywords: Nutrient limitation, stream biofilms, urbanisation, microbial enzymes, earthquake.

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Chapter 1
General Introduction

Land-use activities within a catchment exert a strong influence on water quality with streams described as "gutters down which flow the ruins of continents" (Leopold *et al.*, 1964). Any nutrients and sediments within a given catchment drain into stream networks compromising their ability to provide vital environmental services (Postel and Carpenter, 1997). Such services include in-stream physical and biological processes which are important for the storage, transformation and utilisation of nutrients preventing degradation of water quality (Covino *et al.*, 2010). In New Zealand damaging land-use activities include horticulture, agriculture, and urbanisation. Land was initially widely developed for horticultural and agricultural purposes and more recently urban centres have expanded to accommodate a growth in population size and density (Figure 1.1) (Statistics New Zealand, 2009).

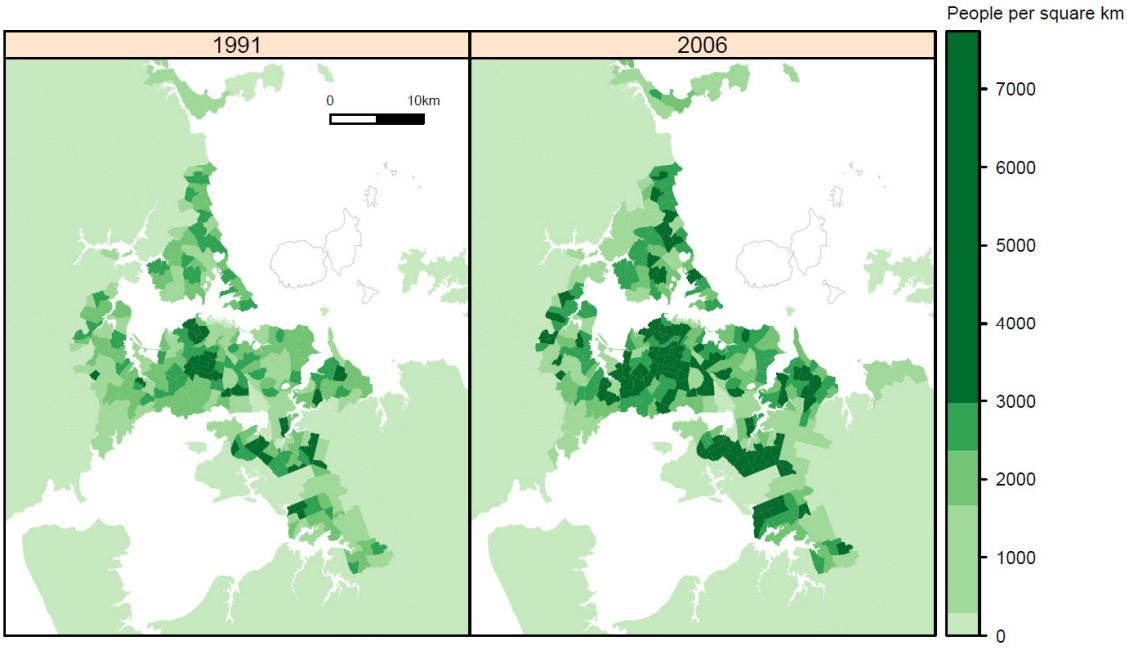


Figure 1. 1. Urban sprawl in Auckland from 1991 to 2006, change in colour darkness indicates changes to population density with white being water (Statistics New Zealand, 2009).

Water quality across New Zealand has declined over time as a result of land conversion for urbanisation and agriculture, with lowland areas the most severely affected (Quinn *et al.*, 1997; ANZECC and ARMCANZ, 2000; Larned *et al.*, 2004; Scarsbrook, 2006; McDowell *et al.*, 2009). Approximately 50% of New Zealand's land, or 70% of the lowland, has been developed for anthropogenic uses (Larned *et al.*, 2004). Due to this development, many of the country's

waterways do not meet regulatory criteria for recreational uses and exceed ecological health guidelines (Ministry for the Environment, 2007, 2006). Agricultural land-use is well documented as being a cause for the degradation of many rural waterways in New Zealand. There is a growing recognition of this issue, such as a second water accord released in July 2013 for improved stream management in dairying catchments (Quinn *et al.*, 1997; Quinn and Stroud, 2002; DairyNZ, 2013). In contrast, streams draining urban catchments in New Zealand have received much less attention despite their often poor condition (Larned *et al.,* 2004). Urban land-use only covers 1% of New Zealand's land area but contains 86% of the population (Ministry for the Environment, 2007). This land-use intensity has caused urban streams to be amongst the most degraded of any land-use classification (Ministry for the Environment, 2007; Larned *et al.,* 2004). The detrimental effects of urbanisation on freshwater has gained attention in the past ten years as urban populations globally have expanded (Paul and Meyer, 2001; Allan, 2004; Meyer *et al.*, 2005).

The 'urban stream syndrome' describes the consistently observed detrimental effects of urban land-use on stream ecosystems (Allan, 2004; Walsh *et al.*, 2005). Increasing impervious surface area and urban infrastructure within a catchment produces characteristic responses in streams (Walsh *et al.,* 2005). Such responses include increases in flow rates, sediment deposition, and contaminant runoff; namely nutrients and heavy metals. After picking up contaminants (including sediments) water is channelled down gutters, into stormwater outlets, and is discharged into nearby waterways elevating levels of chemicals in these environments and causing irregular flow regimes (Paul and Meyer, 2001; Allan, 2004). Other effects of urbanisation include increases in the volume and forms of nutrients and heavy metals entering waterways, with common sources including impervious surface run-off and wastewater leakages. Channel morphology often becomes homogenised reducing in-stream habitat variation and availability. Community composition also changes to one favouring pollutant tolerant species, causing a decrease in species richness, and an increased density of pollutant tolerant organisms (e.g. oligochaetes, gastropods). Increases in stream temperatures due to the loss of riparian vegetation, changes in sediment inputs due to bank destabilisation and overland flow, and decreases in organic matter retention are also common effects of urban land-use (Walsh *et al.*, 2005). Urbanisation has dramatically increased in New Zealand in the last century, and will inevitably keep increasing as populations continue to rise (Statistics New Zealand, 2009). Consequently, it is important to understand how streams respond to increases in urbanisation.

The exact relationship between urbanisation intensity and stream integrity is unclear, with several suggested models (Figure 1.2). Some authors have suggested a stepped threshold,

with systems having a tipping point beyond which they become degraded whilst others have hypothesised a linear decrease with increasing urbanisation (Walsh *et al.*, 2005). The '10% rule' was suggested by Beach (2001) as a threshold of urbanisation beyond which there would be negative impacts to streams, this has been supported by many studies since with findings of decreased in the richness of diatom, macroinvertebrate and fish assemblages at urbanisation levels of 6% to 15% (Morse *et al.*, 2003; Newall and Walsh, 2005; Walsh *et al.*, 2005; Jinyu, 2009; Schwartz *et al.*, 2009). It is, however, unknown whether this stepped threshold which has been observed is universal or whether regional differences exist (Walsh *et al.*, 2005). Additionally, it is not yet known whether New Zealand systems have a consistent threshold for urbanisation, however negative effects of urban land-use on New Zealand streams are well documented and in catchments with high levels of urbanisation streams have lower water qualities than any other land-use (Quinn and Hickey, 1990; Larned *et al.*, 2004; Ministry for the Environment, Environment, 2007; Scarsbrook *et al.*, 2007; Neale, 2012). State of the Environment reporting in New Zealand typically ranks water quality in predominately urban catchments lower than agricultural due to high nutrient concentrations, increases in heavy metals, increased suspended sediments, decreased macroinvertebrate community richness, and elevated temperatures all common symptoms of New Zealand's urban streams (Ministry for the Environment, 2007; Neale, 2012).

Elevated nutrient levels are a serious threat to freshwater ecosystems, often leading to their degradation (Ministry for the Environment, 2007). Levels of biologically available nitrogen around the world have more than doubled since human colonisation, with some regions experiencing levels twenty times higher than natural levels (Allan and Castillo, 2007). Nutrients are used by macrophytes, periphyton, and microbes (fungi, bacteria, eukaryotes) for growth, however at elevated levels (eutrophication) streams become saturated and can no longer process nutrients (Allan and Castillo, 2007). Eutrophication has been linked to blooms of periphyton related to increased inorganic nutrients and light levels which stimulate net primary production, essentially 'choking' waterways (Ministry for the Environment, 2007). Blooms tend to occur in Spring, coinciding with high inorganic nutrient inputs through winter due to the release of terrestrial and aquatic organic matter and increasing sunlight in Summer (Kirchman, 2012). Eutrophication causes large diurnal swings in dissolved oxygen due to increased bacterial respiration, leading to decreased habitat availability for biota and decreased light availability. This makes streams unsuitable for many species, can cause fish kills if oxygen levels plummet far enough, and may lead to a bottom-up alteration of stream food web dynamics (ANZECC and ARMCANZ, 2000; Gucker and Pusch, 2006; Hoellein *et al.*, 2010). Blooms of cyanobacteria

(blue-green algae) can also be potentially toxic to humans and animals, causing the closure of waterways and coastal areas as nutrients are deposited in estuaries (Tank *et al.*, 2008). In addition eutrophication is aesthetically unpleasing, it can increase the costs of water treatment and can increase flood risk with the increased growth of rooted plant species (Hilton *et al.*, 2006).

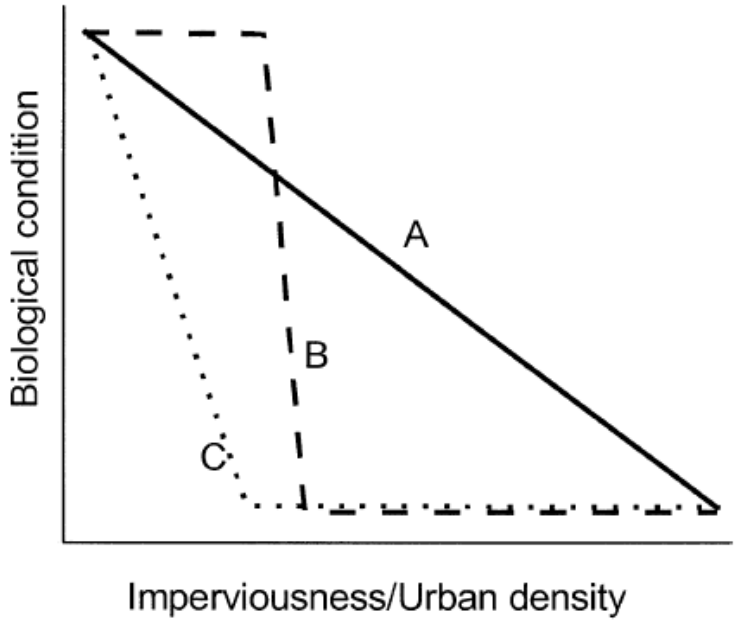


Figure 1. 2. Possible relationships between urbanisation intensity (as imperviousness or urban density) and biological condition. Line A) shows a linear decline with increasing urbanisation, B) shows a switch threshold relationship, indicating degradation beyond a certain point, and C) shows a sharp linear decrease, to a lower biological condition (Walsh *et al.,* 2005).

Urbanisation increases nutrient input to streams by direct (e.g. wastewater) and indirect (e.g. runoff) inputs (Table 1.1) (Walsh *et al.*, 2005). Nutrients move through a number of different forms when in waterways. They can be assimilated by organisms for growth (anabolic) and metabolic processes, used as energy sources (oxidation reactions) or used as alternative electron acceptors (reduction reactions) in the absence of oxygen, known as dissimilatory processes (Stream Solute Workshop, 1990). The two primary nutrients are nitrogen (N) and phosphorus (P); these occur in both dissolved ionic forms, such as nitrate (NO_3^-), ammonium (NH_4^+) and phosphate (PO_4^{3-}), and are incorporated into organic molecules (e.g. amino acids). Dissolved ionic nutrients are the most biologically available and can be readily assimilated by stream plants (Classens *et al.*, 2010). Nutrients NO_3^- and NH_4^+ are commonly found dissolved in the water column where these can be easily taken up by organisms, whereas P is often found bound to inorganic particles in addition to being dissolved in the water column (PO_4^{3-}) (Withers

and Jarvie, 2008). Nutrients are differentially taken up depending on the degree of nutrient loading, which affects the nutrient requirements of biota (Classens *et al.,* 2010; Kirchman, 2012). Therefore an increase in nutrients with urbanisation is expected to alter nutrient uptake (Johnson *et al.,* 2009a).

Table 1. 1. Sources of nitrogen and phosphorus in streams, items marked with a star (*) indicate anthropogenic sources (Hoellein *et al.,* 2011a; Allan and Castillo, 2007).

Nitrogen sources	Phosphorus sources
• Decomposition of organic matter • Nitrogen Fixation • Atmospheric Diffusion • Lightning • Runoff* • Domestic wastewater* • Municipal wastewater* • Agricultural fertilizers*	• Decomposition of organic matter • Bedrock weathering • Runoff* • Domestic wastewater* • Municipal wastewater*

Nutrient cycling is an important ecosystem service which is provided by streams; understanding the impacts of anthropogenic activities (e.g. nutrient loading) on this service is therefore crucial. Nutrient limitation is one way in which this can be measured (Covino *et al.,* 2010). Nutrients and carbon (C) are essential for growth, the ratios at which these are present (stoichiometry) in aquatic systems can inform us as to which element is limiting growth of in-stream biota (Allan and Castillo, 2007). When an organism is not growing as fast as it is ideally could be it is said to be nutrient limited, it is unusual for all elements to be optimal for a species so many organisms may permanently exist in a state of limitation (Gibson, 1979). Early lake manipulation experiments confirmed nutrient limitation theory; these found that P was most likely to limit primary productivity in freshwater systems (Schindler and Fee, 1974; Schindler *et al.*, 1978). Later studies found patterns of limitation to be more complex than this with limitation patterns shifting in different environments due to anthropogenic impacts and often more than one element limiting growth (Francoeur *et al.*, 1999; Francoeur, 2001; McDowell *et al.*, 2009; Johnson, 2009b; Keck and Lepori, 2012; Sardans *et al.*, 2012). Nutrient limitation can be assessed by nutrient ratios; the Redfield ratio of 108C:16N:1P has become a benchmark for assessing nutrient limitation in freshwaters. This molar ratio was based on the ideal ratio of elements in planktonic algae but has now been used to assess limitation across many aquatic systems (Redfield, 1958). In broad scale ecological studies a bounded ratio of 10:1 to 20:1, for dissolved nutrients (N:P) in water, has been suggested as more appropriate rather than the absolute ratio due to environmental variability and multispecies nutrient requirements (Borchat,

1996; Hill *et al.*, 2010b). This approach has also been applied to nutrient ratios in stream sediments (186C:13N:1P) and ratios of microbial extracellular enzymes (EEA) that target energy and nutrient limitation (60C:7N:1P) (Cleveland and Liptizin, 2007; Hill *et al.*, 2012). Departures in any of these ratios can be used to infer which nutrients are likely to be limiting in a system (Redfield, 1958; Hill *et al.*, 2010a). Nutrient loading to streams in urban systems is likely to alter the identity and magnitude of limiting nutrients, this is important to understand if we are to manage nutrient pollution effectively, and therefore can put in place management controls on the limiting nutrient.

Many studies use water chemistry to infer limitation based on their measured ratios or inorganic or total nutrients. This approach may underestimate the total proportion of the elements in the water column as it does not account for high nutrient turnover related to remineralisation or biotic uptake rates (Dodds, 2003; Allan and Castillo, 2007). Water quality parameters are often poorly correlated to stream ecology to justify their use in solely classifying stream health (Vinten *et al.*, 2011; Keck and Lepori, 2012). Reviews of microbial nutrient limitation established that physiochemical measurements do not consistently predict nutrient limitation; with molar N:P ratios only producing accurate predictions at the extremes of less than 1:1 or greater than 100:1 (Francoeur *et al.*, 1999; Keck and Lepori, 2012). Thus, validation from field experiments, such as bioassays, should be used to provide holistic picture of ecological health. Assays using stream microbial communities may therefore provide a more robust measurement of stream health (Hoellein *et al.*, 2009; Lear *et al.*, 2012). Microbial communities are integrative of a longer time period (approximately two weeks) whereas water samples are 'snapshots in time', subject to constant change (Tank and Dodds, 2003; Hill *et al.*, 2006; Vinten *et al.*, 2011; Hill *et al.,* 2012). Processing of nutrients is achieved in streams primarily though microbial processes, which serve as an interface between abiotic and biotic nutrient acquisition (Chrost, 1991; Bernot *et al.*, 2010; Hill *et al.*, 2010a). The ability of stream microorganisms to take up nutrients determines the biogeochemical cycling of nutrients and C which is important in maintaining a healthy ecosystem. Thus, microbial organisms which sit at the base of the stream food web are ecologically important as they take up nutrients from the water column and sediments; moving this energy up through the food chain, supporting higher tropic levels and stream nutrient processing (Figure 1.3) (Allan and Castillo, 2007; Hoellein *et al.*, 2010).

Little is known about the ability of stream microbial communities to process nutrients in highly urbanised environments. Therefore, the main aim of this study is to determine how urbanisation influences microbial limitation in streams. Limitation patterns were compared across different temporal (Spring, Summer) scales and between two cities (Auckland and

Christchurch). This analysis included streams in Christchurch catchments subjected to earthquake damage to infrastructure damage and liquefaction. In this study nutrient limitation was assessed using several techniques. Chapter two assesses *in situ* nutrient limitation patterns on organic and inorganic microbial biofilms. Chapter three describes microbial nutrient limitation by examining patterns of allocation of microbial extracellular enzymes on stream sediments. Chapter four examines the relationship between nutrient limitation responses on biofilms and sediment enzyme activity. Finally, chapter five summarises the main findings of this research with results informing us on changes to nutrient dynamics with land-use, and providing insight into the agreement of different methodologies used to assess nutrient limitation.

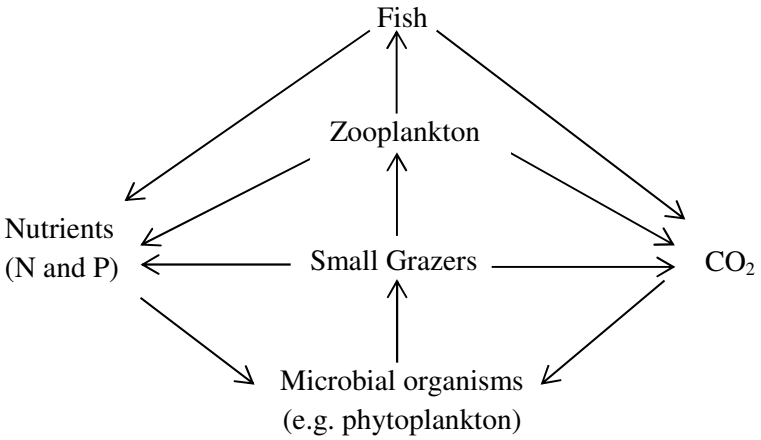


Figure 1. 3. Simplified food chain in an aquatic system. Small grazers include other microbes which would make up the first few links (adapted from Kirchman, 2012).

Chapter 2

Microbial Nutrient Limitation of Organic and Inorganic Biofilms

2.1. Introduction

Microbial organisms (e.g. bacteria, viruses, protozoa) exist as free-living species in the water column or in communities known as biofilms (Findlay, 2010). These biofilms are complex communities of microorganisms living in an extracellular polysaccharide matrix which colonize in-stream habitats and play a key role in the uptake of nutrients (Vinten, 2011; Lear *et al.,* 2012). Biofilms can be divided into two communities; autotrophs which fix carbon from atmospheric CO_2 and heterotrophs which use organic carbon made by other organisms for growth and nutrient uptake (Tank and Webster, 1998; Kirchman, 2012). Autotrophs are most commonly found on inorganic surfaces and heterotrophs on organic surfaces; on inorganic surfaces autotrophs can outcompete heterotrophs as these can fix their own carbon (Tank and Webster, 1998). Biofilms play a pivotal role in the regulation of stream processes such as primary production, community respiration, organic matter decomposition, and nutrient uptake (Tank and Dodds, 2003). Much of the energy entering streams moves through the microbial loop, therefore microbes are ecologically important as they can incorporate nutrients into biomass providing a rich food source for grazers and prevent downstream nutrient export in addition to transforming active compounds into inert forms (denitrification) (Hoellein *et al.*, 2011a; Lear *et al.*, 2012).

The availability of nutrients often limits autotrophic and heterotrophic components of biofilms (Borchat, 1996; Findlay, 2010). Despite the ability of biofilms to take up nutrient from the water column they are often limited by N or P (Tank and Dodds, 2003). Differences in nutrient limitation among sites are thought to be related to factors such as light, temperature, discharge, and nutrient inputs (Johnson *et al.,* 2009; Hill, 1996; Francoeur *et al.,* 1999; Borchat, 1996). Many studies have focused on the autotrophic components of stream biofilms as these drive in-stream primary production (Cheeseman *et al.,* 1992; Mosisch *et al.,* 1999; Francoeur, 2001). Heterotrophs however play an important role in streams dominated by allochthonous matter; determining the cycling of nutrients and carbon to higher trophic levels (Tank and Webster, 1998). Autotrophs in particular are heavily regulated by light availability, as this stimulates primary production by algae (Hoellein *et al.,* 2010). Streams therefore range from

unshaded autotrophic communities in which photoautotrophs provide an energy source, to shaded heterotrophic streams which are reliant on detrital and allochthonous matter (Tank and Webster, 1998). Additionally, biofilms can be disturbed by high flows or grazing insects which can scour biofilms and reduce biomass (Borchat, 1996). Consequently, there are seasonal implications for biofilm functioning. Increased light intensities and temperatures in summer and higher flows in winter, all influence stream processes and nutrient limitation.

Urbanisation can affect the structure and functioning of biofilms and their ability to take up nutrients from the water column (Hoellein *et al.,* 2011a; Lear *et al.,* 2012). Human activities often lead to increases in nutrient concentrations in addition to changes in temperatures, flow, geomorphology, and light which all influence biofilms (Paul and Meyer, 2001). We can therefore expect changes to biofilms, and their ability to remove water column nutrients with increases in urbanisation, leading to variations in nutrient limitation across different land-uses. Additionally, disruption of infrastructure may alter nutrient limitation, for example, earthquake damage can increase leakage of wastewater from the sewerage system and alter inputs of terrestrial soils and sediments (ESR, 2012a). Christchurch was therefore a unique opportunity to address earthquake disturbance in an urban system. The effects of urbanisation or disturbance on microbial nutrient limitation are not well understood with few studies on this topic (Hoellein *et al.,* 2011a). In addition, microbial nutrient limitation studies have generally only focused on inorganic substrates (Francoeur, 2001). The need to focus on both autotrophic and heterotrophic communities has only recently been acknowledged following studies which have found heterotrophs to have distinctly different nutrient requirements to autotrophs (Johnson *et al.,* 2009; Tank and Dodds, 2003; Hoellein *et al.,* 2011a). Understanding stream nutrient limitation patterns therefore requires the assessment of both components. Water chemistry ratios are frequently used to assess limitation; however these do not describe the ability of biofilms to take-up nutrients and are not reliable indicators of biological condition (Francoeur *et al.*, 1999; McDowell *et al.*, 2009; Hoellein *et al.*, 2011a; Keck and Lepori, 2012). Measuring microbial activity can inform us about the impacts of land-use changes on the ability of microbial organisms to take up nutrients; with changes in metabolic rates (e.g. community respiration) and primary production potentially giving insight into anthropogenic impacts. No studies, to my knowledge, have used inorganic or organic substrates to assess limitation in urban centres in New Zealand.

This study intends to describe how nutrient limitation responses differ with changes in land-use intensity across two distinct eco-regions, Auckland and Christchurch. The goal of this chapter is to compare *in situ* nutrient limitation trends for autotrophic and heterotrophic biofilms

across a range of land-uses, focusing particularly on urbanisation. The main aims of this chapter are therefore to:

a. Describe the pattern of identity and magnitude of nutrient limitation with increasing urbanisation,
b. Establish whether if urbanisation has similar effects on nutrient limitation trends in different regions,
c. Asses the relationship between water chemistry and biofilm limitation,
d. Understand the impacts of earthquake damage on nutrient processing in the Christchurch region.

I hypothesised that streams subjected to human land-use pressures (urban, agricultural) would demonstrate different nutrient limitation patterns due to the increased nutrient loads associated with these land-uses. Between regions responses are expected to be similar, concordant with globally common observations in urban streams (i.e. a coherent urban stream syndrome). Changes to nutrient demand by stream biofilms will shift according to the Redfield ratio, such that increases in water column nutrients will be accompanied by decreases in biofilm responses. And finally, that earthquake impacts would change nutrient limitation patterns in Christchurch streams due to inputs of nutrients and fine sediments from liquefaction and infrastructure damage, altering in-stream nutrient processing.

2.2. Methodology

2.2.1. Study design

Nutrient levels in aquatic environments differ between regions and fluctuate with seasonality (Tank and Dodds, 2003; Johnson *et al.*, 2009a). In order to gain a more accurate understanding of nutrient limitation in New Zealand, experiments and sample collections were carried out in two ecologically distinct regions, Auckland and Christchurch, over two seasons to capture spatial and temporal variation in nutrients. Comparing these two regions allowed me to test whether nutrient limitation patterns across different land-uses hold in different locations within New Zealand. The post-earthquake effects of liquefaction from the 2010 and 2011 earthquakes on Christchurch waterways will also be examined in this study.

Christchurch differs from Auckland in several ways including differences in geology and natural environments. Auckland in New Zealand's North Island is situated on a narrow isthmus and has a complex geology due, in part, to the large number of volcanoes spread across the

region (Searle, 1981). In general, the geology of Auckland is dominated by softer sandstone and siltstone and its groundcover was mixed forest pre-human settlement (Harding and Winterbourn, 1997). There are however a few notable differences in geology across the Auckland region for instance in West Auckland harder rock such as basalt dominates, giving rise to the Waitakere ranges as the surrounding silt and sandstone has eroded over time (Gage, 1980; Searle, 1981). Southern parts of Auckland, notably the Franklin district, were historically dominated by estuarine and lacustrine conditions, giving this area soil suitable for agriculture and horticulture which is the predominant land-use in the region today (Waikato Regional Council, 1991). In contrast, Christchurch is dominantly composed of glacial gravels, glacier deposits, and superficial sands with grasslands and swamplands dominating in the natural environment (Gage, 1980; Harding and Winterbourn, 1997). The geology of Christchurch can be divided into three main landforms, volcanic rock at Banks Peninsula, alluvial flood plains and terraces, and coastal plains, the plains are composed of soft, easily reworked materials giving the area a small degree of relief (Christchurch City Council, 2003). The majority of the rivers in Christchurch are Spring fed, something not seen in Auckland, in addition to receiving overland flow and lateral inputs (Christchurch City Council, 2005). Geology can influence in-stream nutrient concentrations through the weathering of bedrock in volcanic areas (e.g. the Waitakere ranges Auckland) which phosphorus is derived from (Death *et al.,* 2007).

Development intensity differs considerably between the two cities; Auckland is New Zealand's largest city followed by Christchurch (Statistics New Zealand, 2009). Auckland has a population of 1,469,900 covering 4894km^2 whereas Christchurch city has a population of 348,435 covering 1494km^2 as of 2010 (Christchurch City Council, 2007; Statistics New Zealand, 2009; Christchurch City Council, 2010; Auckland Council, 2011). Peak densities also differ between the regions with approximately 4,000 people per square kilometre in Auckland in contrast to 1,200 in Christchurch in the central city suburbs (Christchurch City Council, 2007; Statistics New Zealand, 2009). Many urban streams in both regions were channelized during city expansion to decrease the risk of disease; this was done by piping them underground, lining them with concrete, or converting them to box-culvert channels (Blakely and Harding, 2005; Humphris, 2013).

Recent earthquakes in the Canterbury region have caused changes to stream functioning, through changes to sediment budgets, biological communities, and wastewater contamination (James and McMurtrie, 2011; Rutherford and Hudson, 2011; Taylor and Blair, 2011; ESR, 2012a). The September 4 2010 earthquake had a magnitude (M_W) of 7.1; this caused the

strongest ground movements ever recorded in New Zealand, and was centred at Dartfield 44km from Christchurch city (Cox *et al.,* 2012; The Royal Society of New Zealand, 2012). This was followed by another on the 22nd February 2011 M_W 6.3, centred only 4km from Christchurch; this second earthquake caused extensive damage to infrastructure around the city (The Royal Society of Zealand, 2012). Earthquakes affected waterways through an upwelling of liquefaction (silt/fine sands) and groundwater into Canterbury streams, with those in the eastern suburbs the worst affected (Cox *et al.*, 2012). Liquefaction was often contaminated with sewage from the damaged infrastructure, causing streams to become smothered in fine sediments and nutrient levels to increase (ESR, 2012b). The 2011 earthquake caused an estimated 500m^3 of sewage to be discharged into the Avon River every day for six months until infrastructure was repaired (ESR, 2012a). Subsequent aftershocks have meant a slow recovery for streams with further damage to infrastructure causing influxes of sediment and nutrients (Christchurch City Council, 2011; Environment Canterbury, 2013). The effects of liquefaction on nutrient limitation patterns are something which, to my knowledge, has not been previously described. The inclusion of sites affected by liquefaction therefore provides an interesting research opportunity to understand how disasters affect stream functioning in human-dominated landscapes.

2.2.2. Study sites

Within Auckland and Christchurch 24 (n=12/region) sites were chosen to assess *in situ* nutrient limitation of inorganic and organic biofilms (Figure 2.1). Sites are a subset of those used for water and sediment sampling as described in chapter three (Appendix B.1 & B.2). Geographical distribution of sites was considered during sample design to ensure that geographical variations (e.g. geology, development intensity) across both cities were captured. Sites in both regions cover a number of different land-uses at varying intensities so changes in nutrient dynamics could be assessed along a gradient of land-use intenisty.

All Auckland sites are routinely monitored as part of Auckland Councils freshwater monitoring programme. Selected sites were chosen based on analysis of nutrient data supplied by Auckland Council. Data analysis included monthly nutrient analysis over the time period 2002 to 2012 and between site comparisons of nutrient levels over the same time period. Variables used in site selection included average total N and P, the ratio of TN:TP, and the percentage land cover of native, pastoral, or urban land within each sites catchment. Sites were ranked by both their nutrient ratios and land-uses; ensuring that the chosen sites were representative of all land-uses and had contrasting ratios of nutrients for comparison. All sites were visited in order to

assess their suitability for this project. Sites needed to be accessible, wadeable, have riffle habitat or fast flowing reaches, and be secluded to minimise the chances to vandalism.

The final twelve sites used for this experiment can be broadly divided into four land-use categories; native, urban, suburban, and pastoral. These categories are not completely isolated from one another, with overlaps in land-uses present, allowing for a land-use gradient approach. Urbanisation intensity ranges from 0% to 100%; and covers a number of different stream types such as impounded (box-channel), with or without riparian corridors, and variations in urban use (industrial, residential, city). Additionally, sites have different substrates and underlying geology's. Reference sites in New Zealand are often upland and of volcanic origin. These are often considered N limited as volcanic geology provides a natural P source (Death *et al.,* 2007). This was considered in study design and another reference site, with silt/sand stone geology was included (West Hoe).

Sites in Christchurch were chosen based on land-use and earthquake impact. The chosen sites can be split into three categories: rural-suburban, urban-wetland, and urban. Like in Auckland, urban sites included a range of types including channelized streams, streams with riparian buffers, and variations of stream width and depth. Earthquake effects were also described at each site by the presence of liquefaction as heavy, light, or none. A number of sites from each category within urban areas were chosen to be compared to urban sites with no liquefaction impact. Due to the lack of reference sites in Christchurch, sites with the least disturbed conditions were used, in this instance rural-suburban sites (Stoddard *et al.*, 2006).

All sites used in this study were assessed using ArcView GIS 3.3 with River Environment Classification (REC) and Freshwater Environments New Zealand River Classification (FEWNZ) databases to gather basic catchment characteristics (Harding *et al.*, 2009) (Tables 2.1 & 2.2).

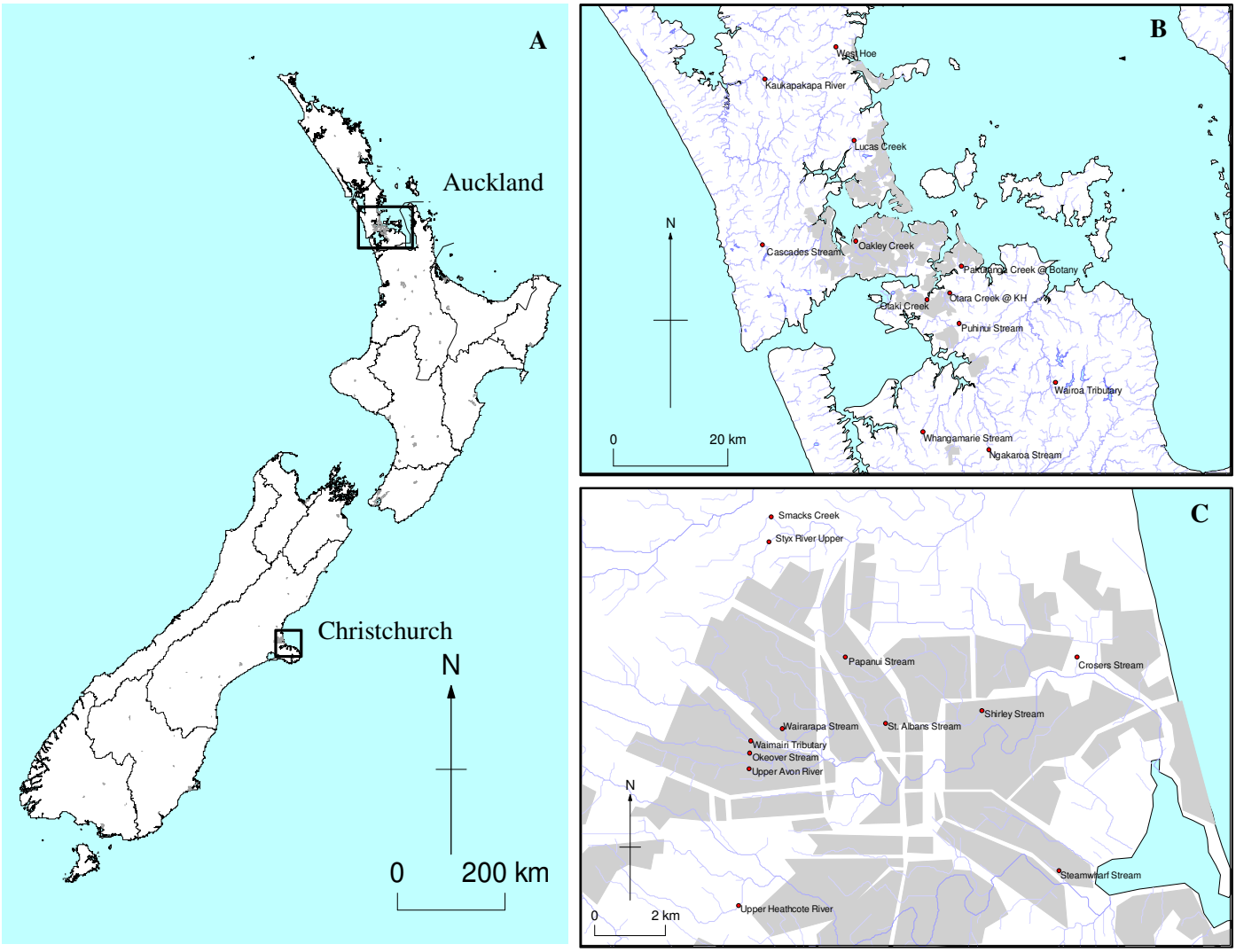


Figure 2. 1. Location of nutrient diffusing substrates study sites. Map A shows the location of the two study regions within New Zealand, Maps B and C show the Auckland and Christchurch regions respectively with all sites marked and labelled. Grey areas represent urban development; blue lines are rivers and streams (Adapted from the New Zealand Freshwater Fish Database, 2009).

Table 2. 1. Characteristics of Auckland NDS sites, nutrient data were provided by Auckland Council and covers 2002-2012, other descriptors are from GIS based databases. Nutrients are described as mean (±SE) total nitrogen (TN) and total phosphorus (TP) (Auckland Council, 2012; REC and FWENZ databases).

Site Name	Land-use	Substrate	Mean TN (µgN/L)	Mean TP (µgP/L)	Catchment Area (ha)	Stream Order	Distance from sea (km)	Elevation (m)	GPS (NZMG) Easting	GPS (NZMG) Northing
Otaki Creek	Urban	Soft	1983 (365)	110 (16)	160	2	0.5	2.6	6468910	2674710
Pakuranga @ Botany Rd	Urban	Hard	1235 (71)	97 (10)	738	3	0.7	15.5	6474688	2680726
Oakley Creek	Urban	Hard	1791 (77)	68 (3)	1224	3	1.6	21.4	6479023	2662295
Puhinui Stream	Rural-suburban	Hard	952 (79)	75 (4)	624	3	9.5	37.1	6464804	2680411
Lucas Creek	Rural-suburban	Hard	591 (56)	70 (5)	609	3	2.7	19.9	6496265	2661870
Otara @ Kennell Hill	Rural-suburban	Hard	759 (49)	85 (5)	190	3	2.9	10.4	6470056	2678735
Whangamarie Stream	Rural	Soft	14581 (635)	33 (7)	790	3	9.7	38.9	6446266	2673997
Kaukapakapa River	Rural	Soft	808 (55)	75 (4)	6163	5	12	17.2	6506757	2646355
Ngakaroa Stream	Rural	Soft	3542 (210)	31 (2)	449	2	7	35	6443280	2655825
Wairoa Tributary	Native	Hard	209 (38)	42 (2)	216	2	27.4	92.6	6454464	2697094
West Hoe Stream	Native	Soft	593 (381)	27 (1)	53	1	0.5	51.5	6512307	2658832
Cascades Stream	Native	Hard	148 (25)	37 (3)	1390	2	13.5	125.6	6478297	2645984

Table 2. 2. Characteristics of Christchurch NDS sites, land-use and earthquake influence as described by a collaborator, other descriptors are from GIS based databases (REC AND FWENZ databases).

Site Name	Land-use	Earthquake Influence: Liquefaction	Substrate	Catchment Area (ha)	Stream Order	Distance from sea (km)	Elevation (m)	GPS (NZMG) Easting	GPS (NZMG) Northing
Smacks Creek	Rural-suburban	None	Hard	264	2	23.0	21.2	5749544	2476842
Styx River Upper	Rural-suburban	None	Hard	298	2	22.2	20.0	5748840	2476782
Crosers Stream	Urban-wetland	Heavy	Soft	43	1	5.1	2.8	5745567	2485502
Okeover Stream	Urban	None	Hard	7861	3	24.8	17.9	5742824	2476321
Papanui Stream	Urban	Light	Hard	88	1	17.2	10.6	5745549	2478948
Shirley Stream	Urban	Heavy	Soft	134	1	14.0	6.3	5744081	2482817
St. Albans Stream	Urban	Heavy	Soft	427	1	16.8	8.2	5743675	2480092
Steamwharf Stream	urban	Heavy	Hard	66	1	3.1	0.2	5739503	2484969
Upper Avon River	Urban	None	Hard	495	2	24.2	15.0	5742382	2476239
Upper Heathcote River	Urban	None	Hard	521	2	20.8	19.4	5738513	2475922
Waimairi Stream Tributary	Urban	None	Hard	32	1	23.4	14.8	5743170	2476253
Wairarapa Stream	Urban	Light	Soft	856	3	24.1	12.1	5743547	2477158

2.2.3. Experimental design

Bioassays were used to assess nutrient limitation; experiments were carried out twice in Auckland and once in Christchurch. Historical Auckland Council data spike in nutrient levels in streams over January to February and lower nutrient levels present September to December (Figure 2.2). Consequently, experiments were carried out in Auckland streams during Spring (4th October to the 1st November 2012) and Summer (14th January to the 10th of February 2013). For logistical reasons assays were carried out only once in Christchurch over the Summer season (4th March to the 27th March). Seasonal variation was also intended to capture variation in stream physiochemical variables such as temperature and differences in riparian cover. Water sampling, sediment sampling, and the measurement of physiochemical variables, described in detail in chapter three, were carried out during bioassay incubation. During these periods, there was some heavy rainfall in Auckland over Spring and a lower than average rainfall in Auckland and Christchurch over Summer (Weekes, 2012; Law, 2013).

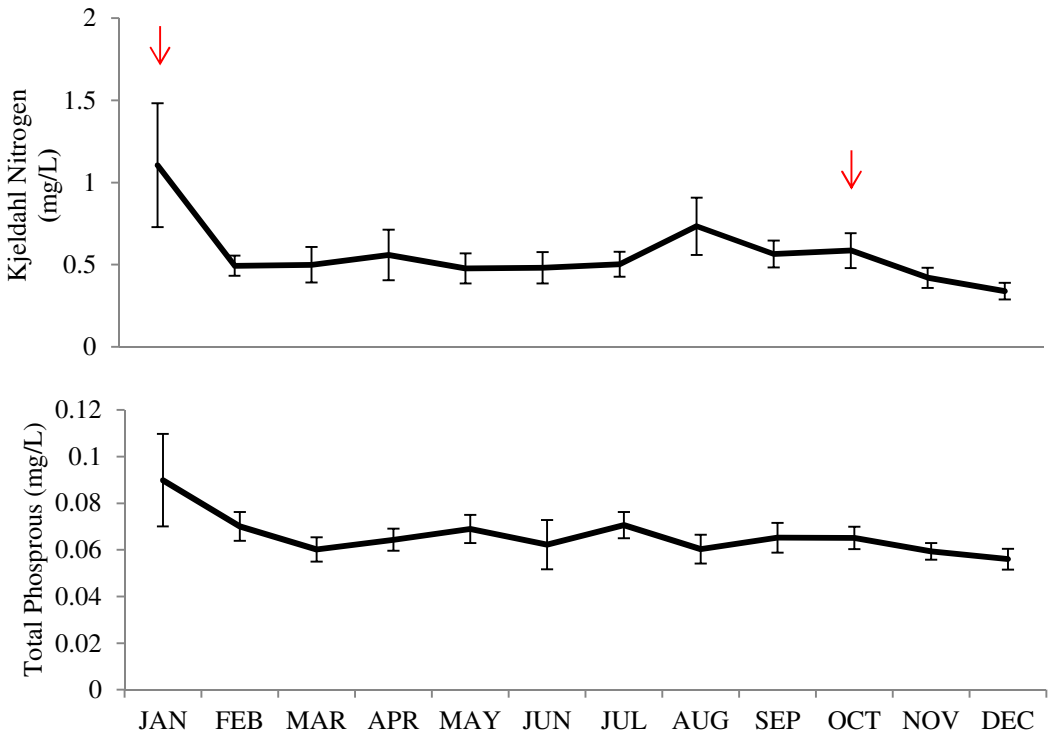


Figure 2. 2. Monthly average (±SE) nutrient trends across Auckland NDS sites from 2002 to 2012. Red arrows indicate the two seasons in which the experiment was carried out in this region.

2.3.4. Bioassays

Bioassays were carried out using nutrient diffusing substrata (NDS), a commonly used methodology for testing *in situ* nutrient limitation of stream biofilms (Tank and Dodds, 2003). NDS were constructed and deployed according methodology as set out in Tank *et al* (2006) with some modifications as described below.

Construction

NDS were 30mL polystyrene plastic containers filled with nutrient amended agar with a total of four treatments: control (agar only), +N (0.5 M KNO_3), +P (0.5 M KH_2PO_4), +NP (0.5 M KNO_3 + 0.5 M KH_2PO_4). Containers were topped with porous surfaces; either glass fritted disks or cellulose sponge cloth to mimic inorganic (rocks) and organic (woody debris) stream substrates respectively. Glass frits were heated on a hot plate before being fused to the openings of the plastic containers filled with agar. When the agar had cooled the containers were flipped upside down leaving the agar to set resting against the glass frit. Cellulose capped containers were made by drilling a 22mm hole into the lids of 28mL polypropylene containers then inserting a 28mm cut out of untreated Wettex© cellulose sponge cloth and filling the container with nutrient amended agar. Sponge was rinsed and soaked in deionized water before use. All containers were marked with coloured tape to indicate the nutrient treatment.

Deployment

At all sites five replicates of each of the four nutrient treatments for both the sponge and glass substrates (n=480/experiment) were attached to a metal rack in a random order using cable ties (Figure 2.3). The racks were the placed in the streams parallel to the flow in riffles, or fast moving areas if riffles were absent, at an area with representative ambient light conditions. Racks were secured to the streambed using re-bar and wire and left in the streams for 21 days to allow time for biofilm colonization (Francoeur *et al.*, 1999; Tank and Dodds, 2003; Tank *et al.*, 2006; Marcarelli and Wurstsbaugh, 2007). NDS incubation times can be shorter or longer, depending on biofilm colonization rates, racks were therefore checked after two weeks of incubation to check on biofilm growth. In Auckland, placement of the racks in the streams was staggered over a week, with two or three placed in per day, depending on site accessibility. This was done to allow time for respiration assays upon removal from the streams. In Christchurch nutrient diffusers were placed in four sites per day over three days.

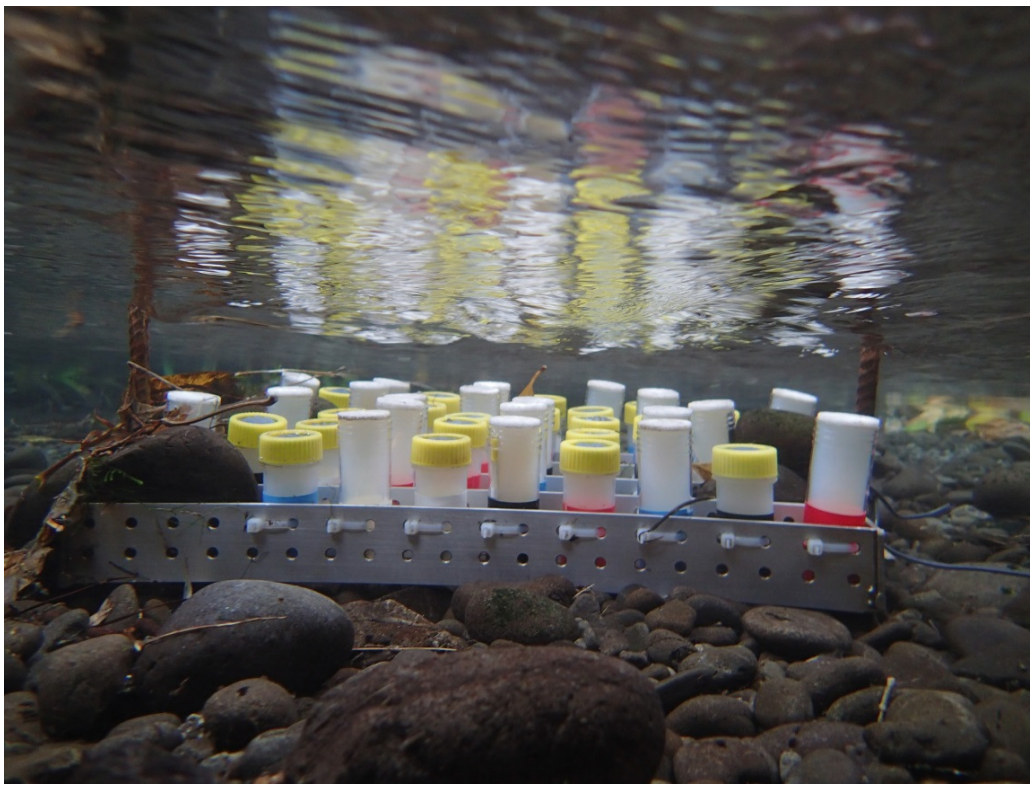


Figure 2. 3. NDS (Nutrient diffusing substrata) sitting on the stream bed of Okeover Stream, Canterbury in March 2013.

Collection

Following in-stream incubation, glass frits and sponges were removed from the containers. Both substrates were placed into labelled bags, and kept on ice until arrival at the laboratory. Cellulose sponges were taken to the lab and respiration assays were immediately carried out. Glass frits were frozen until subsequent chlorophyll *a* analysis. A 2-L water sample was also taken from each site to be used during bioassays and also kept in a chilly bin until use the same day.

2.2.5. Physiochemical measurements

Physiochemical parameters were measured mid-way through NDS incubation and at collection, including measurement of water temperature, dissolved oxygen, pH and conductivity. Water samples were taken once during the NDS incubation and were analysed nitrate + nitrite (NO_X), ammonium (NH_4^+) and phosphate (PO_4^{3-}). Detailed collection and analysis methodologies are as described in chapter three.

Stream habitat was characterised following a modification of habitat assessment protocols P3 and P2 as described by Harding *et al* (2009). Briefly, nine transects were measured at each stream over a reach ten times the width of the stream. Different aspects of river morphology, e.g. riffles, runs, and pools, were explicitly included in this assessment to gauge variation. At each transect stream wetted width, water depth, and velocity were measured at nine

points across the stream, these measurements were used to calculate stream discharge. Velocity was measured using a Marsh McBirney current meter in Auckland. In Christchurch stream velocity was assessed by measuring the time it took a floating object to travel one metre through a run, this was repeated ten times to get an average. This method was used in Christchurch due to equipment interference thought to be caused by copper piping or wiring near the stream. Travel times were converted to surface velocities ($V_{surface}$), calculated as, $V_{surface}$ = travel distance/travel time. Mean velocity was then obtained using a correction factor (k) of 0.85, where V_{mean}= k $V_{surface}$. Sediment size was characterised at the same time as stream depth and velocity measurements were taken, grab samples were taken at each point and characterised using the Udden-Wentworth scale (-12 Φ = boulder to 14 Φ = clay). At each site a reach assessment and a riparian assessment were also carried out. The reach scale assessment involved identifying the proportion of habitats within each reach and noting down the presence of any organic materials such as macrophytes, algae, and woody debris. Semi-quantitative analysis was used to measure riparian habitat, eleven attributes were scored from one to five and an average score calculated. Habitat assessments were carried out in Auckland from the 4th of March to the 8th of March and in Christchurch from the 14th March to the 18th of March.

2.2.6. Sample analysis

Chlorophyll *a* is the main light harvesting accessory pigment present in all green plants and algae, the measurement of this on glass frits gives an indication of algal biomass or primary productivity across nutrient treatments (Kirchman, 2012). Glass frits were analysed for chlorophyll *a* content in accordance with EPA Standard Method 10200 H.3 (APHA, 2000). Chlorophyll was extracted from frits with 10mL of 95% ethanol in a film canister. Canisters were heated to 75˚C in a water bath for two minutes then refrigerated overnight. Extracts were warmed to room temperature then filtered (Whatman® GF/F glass microfiber filter) into a 1cm cuvette and read on a GBC Cintra 2020 UV visible spectrophotometer. The sample was then acidified in the cuvette with 0.1 ml O.1*N* HCL for 90 seconds, to correct for phaeopigments, before being read again. All chlorophyll analysis to extraction was carried out in dim light conditions to avoid changes in absorbance due to photo degradation. Data were expressed as chlorophyll *a* mg/m^3 by relating extracted chlorophyll data to the area of the glass frit (5.73cm^2).

Respiration assays were carried out on sponge biofilms within six hours of removal of the nutrient diffusers from streams by measuring oxygen consumption in closed containers incubated in the laboratory (Niyogi *et al.*, 2003). To measure respiration rate the sponge biofilms were transferred to numbered glass vials, aerated stream water from the same site was added, and the vials were sealed taking care to avoid any oxygen bubbles. Vials were then placed into a

temperature controlled room and left for two hours. An incubation duration of 2 hours was used based on a pilot test using spare diffusers from two sites, one 100% native and the other 100% urban. Incubation temperature was set to at the average temperature of the stream sites at the time of incubation, Auckland Spring and Summer samples were incubated at 15˚C and 20˚C respectively, and Christchurch Summer samples were incubated at 15˚C. Four blank vials, containing stream water only, were included in the respiration assays for each site to account for changes in dissolved oxygen not attributed to the sponge biofilm (e.g. free living microbes). Respiration rates were adjusted for the final surface area of sponge ($\mu g\ O_2/cm^2/h^{-1}$). It was observed that some sponges had holes in, to correct for this sponges were photographed for measurement and analysed using the software Image J (Rasband, 2012). There have been no differences found between expressing respiration rate per unit area or per gram weight (Hoellein *et al.,* 2010).

2.2.7. Statistical analysis

All statistical analyses were completed using the statistical software packages SPSS version 17.0 or SigmaPlot version 12.0. Negative measurements which infer negative biomass or productivity were removed prior to analysis in addition to removing any outliers, which was determined by creating a dot plot of responses for each of the nutrient treatments. To test whether biofilms were significantly affected by N, P, or NP enrichment a two factor analysis of variance (ANOVA) was used, with the addition of either N or P nutrient used as factors (Tank and Dodds, 2003). Following a significant interaction term, a *post-hoc* least-squares mean (LSM) test was carried out to determine which treatments differed significantly, with significance indicated at the $\alpha = 0.05$ level. If data were non-normally distributed (Shapiro-Wilk test $P < 0.05$) it was log transformed prior to analysis. Significance was interpreted following Tank and Dodds (2003). N or P limitation was indicated when either N or P addition had a positive response relative to the controls used. Colimitation was indicated when there was a significant response to N+P enrichment, the N and P additions separately, or if all three of the nutrient additions had a significant response. Primary and secondary limitation was indicated by a positive response from either N or P and a positive response from N+P, where the positive response from N or P determines which of the nutrients was primarily limiting growth. If no significant responses were observed this was classified as not limited by either N or P. Suppression was also noted, where a response to nutrient amendment was significantly less than the control response.

In order to compare limitation results between sites and substrates, the relative magnitude of nutrient limitation was expressed as a nutrient response ratio (RR) (Tank and Dodds, 2003).

NRR's were calculated as the logarithmic ratios (log_{10}) of the nutrient amended values to the average values of the controls (e.g. RR_N = +N/control). Using a log ratio normalises the responses across sites and substrates by expressing the response to nutrient enrichment relative to the controls. Log normalisation also scales no response to 0 while a positive response ratio indicates a stimulatory effect of nutrient enrichment and a negative value indicates suppression by nutrient enrichment.

All physiochemical parameters recorded during NDS incubations along with habitat assessment variables were summarised, and ANZECC trigger values marked where appropriate (ANZECC and ARMCANZ, 2000). Riparian condition was characterised on a 1 (low)-5 (high) scale across multiple parameters. These scores were averaged to an overall riparian score for each site. The inorganic nutrients NO_X and NH_4^+ were added together for analysis as dissolved inorganic nitrogen (DIN) in addition to being analysed separately. Phosphate is presented as soluble reactive phosphate (SRP). Spearman correlations were used to assess relationships between these physiochemical variables, land-use, and magnitude of nutrient limitation (i.e. RR's). Differences in physiochemical parameters between seasons in Auckland were compared using a paired *t*-test. One way analysis of covariance (ANCOVA), with percentage human influence (%urban and % pastoral) as the covariate, was then used to examine if eco-regions had an effect on stream physiochemical characteristics between Auckland and Christchurch Summer incubations, significance was noted from the interaction term.

Simple linear regression was used to quantify relationships between RR's and independent variables such as land-use and stream nutrient concentrations. Sites which showed nutrient suppression across all treatments (+N, +P, +NP) were excluded from analysis as these caused the data to become skewed. Data were log-transformed where appropriate to meet the assumptions of linear regression, namely constant variance (homoscedasticity). In some cases distinct breakpoints were apparent in regressions. These were statistically analysed using piecewise linear regression to denote change points in responses.

Christchurch data were not analysed using linear regressions as there was no continuous gradient in land-use intensity. Land-use data were therefore analysed categorically for both Auckland and Christchurch by splitting sites into land-use categories based on catchment characteristics data and calculating mean response ratios and standard errors. To test for significant differences in RR's between land-use categories a one way ANOVA was used with land-use as a fixed factor and the response ratios (RR_N, RR_P, RR_{NP}) as dependant variables, with significance indicated at the $\alpha = 0.05$ level. In Christchurch where there were only two land-uses

(urban and agricultural) independent sample *t*-tests with non-normal variance were used to compare means between land-use categories ($P < 0.05$). Urban sites in Christchurch were also divided by liquefaction impact as heavy, light, or none. RR's for each of these categories were plotted for comparison along with the RR's from reference sites, significance was determined using a one-way ANOVA and *post-hoc* LSM where the interaction term was significant ($\alpha = 0.05$).

In order to determine if the impact of urbanisation was consistent between Auckland and Christchurch sites categorically defined as urban were isolated and the RR's plotted to compare responses to urbanisation. These were tested for significant differences using an independent samples *t*-test with equal or non-equal variance dependant on the outcome of a Levene's test of equal variance.

2.3. Results

2.3.1. Physiochemical parameters

Physiochemical parameters differed between Spring and Summer incubations in Auckland. At all sites temperature was highest over Summer (19 ± 0.6˚C) compared to Spring (14 ± 0.5˚C) (*t*-test, $P < 0.0001$), coinciding with a drop in dissolved oxygen from Spring (9.5 ± 0.6 mg/L) to Summer (6.7 ± 0.6 mg/L) (*t*-test, $P = 0.024$). Stream pH was significantly higher in Spring (7.5 ± 0.07) compared to Summer (7.3 ± 0.08) (*t*-test, $P = 0.012$). SRP levels were 64% higher in Summer (14.3 ± 2.0 µgP/L) than Spring (9.2 ± 3.2 µgP/L) (*t*-test, $P = 0.026$). However, concentrations of NO_X were similar in both seasons (*t*-test, $P = 0.154$).

Between regions in Summer temperature and conductivity were significantly higher in Auckland than in Christchurch (one-way ANCOVA, $P < 0.0001$ and $P < 0.009$ respectively) (Table 2.3 & 2.4). Whereas, stream discharge was significantly higher in Christchurch than in Auckland (one-way ANCOVA, $P = 0.017$); with a mean of 102 L/s in Christchurch and 37 L/s in Auckland. Nutrient concentrations did not significantly differ between regions, as both included a large range of concentrations (ANCOVA, DIN: $P = 0.556$, SRP: $P = 0.683$).

Auckland NO_X concentrations ranged from <5 to 13950µgN/L in Spring and <5 to 1170µgN/L in Summer; in both seasons the highest concentration was found at Whangamarie stream. Christchurch concentrations ranged between 96 and 521µgN/L, with the maximum concentration coming from the Upper Avon River. Whilst SRP concentrations reached maximum values of 28 and 31µgP/L in Auckland Spring and Summer respectively, and 34µgP/L in Christchurch. Ratios of DIN:SRP ranged from 1:1 to 4906:1 in Auckland, with ratios

generally increasing with human land-use. An exception was Kaukapakapa (pastoral) which had low DIN:SRP ratios compared with other pastoral sites (Table 2.3). Christchurch DIN:SRP ratios ranged from 31:1 to 2409:1. In Christchurch, higher DIN:SRP ratios were associated with a greater land-use intensity; with the lowest ratios from reference sites (rural-suburban).

Trigger values were most often exceeded by NO_X concentrations in Auckland; all of these came from urban or pastoral land-uses (Table 2.3). SRP exceeded trigger values more often in Summer than in Spring. Christchurch showed the same number of exceedances for both NO_X and SRP with reference sites (rural-suburban) never exceeding guideline values (Table 2.4). Concentrations of NH_4^+ only exceeded guidelines in urban sites, with values reaching up to 363µgN/L in Christchurch and 118µgN/L in Auckland. In both regions pH was circumneutral with an average of 7.3 in both cities, there were however a few sites with pH values outside of the suggested guidelines.

Table 2. 3. Summary of physiochemical variables in Auckland during NDS incubation, with standard error in parentheses, <5 indicates that the sample was under the instrument detection limit. Sites are coded with land-uses as either reference (REF), urban (U), suburban (SU), pastoral (P). Dashed lines (-) indicates no available data. Values marked with a star (*) are over ANZECC & ARMCANZ (2000) trigger values; NO_X=444 µg/l, SRP =10 µg/l, NH_4^+=21 µg/l, pH= 7.2-7.8).

Site Name	Season	DO (mg/L)	Temp (˚C)	pH	Conductivity (µS/cm)	Discharge (L/s)	SRP (µgP/L)	NO_X (µgN/L)	NH_4^+ (µgN/L)	DIN: SRP
Cascades Stream (REF)	Spring	11.05(0.06)	11.7(0.2)	7.9(0.1)*	128(3)	-	8	<5	<5	1
	Summer	9.60(0.05)	17.5(0.9)	8.1(0.8)*	95(35)	89(19)	11*	<5	<5	1
West Hoe (REF)	Spring	9.55(0.01)	13.0(0.6)	7.2(0.0)	161(10)	-	<5	<5	<5	5
	Summer	8.40(0.19)	15.1(0.3)	7.1(0.2)*	151(11)	0.3(0.3)	6	<5	<5	2
Wairoa Trib (REF)	Spring	10.19(0.42)	12.2(0.0)	7.4(0.2)	115(5)	-	28*	52	<5	4
	Summer	9.62(0.22)	15.4(0.5)	7.4(0.1)	107(13)	29(16)	41*	95	<5	5
Otaki Stream (U)	Spring	5.05(2.35)	13.2(0.2)	7.4(0.1)	726(148)	-	5	2332*	82*	1016
	Summer	8.32(3.53)	22.9(0.6)	7.0(0.4)*	875(132)	0.6(0.1)	13*	477*	89*	97
Oakley Creek (U)	Spring	10.32(0.10)	14.4(0.0)	7.8(0.2)	180(19)	-	16*	984*	<5	138
	Summer	7.93(0.68)	19.5(0.6)	7.2(0.1)	162(60)	17(3)	14*	658*	<5	108
Pakuranga Creek (U)	Spring	8.14(0.85)	16.2(0.1)	7.3(0.0)	227(29)	-	<5	975*	64*	571
	Summer	5.72(0.90)	20.5(0.4)	7.2(0.2)	198(12)	7(4)	10	334	118*	104
Otara Creek (SU)	Spring	7.76(3.16)	17.0(0.0)	7.6(0.1)	390(172)	-	9	92	<5	23
	Summer	4.84(0.35)	20.2(0.7)	7.1(0.1)*	562(386)	11(2)	7	6	<5	3
Puhinui Stream (SU)	Spring	8.72(1.23)	12.2(0.0)	7.3(0.2)	242(67)	-	9	568*	8	145
	Summer	7.66(0.90)	20.5(2.3)	7.2(0.4)	508(356)	2(0.4)	31*	392	10	29
Lucas Creek (SU)	Spring	10.08(0.22)	14.9(0.01)	7.6(0.3)	195(8)	-	5	166	<5	72
	Summer	6.34(0.38)	18.5(0.3)	7.4(0.1)	188(3)	6(1)	11*	64	17	16
Ngakaroa Stream (P)	Spring	9.72(0.04)	14.5(0.0)	7.4(0.1)	297(148)	-	<5	6275*	<5	3173
	Summer	6.83(0.62)	18.8(0.1)	7.1(0.1)*	544(407)	237(234)	<5	7199*	<5	4059
Whangamarie Stream (P)	Spring	14.15(1.95)	12.8(3.4)	7.2(0.2)	242(35)	-	6	13950*	<5	4906
	Summer	2.53(2.16)	19.6(0.2)	7.2(0.1)	207(383)	30(10)	6	11750*	<5	4129
Kaukapakapa River (P)	Spring	9.22(0.03)	14.4(0.0)	7.7(0.1)	196(10)	-	12*	121	10	24
	Summer	5.14(0.73)	18.5(0.6)	7.1(0.2)*	176(24)	2(0.6)	18*	16	9	3

Table 2. 4. Summary of physiochemical variables in Christchurch during NDS incubation, with standard error in parentheses, <5 indicates that the sample was under the instrument detection limit. Sites are coded with land-uses as either reference (REF), rural-suburban (RS), urban-wetland (UW), or urban (U). Liquefaction intensity is noted at HL (heavy liquefaction) or LL (light liquefaction). Values marked with a star (*) are over ANZECC & ARMCANZ (2000) trigger values; NO_X=444 µg/l, SRP =10 µg/l, NH_4^+=21 µg/l, pH= 7.2-7.8.

Site Name	DO (mg/L)	Temp (˚C)	pH	Conductivity (µS25/cm)	Discharge (L/s)	SRP (µgP/L)	NOx (µgN/L)	NH_4^+ (µgN/l)	DIN: SRP
Smacks Creek (REF, RS)	5.23(0.34)	15.3(0.6)	7.0 (0.2)*	113 (0)	85(10)	8	439	<5	118
Styx River Upper (REF, RS)	5.21(0.37)	12.9(0.0)	7.2 (0)	118(1)	117(4)	5	709	<5	303
Crosers Stream (UW, HL)	5.15(1.05)	16.9(0.6)	7.5 (0.3)	395 (107)	334(10)	17*	237	<5	31
Papanui Stream (U, LL)	6.11(0.12)	13.6(0.3)	7.1(0.1)*	99 (32)	109(18)	8	424	25*	448
Shirley Stream (U, HL)	7.95(0.0)	15.9(1.3)	7.6 (0.1)	185 (5)	65(7)	27*	430	143*	48
Okeover Stream (U)	9.14(0.20)	14.1(0.2)	7.2 (0.1)	169 (1)	107(7)	9	4387*	<5	1100
St. Albans Stream (U, HL)	7.76(0.0)	15.2(0.6)	7.4 (0.1)	274(8)	33(3)	<5	96	363*	266
Steamwharf Stream (U, HL)	7.22(0.59)	14.9(0.5)	7.6 (0.1)	131(38)	57(8)	14*	752*	8	1212
Upper Avon River (U)	6.44(0.37)	13.8(0.2)	6.9(0.1)*	173(1)	131(22)	5	5214*	6	2409
Upper Heathcote River (U)	10.96(1.25)	16.3(1.3)	7.7(1.3)	277(6)	92(8)	30*	2513*	23*	184
Waimairi Stream (U)	8.41(0.09)	14.9(10.4)	7.1(0.4)*	173(1)	103(3)	34*	1802*	21	119
Wairapapa Stream (U, LL)	7.85(0.34)	13.8(0.4)	7.3(0.4)	146(1)	82(7)	10	886*	15	196

2.3.2. Land-use correlations

Auckland native and urban land-uses demonstrated the strongest relationships to nutrient concentrations (Table 2.5). Most physiochemical variables, with the exception or riparian condition, were negatively correlated with the extent of native vegetation. These sites showed strong negative correlations with variables; DIN, DIN:SRP, conductivity, sediment size, and a positive correlation with riparian condition; consistent with the expectations of reference sites. In contrast, increasing urban land-use percentage was associated with increased DIN, DIN:SRP, conductivity, sediment size, and negative correlation with riparian condition. Pastoral land-use showed similar trends to urban, there were however fewer significant trends in this category. Variables showed weaker correlations with pastoral land-use than urban with the exception of riparian condition, this decreased with increasing pastoral land-use.

Christchurch correlations are not shown for land-use percentages due to there being few instances of significance, likely related to the lack of gradient within the data and the dominance of rural land-use at reference sites. There were only two significant correlations between land-use and physiochemical variables in Christchurch. These were between the extent rural-suburban (reference) land-use and discharge (r_s = 0.683, P = 0.0126) and depth (r_s = 0.599, P = 0.0359).

Table 2. 5. Spearman correlations (r_s) between land-use (%) and physiochemical variables for Auckland, non-significant variables not shown, significance is noted as: $P < 0.05$=*, $P < 0.005$, $P < 0.0005$, ns= not significant.**

Variable	Land-use		
	% Native	% Urban	% Pastoral
DIN (µgN/L)	-0.821***	0.852***	0.529*
NH_4^+ (µgN/L)	-0.453*	0.727***	ns
NOx (µgN/L)	-0.813***	0.835***	ns
SRP (µgP/L)	ns	ns	ns
DIN:SRP	-0.809***	0.852***	0.481*
Conductivity (µS/cm)	-0.767***	0.696**	0.716**
Sediment size (Φ)	-0.594**	0.542*	ns
Riparian condition	0.599**	-0.763***	-0.840***

2.3.3. Nutrient limitation as indicated by community respiration and chlorophyll *a*

Heterotrophic activity (respiration) on organic substrata consistently indicated nutrient limitation (Table 2.6). In Auckland N limitation was dominant at reference sites and P limitation at urban and pastoral sites, suggesting a switch in limitation with modifications to land-use. Secondary limitation was the most common response in Auckland, this was more common in Spring than in Summer when seven of twelve sites showing this. There were two instances of no limitation of organic substrates in both Spring and three in Summer, these came from the same two sites; one urban (Otaki) and one pastoral (Kaukapakapa). Notably, Kaukapakapa had very low DIN:SRP ratios compared to other sites in the same land-use classification (Spring: 24:1; Summer: 3:1). Colimitation was common amongst Auckland suburban streams in both seasons, becoming more prominent in Summer. Puhinui (suburban) and Ngakaroa (pastoral) Streams switched from primarily P limited to co-limited in Summer, possibly related to increased water column N in Summer (Table 2.3).

Nutrient limitation patterns were less coherent in Christchurch where there are no true reference systems (Table 2.6). Christchurch reference (rural-suburban) sites both showed some form of P limitation on organic substrates (P limited or colimited). There were four instances of colimitation in Christchurch, most common in urban sites although a reference site also exhibited this response. The most common response in Christchurch was no limitation, interestingly four of the five sites which displayed this response were also affected by heavy liquefaction Interestingly, suppression effects (N, P, or both) were reasonably common in human-dominated catchments and liquefaction sites, but not in reference catchments in Auckland and Christchurch.

Autotrophic activity (chlorophyll *a*) on inorganic substrates showed few instances of nutrient limitation, with only 10 of a potential 36 sites showing any form of limitation (73% not limited)

(Table 2.6). Where limitation did occur N limitation was the most common response. Nutrient suppression was less common on inorganic substrates, with P suppression the most common response. Few sites showed the similar limitation patterns on both inorganic and organic substrates, Cascades Stream in Spring (native) and Wairoa Tributary (native) in Summer demonstrated N limitation on both surfaces, and the Styx River (rural- suburban) in Christchurch was consistently P limited.

Table 2. 6. Nutrient limitation indicated by nutrient diffusing substrata for respiration (organic cellulose sponse) and chlorophyll *a* (inorganic glass frits) in Auckland (Spring and Summer) and Christchurch (Summer), no limitation is indicated by a dashed line (-). Sites are coded with land-uses as either reference (REF), native (N), suburban (SU), urban (U), Rural-suburban (RS), Urban-wetland (UW), or pastoral (P). Liquefaction sites are also noted at heavy liquefaction (HL) or light liquefaction (LL).

Region/ Season	Stream	Respiration/ Organic substrata		Chlorophyll *a*/ Inorganic substrata	
		Limitation	Suppression	Limitation	Suppression
Auckland Spring	Cascades Stream (REF, N)	1˚N, 2˚P limited	-	N limited	-
	West Hoe (REF, N)	1˚N, 2˚P limited	-	-	-
	Wairoa Tributary (REF, N)	1˚N, 2˚P limited	-	-	-
	Otaki Stream (U)	-	-	-	
	Pakuranga Creek (U)	1˚P, 2˚N limited	-	-	-
	Oakley Creek (U)	Colimited	-	-	-
	Puhinui Stream (SU)	1˚P, 2˚N limited	-	-	-
	Lucas Creek (SU)	Colimited	-	N limited	-
	Otara Creek (SU)	Colimited	-	-	-
	Whangamarie Stream (P)	1˚P, 2˚N limited	-	Colimited	-
	Kaukapakapa River (P)	-	N, P, and NP	-	-
	Ngakaroa Stream (P)	1˚P, 2˚N limited	-	-	-
Auckland Summer	Cascades Stream (REF, N)	Colimited	-	N limited	-
	West Hoe (REF, N)	1˚N, 2˚P limited	-	-	-
	Wairoa Tributary (REF, N)	1˚N, 2˚P limited	-	N limited	-
	Otaki Stream (U)	-	N, P, and NP	-	-
	Pakuranga Creek (U)	-	-	-	P
	Oakley Creek (U)	P limited	-	-	-
	Puhinui Stream (SU)	Colimited	-	-	-
	Lucas Creek (SU)	Colimited	-	N limited	-
	Otara Creek (SU)	Colimited	-	-	N
	Whangamarie Stream (P)	P limited	-	-	-
	Kaukapakapa River (P)	-	N and P	Co-limited	-
	Ngakaroa Stream (P)	Colimited	-	-	-
Christchurch Summer	Styx River Upper (REF, RS)	P limited	-	P limited*	-
	Smacks Creek (REF, RS)	Colimited	-	-	-
	Papanui Stream (U, LL)	1˚P, 2˚N limited	N	-	-
	Okeover Stream (U)	Colimited	-	-	P
	Steamwharf Stream (U, HL)	-	N and P	N limited	-
	Crosers Stream (UW, HL)	-	N	-	P
	Shirley Stream (U, HL)	-	N	N limited	-
	St. Albans Stream (U, HL)	-	N	-	-
	Upper Avon River (U)	Colimited	-	-	-
	Waimairi Stream (U)	P limited	-	-	P
	Wairapapa Stream (U, LL)	Colimited	-	-	-
	Upper Heathcote River (U)	-	P	-	-

2.3.4. Response ratios and land-use: categorical relationships

Auckland

Seasonal differences existed in the magnitude of biofilm N and P limitation, with RR's typically higher in Spring on organic substrates (Figure 2.4). When analysed across land-use categories the magnitude of P limitation (RR_P) (*t*-test, $P = 0.032$) and the magnitude of colimitation (RR_{NP}) (*t*-test, $P = 0.038$) were higher in Spring than in Summer. No significant difference existed between the magnitude of N limitation (RR_N) between Spring and Summer (*t*-test, $P > 0.05$). In contrast, on inorganic substrata there were no significant differences in response ratios between Spring and Summer.

In Spring RR_N was strongly related to percentage native land-use compared to all other land-use categories where RR_N was close to zero (no response) (ANOVA, urban: $P = 0.025$; suburban: $P = 0.023$; pastoral: $P = 0.004$). In contrast, RR_P was weakly related to native land-use (response at 0) but strongly related to pastoral land-use (ANOVA, $P = 0.014$). Urban, suburban, and native land-uses demonstrated similarly strong limitation response to RR_{NP} than any other treatment (ANOVA, $P > 0.05$). There were no significant differences in RR_{NP} among any of the categories (ANOVA, $P > 0.05$), with this treatment showing consistently positive responses.

In Summer RR_N was also strongly related to the percentage native land-use in the catchment compared to all other land-use categories (ANOVA, urban: $P = 0.011$, suburban: $P = 0.004$, pastoral: $P = 0.004$) (Figure 2.4). In contrast, pastoral, suburban, and urban land-uses were weakly related to RR_N, with no significant differences between these (ANOVA, $P > 0.05$). Pastoral land-use demonstrated strongest P limitation this was significantly stronger than P limitation at suburban (ANOVA, $P = 0.004$) and native sites (ANOVA, $P = 0.010$), but not urban sites (ANOVA, $P = 0.092$). Organic RR_P was significantly stronger at urban than suburban sites (ANOVA, $P = 0.035$). Biofilm RR_P and RR_{NP} produced equally strong responses between pastoral and urban land-uses (ANOVA, $P > 0.05$). Within categories RR_{NP} was significantly stronger than RR_N in native (ANOVA, $P = 0.004$), suburban (ANOVA, $P = 0.028$), and pastoral (ANOVA, $P = 0.041$), land-use categories. Additionally, RR_{NP} is stronger than RR_P in suburban (ANOVA, $P = 0.026$), native (ANOVA, $P < 0.0001$), and urban (ANOVA, $P = 0.039$) land-use categories.

On inorganic substrates land-use categories were not significantly related the magnitude of N or P limitation in Spring or Summer (ANOVA, $P > 0.05$) (Figure 2.4). In both seasons urban land-use showed little response to any of the nutrient treatments, with averages centred at approximately 0. Like on organic substrata RR_{NP} was consistently strong across land-uses in

Spring, and native sites demonstrated their strongest limitation response to RR_N. Over Summer only native land-use demonstrated strong N and P limitation responses compared to all other categories, which demonstrated no or negative responses.

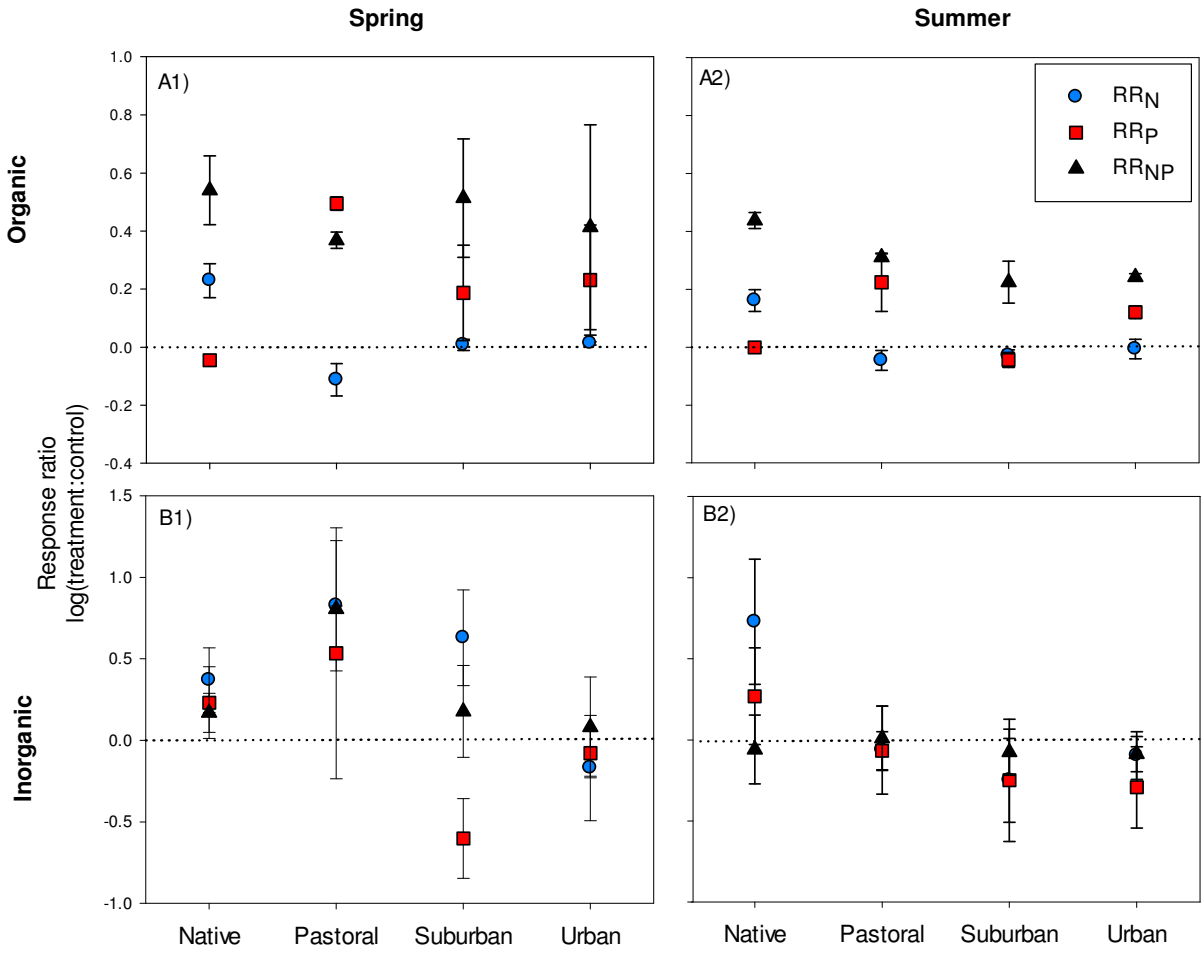


Figure 2. 4. Average (±SE) response ratios on organic (A1, A2) and inorganic substrates (B1, B2) by land-use category in Auckland Spring and Summer. Response ratios are noted in the legend for nitrogen (RR_N), phosphorus (RR_P), and nitrogen and phosphorus enriched biofilms (RR_{NP}). Dashed line at the response ratio of 0 indicates no response. Note the difference in scale on the Y-axis.

Christchurch:

Between land-use categories in Christchurch organic RR_N was always negative, but was weaker in urban sites compared to rural-suburban (*t*-test, $P = 0.024$) (Figure 2.5). There were no differences in RR_P between land-use categories (*t*-test, $P = 0.218$). RR_{NP} demonstrated the strongest responses across both of the categories, this response was significantly stronger in rural-suburban sites (*t*-test, $P = 0.024$). Within each of the categories, urban and rural-suburban, RR_{NP} was stronger than RR_N (*t*-test, $P = 0.002$, $P = 0.05$ respectively). Urban land-use demonstrated a weak RR_P, with a mean value of -0.008.

The RR_P on inorganic biofilms was strongly related to the percentage rural-suburban land-use in the catchment, and was weakly related to urban land-use (*t*-test, $P = 0.024$). Christchurch urban sites showed no positive responses for any of the nutrient additions, RR_P was negative (suppressed); this was weaker than RR_N and RR_P (*t*-test, $P = 0.029$). Respiration results show clearer trends in the data, with few significant results coming from chlorophyll *a* analysis.

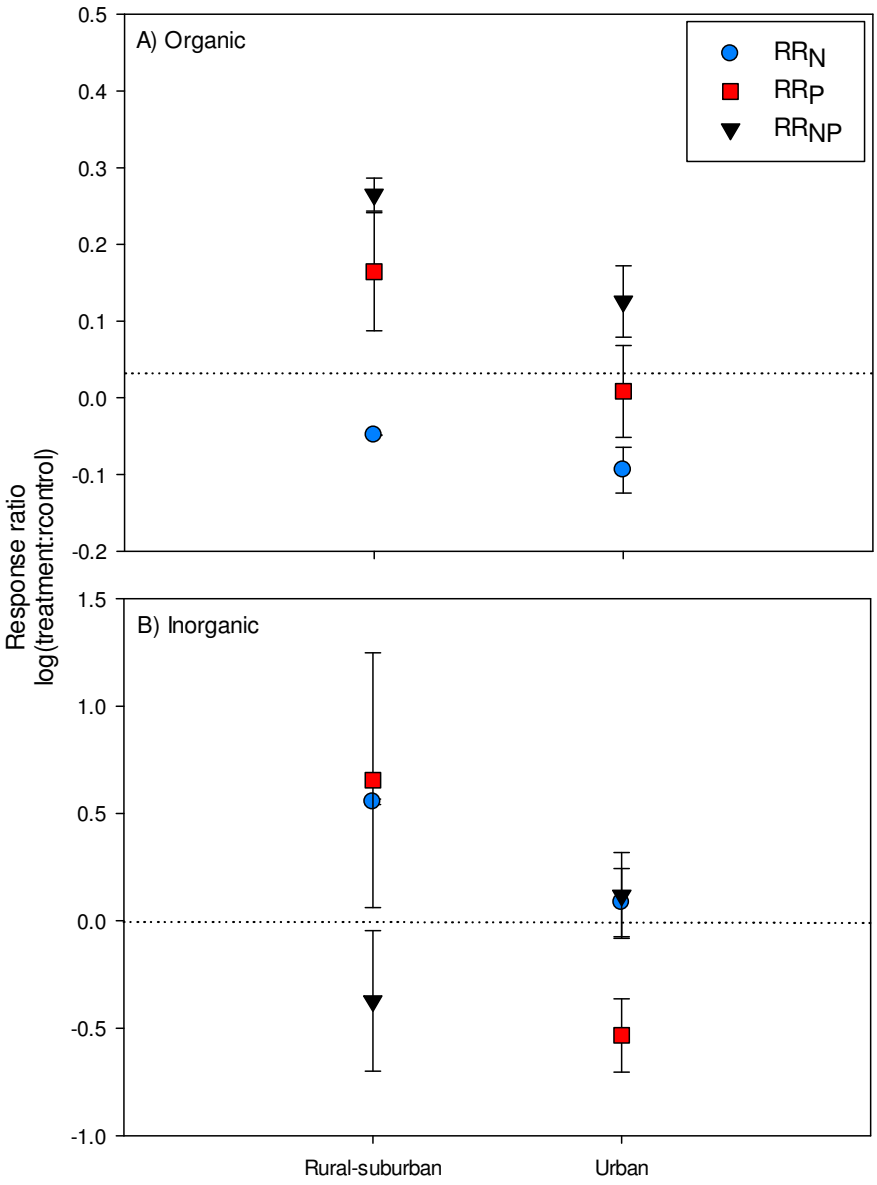


Figure 2. 5. Average (±SE) response ratios grouped by land-use category in Christchurch on organic (A) and inorganic (B) substrates. Dashed line at the response ratio of 0 indicates no response. Note the difference in scale on the Y-axis.

2.3.5. Response ratio correlations

Auckland

The magnitude of N and P limitation of microbial biofilms on organic substrates was strongly related to the amount of native vegetation in the catchment in both seasons. In particular, RR_N increased with increasing native land cover (Table 2.7). At the same time, RR_P declined with increasing native land cover. Trends shown by urban land-use are in opposition to those shown by native. Urban land-use was negatively correlated with RR_N, showed a positive RR_P in Spring ($r_s = 0.778$, $P = 0.025$) and RR_{NP} in Summer ($r_s = -0.712$, $P = 0.025$). In contrast, sites with stronger RR_P tended to have increased DIN in Spring and Summer, conductivity in Spring, and a decrease in stream riparian quality in Spring. Pastoral land-use was also positively correlated with RR_P in Spring and negatively with RR_N in Spring and Summer. Temperature was positively correlated with RR_{NP} in Summer, but not Spring. Responses differed between seasons, with correlations of a higher significance in Spring. The magnitude of N or P limitation on inorganic substrates was not significantly correlated with physiochemical variables or land-use in Auckland Spring (Table 2.7). In Summer inorganic RR_N was inversely related to the amount of pastoral land-use in the catchment, temperature, and positively related to stream pH.

Christchurch

Organic RR_N was positively related to water column NO_X and DIN:SRP, contrary to Auckland (Table 2.8). Temperature was negatively correlated with both RR_P and RR_{NP} and stream pH was negatively correlated with RR_N, RR_P, and RR_{NP}. On inorganic substrates pH and temperature were positively correlated with RR_{NP}; there were no other significant correlations.

Table 2. 7. Spearman correlations (r_s) in Auckland between nutrient response ratios (NRR) and physiochemical variables, non-significant variables not shown, significance is noted as: $P < 0.05$=*, $P < 0.005$, $P < 0.0005$, ns= not significant. Figures in bold indicate those that are only significant in that season. No significant correlations existed between chlorophyll *a* and stream variables in Spring.**

Parameter	Season	Variable	Response Ratio		
			RR_N	RR_P	RR_{NP}
Respiration	Spring	% Urban	-0.852**	0.778*	ns
		% Native	0.667*	-0.717*	ns
		% Pastoral	-0.707*	0.763*	ns
		%Horticulture	-0.707*	0.707*	ns
		NH_4^+(µgN/L)	ns	ns	ns
		NO_X (µgN/L)	-0.867***	0.883**	ns
		DIN (µgN/L)	-0.833***	0.917***	ns
		SRP (µgP/L)	ns	ns	ns
		DIN:SRP	-0.883***	0.867***	ns
		DO (mg/l)	ns	ns	ns
		Temperature (˚C)	-0.650*	ns	ns
		Conductivity (µS/cm)	-0.783*	0.767*	ns
		Riparian score	0.962***	-0.812**	ns
		Sediment size (Φ)	ns	ns	ns
	Summer	% Urban	-0.780*	ns	-0.712*
		% Native	0.745*	-0.697*	ns
		% Pastoral	-0.693*	ns	ns
		% Horticultural	ns	ns	ns
		NH_4^+ (µgN/L)	-0.636*	ns	ns
		NO_X (µgN/L)	-0.636*	0.685*	ns
		DIN (µgN/L)	-0.624*	0.745*	ns
		SRP (µgP/L)	ns	ns	ns
		DIN:SRP	-0.636*	0.697*	ns
		DO (mg/l)	0.818**	ns	ns
		Temperature (˚C)	-0.888***	ns	-0.900***
		Conductivity (µS/cm)	-0.758**	ns	ns
		Riparian score	0.675*	ns	ns
		Sediment size (Φ)	-0.602*	ns	ns
Chlorophyll *a*	Summer	% Pastoral	-0.632*	ns	ns
		Temperature (˚C)	-0.717*	ns	ns
		pH	0.745*	ns	ns
		Conductivity (µS/cm)	-0.830**	ns	ns
		Discharge (L/s)	ns	-0.697*	ns
		Width (m)	ns	-0.758*	ns
		Depth (m)	ns	-0.681*	ns

Table 2. 8. Spearman correlation (r_s) in Christchurch between nutrient response ratios (NRR) and physiochemical variables, non-significant variables not shown, significance is noted as: $P < 0.05$=*, $P < 0.005$, $P < 0.0005$, ns= not significant.**

Parameter	Variable	Response Ratio		
		RR_N	RR_P	RR_{NP}
Respiration	NH_4^+ (µgN/L)	ns	ns	ns
	NO_X (µgN/L)	0.601*	ns	ns
	SRP (µgP/L)	ns	ns	ns
	DIN:SRP	0.643*	ns	ns
	DO (mg/l)	ns	ns	ns
	pH	-0.608*	-0.713*	-0.699*
	Conductivity (µS/cm)	ns	ns	-0.741**
	Temperature (ºC)	ns	-0.712*	-0.758**
	Sediment size (Φ)	-0.570*	ns	ns
Chlorophyll *a*	NH_4^+ (µgN/L)	ns	ns	ns
	NO_X (µgN/L)	ns	ns	ns
	SRP (µgP/L)	ns	ns	ns
	DIN:SRP	ns	ns	ns
	DO (mg/l)	ns	ns	0.580*
	pH	ns	ns	0.713*
	Conductivity (µS/cm)	ns	ns	ns
	Temperature (ºC)	ns	ns	ns
	Sediment size (Φ)	ns	ns	ns

2.3.6. Response ratios across a land-use gradient

Analysis of Auckland land-use data revealed significant relationships between land-use percentage and RR_N for urban and pastoral land-uses. The relationship between N limitation and extent of urban land-use was distinctly non-linear (Figure 2.6A). At low percentages of urban land-use (native/references sites) RR_N shows positive responses, dropping to 0 (no response) at an urbanisation of 30% after which there was no indication of N limitation ($r^2 = 0.7699$, $P = 0.0008$). The regression model predicts a breakpoint at 17% urbanisation. However, there are no sites between 10-30% urbanisation so 30% may be a more reliable estimate. In contrast, at low percentages of urbanisation RR_P shows no response (sitting at an RR of 0) this increases with urbanisation percentage (Figure 2.7).

At low percentages of pastoral land-use N limitation was stronger and limitation became weaker and negative with increasing pastoral land-use ($r^2 = 0.6306$ $P = 0.0001$) (Figure 2.6B). When fitted with a piecewise regression model a breakpoint is predicted at 25% pastoral land-use after which there is no indication of N limitation ($r^2 = 0.6977$, $P = 0.0011$). Native land-use did not cover a gradient as therefore couldn't be fitted with a regression model (but see Table 2.7).

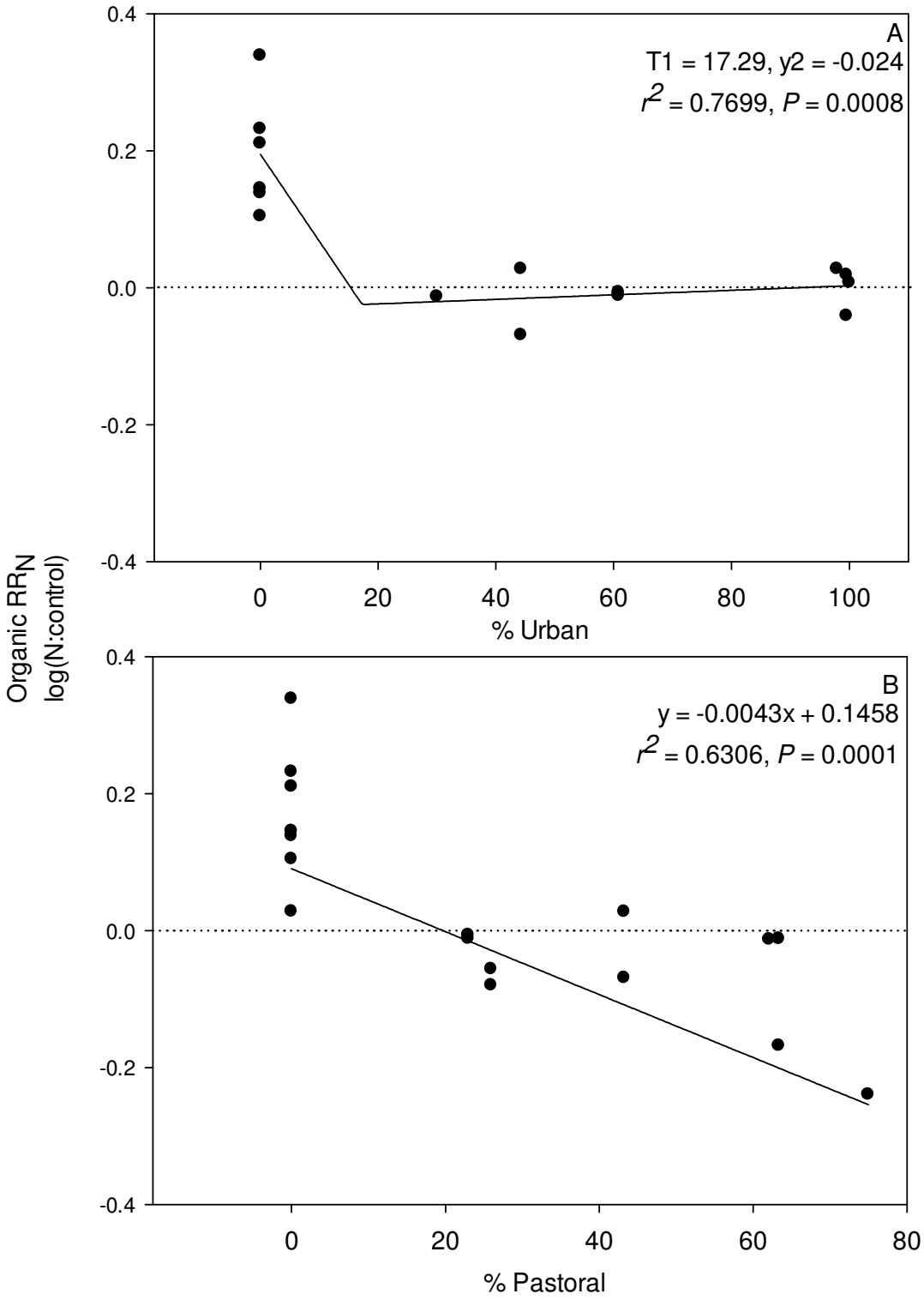


Figure 2. 6. Relationship between land-use and the response ratio of N on organic substrates in Auckland Spring and Summer, with two segment piecewise linear regression models fitted for A) urban (linear fit: r^2 = 0.5166, P = 0.0025) and B) pastoral land-use (piecewise fit: r^2 = 0.6977, P = 0.0011, T1 = 24.95, y2 = -0.025). T1 indicates the breakpoint value on the x-axis and y2 indicates the breakpoint on the y-axis. Dashed line at the response ratio of 0 indicates no response.

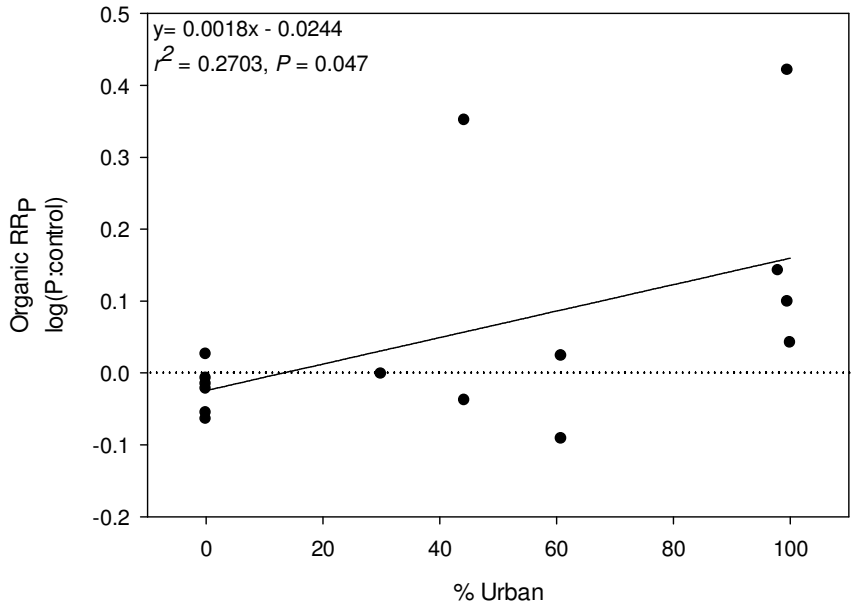


Figure 2. 7. Relationship between urban land-use and the response ratio of phosphorus (RR_P) on organic substrates in Auckland Spring and Summer with a linear regression model fitted. The dashed line at response ratio 0 indicates no response.

2.3.7. Response ratios and water chemistry

In Auckland, RR's on organic biofilms were best explained by water DIN concentrations in both seasons. Sites with low water column DIN showed the strongest responses to RR_N, where concentrations DIN explained 74% of the variation in response ratios in Spring, and 43% in Summer (Figure 2.8, 2.9 A1). Similarly when the molar ratio of inorganic nutrients (DIN:SRP) was low RR_N was higher. In general, sites showing N limitation fell under the Redfield ratio and sites above this ratio showed no response or a supressed response to N (Figures 2.8 & 2.9 A2). This pattern was found in Auckland over both Spring and Summer, but the trend can be explained with a higher degree of confidence in Spring (Figure 2.8, 2.9 A2). In contrast, RR_P was stronger with high water column DIN and molar DIN:SRP ratios in Spring and Summer (Figure 2.8 & 2.9 B1, B2). All sites over the Redfield ratio in Spring showed positive responses, with those below all sitting at around 0 (no response), this was similarly the case in Summer with a few exceptions (Figure 2.8 & 2.9 B2). Water SRP concentrations were not related to RR's in Spring or Summer.

In contrast, Christchurch RR_N was positively related to water DIN concentrations, explaining 53.5% of the variation in biofilm response (Figure 2.10A1). Sites with low water DIN:SRP ratios demonstrated weaker N limitation on organic biofilms, contrary to expectations (Figure 2.10 B1). Notably, all sites were unlimited by N (Okeover and Upper Avon) or demonstrated N suppression. DIN concentrations in Christchurch started much higher (lowest

concentration was 240µgN/L), sites with similarly high DIN concentrations in Auckland also demonstrated suppression. However, the most suppressed sites had the lowest DIN concentrations with the suppression effect weakening within increasing DIN, contrary to expectations. Water column N and P were not significantly related RR_P on organic biofilms (Figure 2.10 A2 & B2).

Water chemistry was a poor predictor of RR's on inorganic biofilms; there were no significant relationships in Auckland Spring or Christchurch Summer. In Auckland Summer sites strongly colimited had low SRP concentrations (r^2 = 0.452, P = 0.033) (Figure 2.11). Suppression was for evident for RR_{NP} in sites with SRP concentrations above approximately 12µgP/L.

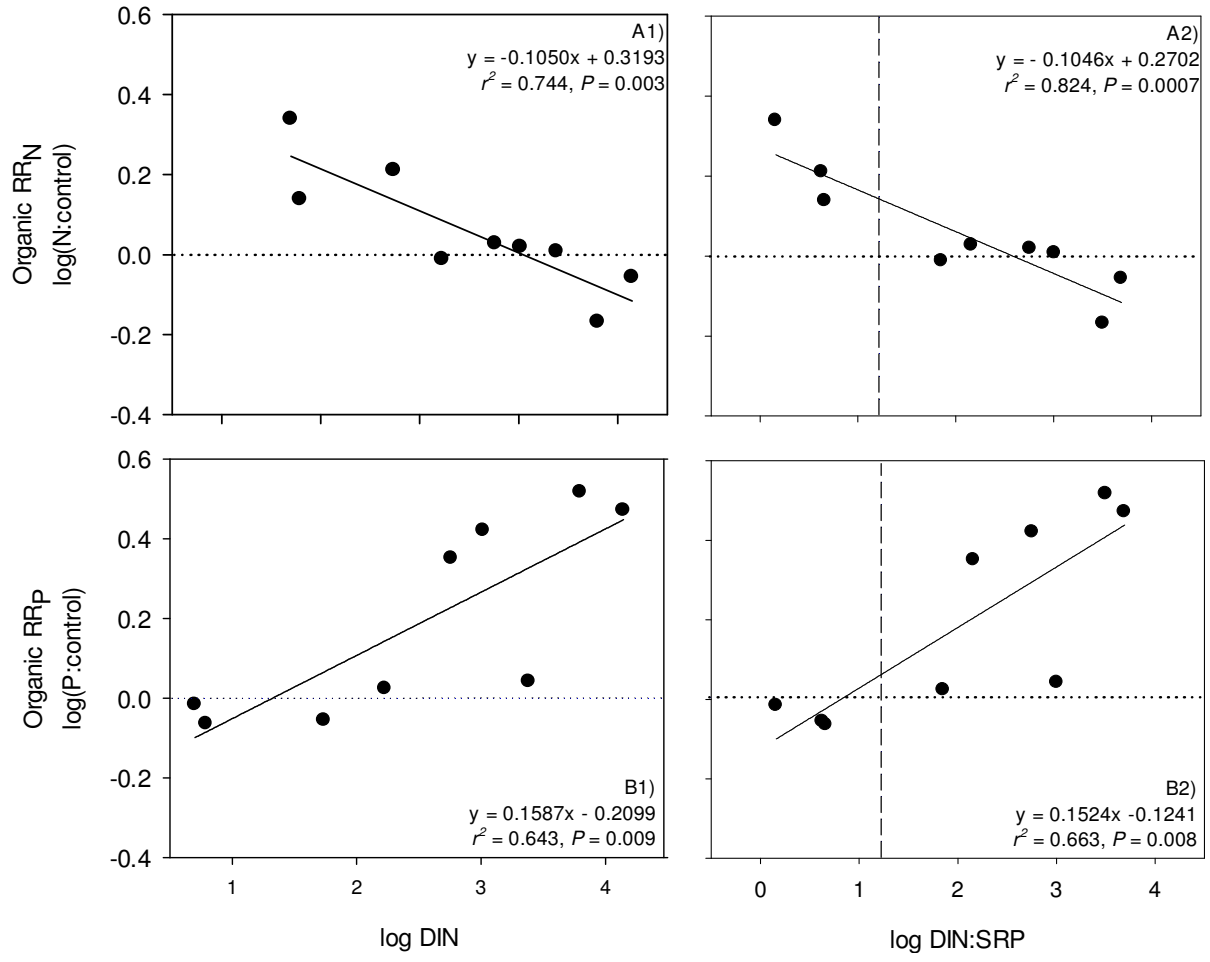


Figure 2. 8. Auckland Spring response ratios on organic substrata showing the relationship between A) RR_N or B) RR_P and water column 1) DIN or 2) DIN:SRP. The dotted lines which intercept the y-axis at 0 indicate no response. The dashed lines on graphs A2 and B2 indicate the Redfield ratio (log[1.2:1] = 16:1).

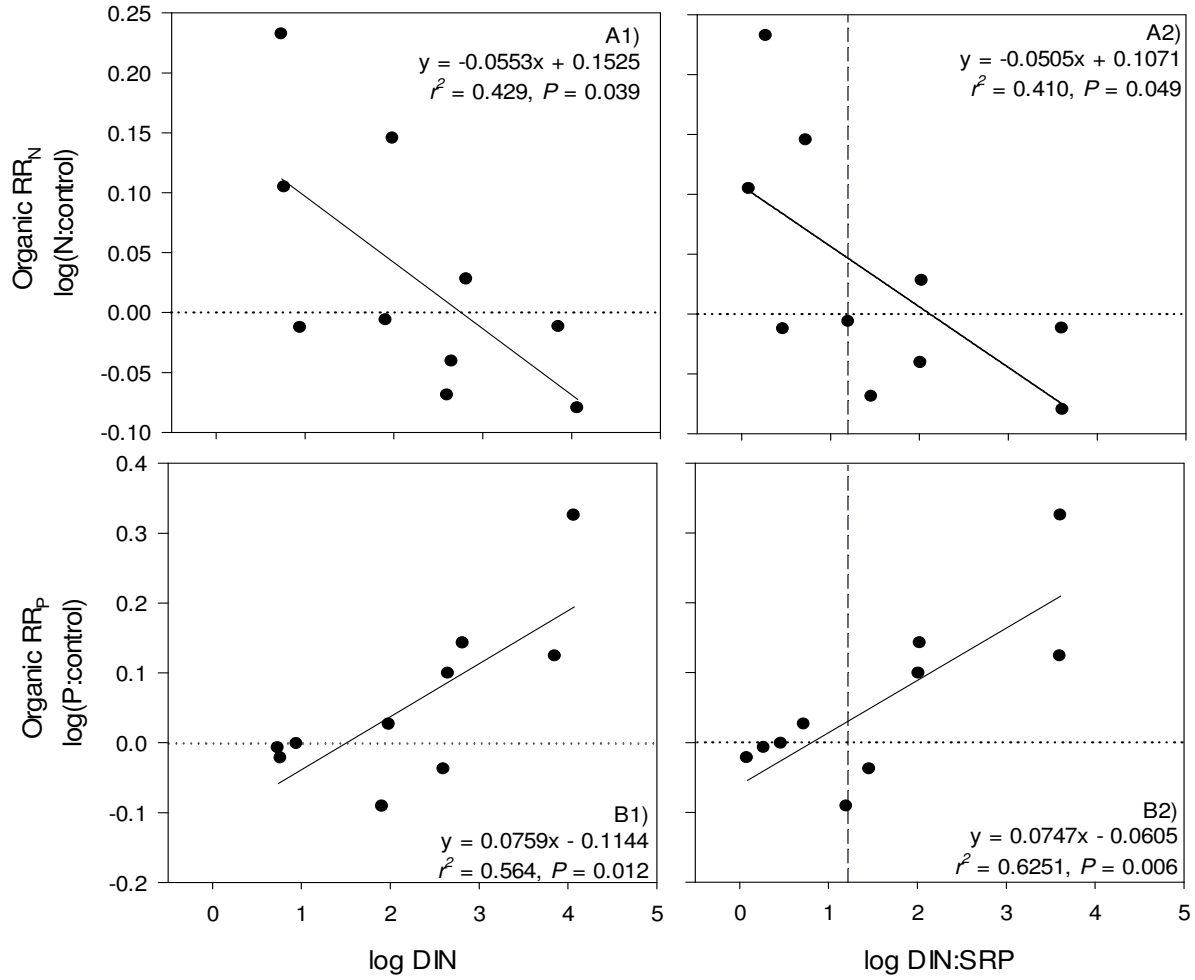


Figure 2. 9. Auckland Summer response ratios and water chemistry on organic substrata, with A) RR_N and B) RR_P against 1) log DIN) 2) log DIN:SRP. The dotted line at the response ratio of 0 indicates no response, and the dashes lines on graphs A2 and B2 show the Redfield ratio (log[1.2:1] = 16:1).

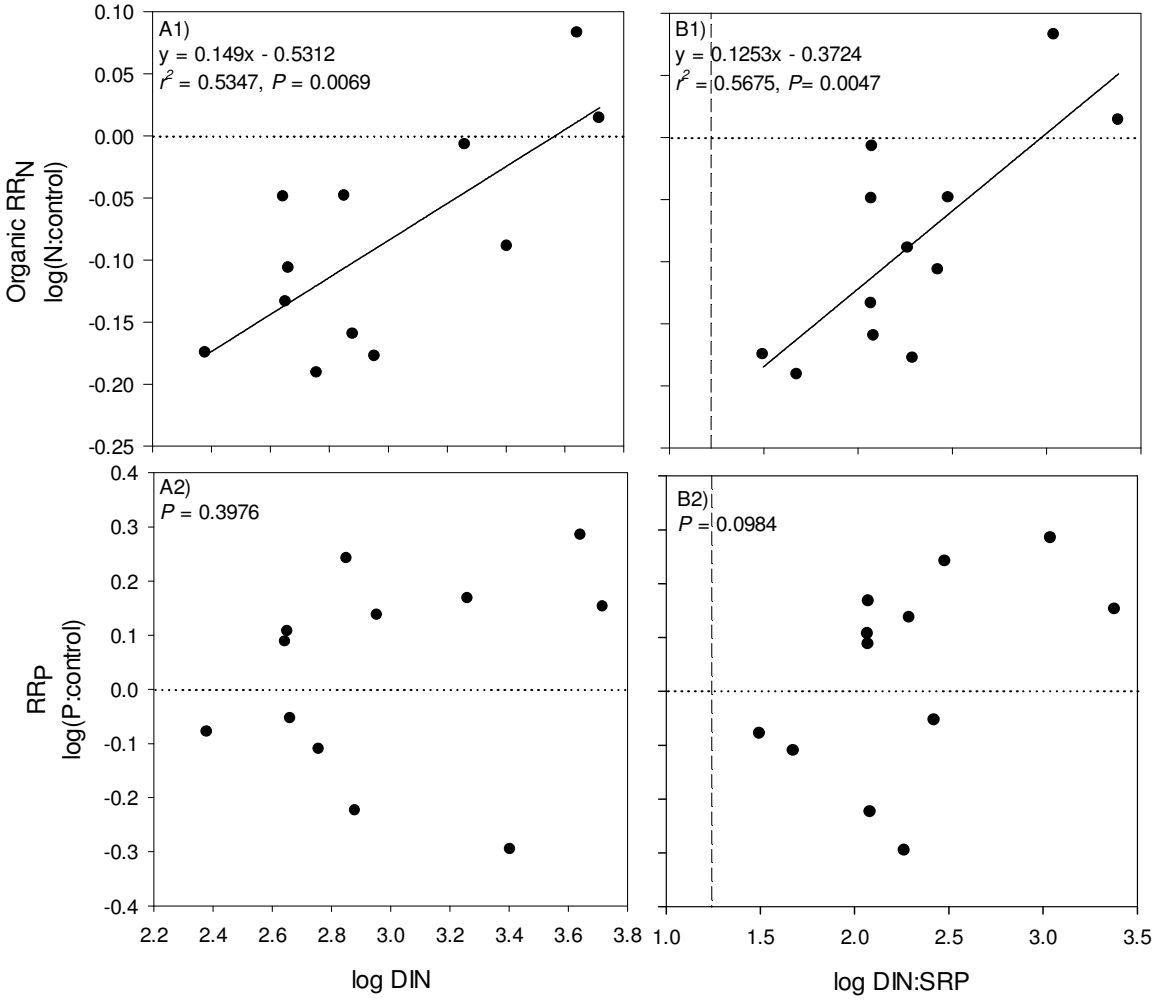


Figure 2. 10. Relationship between RR_N and A) water column DIN and B) the stoichiometric ratio of DIN:SRP on organic biofilms over Christchurch Summer, with a linear regression line fitted. Dashed line at the response ratio of 0 indicates no response. The dashed line on graph B indicates the Redfield ratio (log[1.2:1] = 16:1).

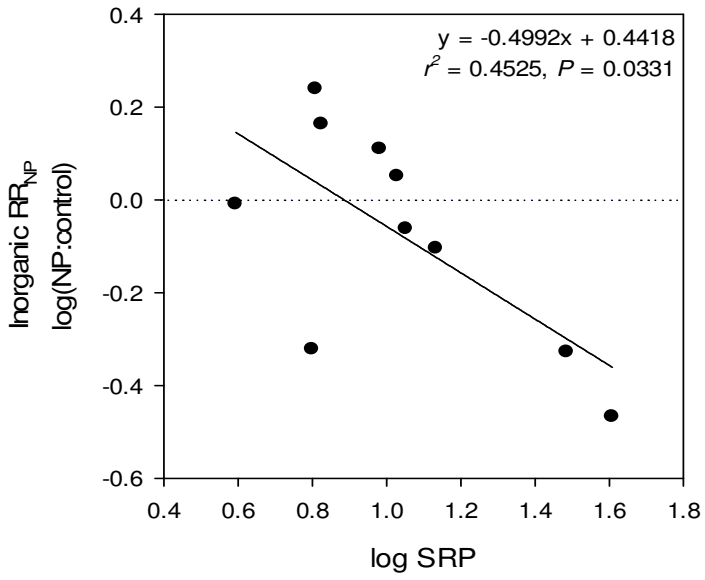


Figure 2. 11. Relationship between water column SRP and inorganic biofilm RR_{NP} in Auckland Summer. The dotted line at the response ratio of 0 indicates no response.

Plotted nutrient ratios and RR's show distinct breakpoints in the data at nutrient concentrations similar to that of the Redfield ratio (Figure 2.12). In Auckland, RR_N of organic biofilms declined to approximately 0 at a molar DIN:SRP ratio of 18.6:1, similar to the Redfield ratio of 16:1 (Figure 2.12A). All sites with a positive response and low water column nutrient ratios are native, supporting previous findings of N limitation in this land-use category. Lucas creek, a suburban site, had a nutrient ratio of 16:1 in Summer with a response ratio of approximately 0 and sits at the breakpoint, demonstrating support for the Redfield ratio. In addition the bioassays in Lucas Creek indicated colimitation by N and P. In contrast, RR_P on organic biofilms stayed stationary at approximately 0 until the molar DIN:SRP ratio of 14.5:1 before responding positively (Figure 2.12 B). Again this predicted breakpoint is similar to the Redfield ratio. Sites with water column nutrient ratios <14.5:1 were reference sites with the addition of suburban sites Otara Creek and Lucas Creek in Summer.

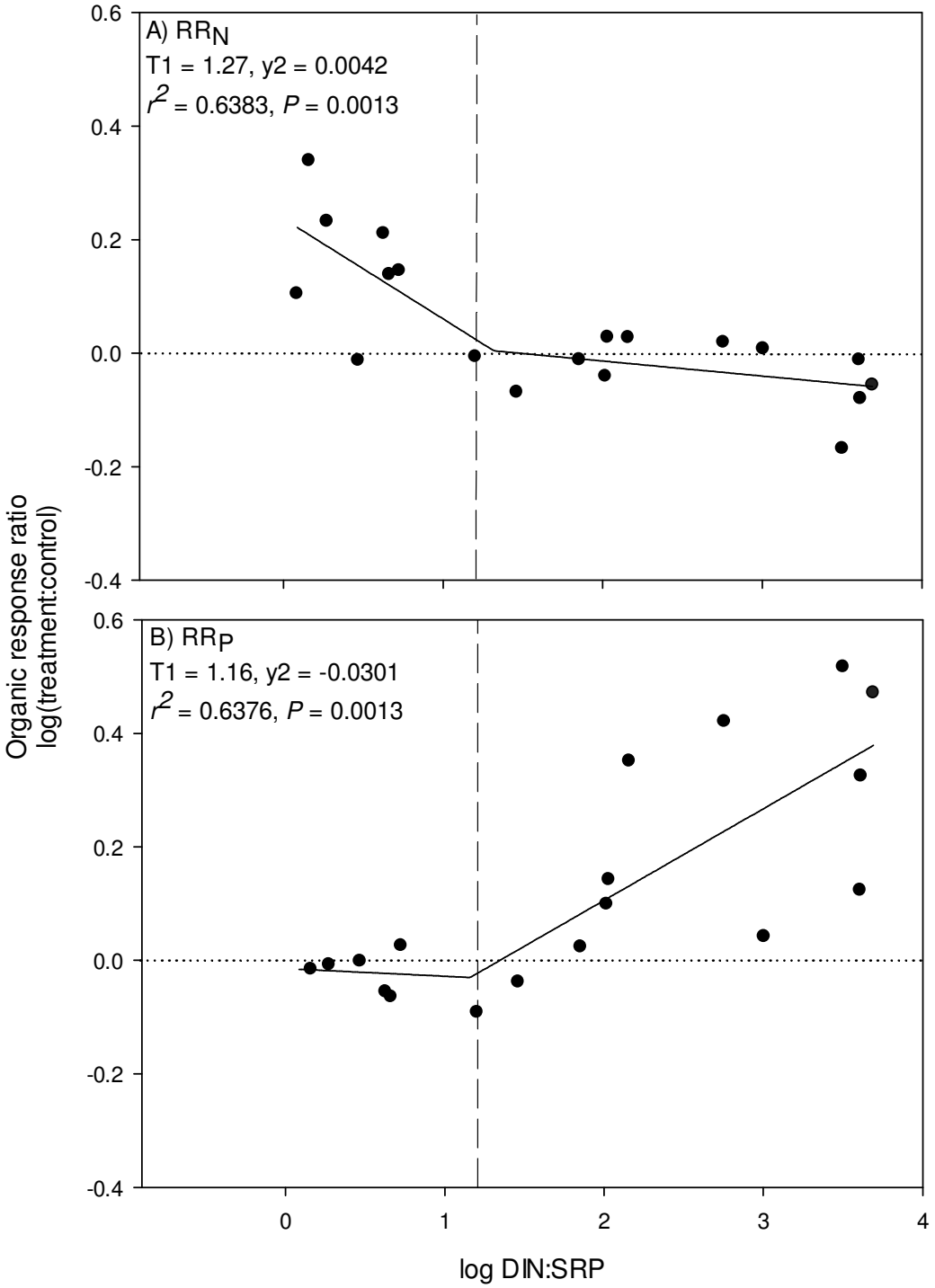


Figure 2. 12. Community respiration responses on organic substrates plotted against the log molar ratio of water column dissolved inorganic nitrogen (DIN) to soluble reactive phosphorus (SRP) concentrations for A) N enriched substrates and B) phosphorus enriched substrates. Both plots are fitted with a two segment piecewise linear regression. T1 indicates the breakpoint value on the x-axis and y2 indicates the breakpoint on the y-axis. The dotted line at 0 on the y-axis indicates no response on organic biofilms. The dashed line intercepting the x-axis indicates the Redfield ratio (log[1.2:1] = 16:1).

2.3.8. Coherence in response to urbanisation across cities

Responses to urbanisation between organic and inorganic substrata followed similar trends in Auckland and Christchurch in Summer (Figure 2.13A). The addition of either P or +NP in Auckland and Christchurch urban streams elicited a positive response on organic biofilms, whilst addition of N only elicited little response. Between cities RR_P (*t*-test, $P = 0.344$) and RR_{NP} (*t*-test, $P = 0.063$) were not significantly different, both demonstrated strong positive limitation patterns. RR_N was negative in both cities, with Christchurch's responses lower than Auckland's (*t*-test, $P = 0.034$). In both cities response to N enrichment was significantly lower than the response to +NP (Auckland: ANOVA, $P < 0.0001$; Christchurch: ANOVA, $P = 0.005$). In Auckland responses to P addition were weaker than responses to +NP (ANOVA, $P = 0.011$). Overall, organic substrates responded the same way to urbanisation between cities.

All three nutrient enrichments produced the same trends to urbanisation on inorganic substrates in Auckland and Christchurch with no significant differences between these (*t*-test, $P > 0.05$) (Figure 2.13B). Responses to +N and +NP were slightly positive whereas responses of +P were negative. This suggests P suppression on inorganic substrates which is consistent with bioassays (Table 2.6). Significant differences were found between RR_P and both RR_N (ANOVA, Christchurch: $P = 0.021$; Auckland: $P = 0.005$) and RR_{NP} (ANOVA, Christchurch: $P = 0.015$; Auckland: $P = 0.026$).

Responses on organic and inorganic substrates were not coherent, with suppression of heterotrophs by N and suppression of autotrophs by P. P limitation was significantly stronger on organic than inorganic substrates in both Auckland (*t*-test, $P = 0.007$), and Christchurch (*t*-test, $P = 0.025$). The addition of P elicited a positive response on organic substrates and a negative response on inorganic substrates in Auckland and Christchurch, differences were not however significant (*t*-test, $P > 0.05$). Organic substrates in Auckland also demonstrated significantly stronger response to +NP compared to inorganic substrates (*t*-test, $P = 0.023$).

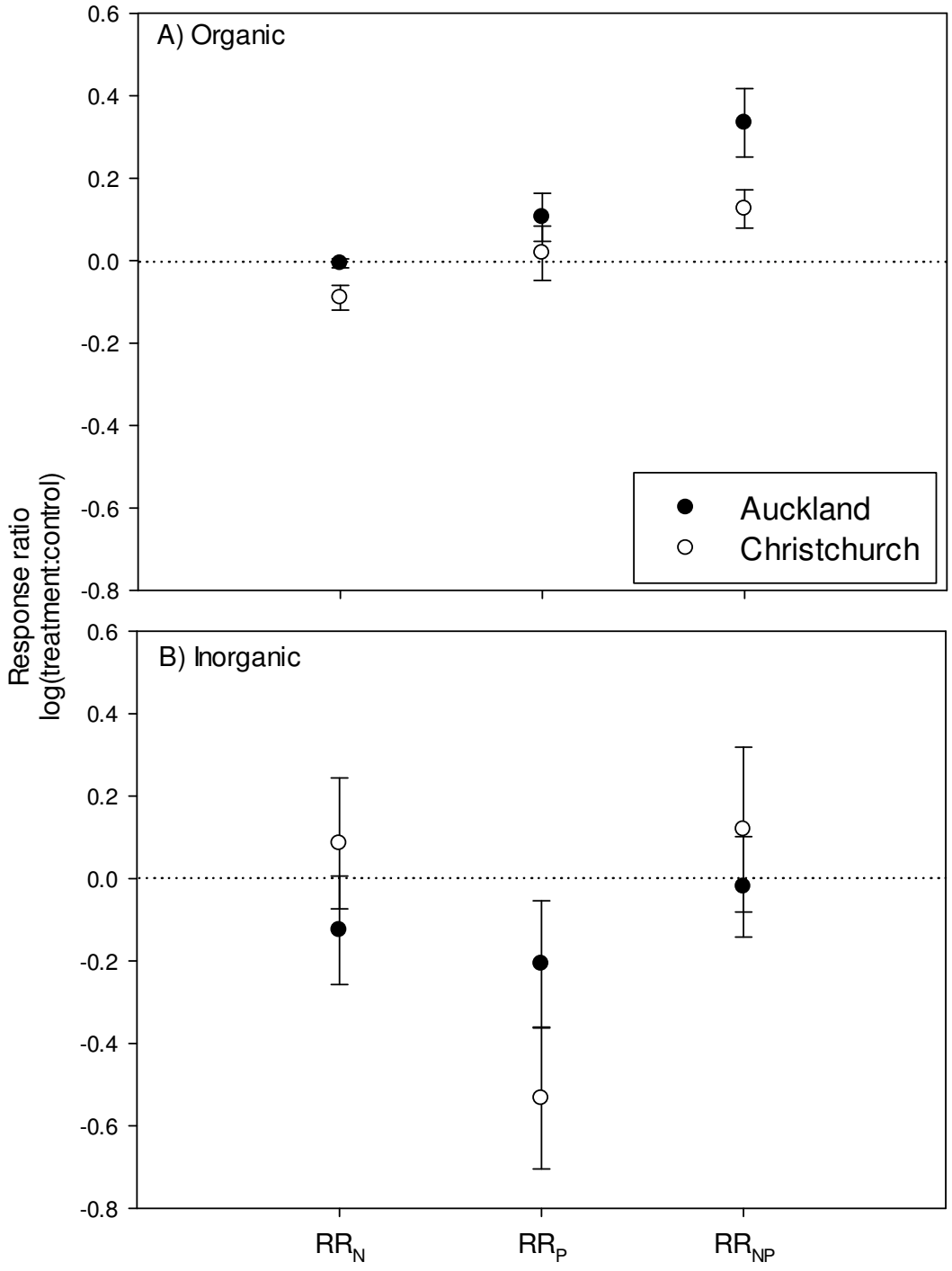


Figure 2. 13. Average (±SE) response ratios in urban sites on A) organic and B) inorganic biofilms in Auckland and Christchurch during Summer. Dashed line at the response ratio of 0 indicates no response.

2.3.9. Effects of earthquake damage - liquefaction

Urban streams impacted by heavy liquefaction showed significantly different trends in nutrient limitation compared to streams with no or only light liquefaction. Sites with heavy liquefaction showed negative (suppressed) responses to both +P and +N enrichments and no response to +NP enrichment on organic substrates (Figure 2.14 A). In contrast, +P and +NP stimulated microbes in the other three liquefaction categories. Addition of N either supressed or had little effect on microbes in streams with little or no liquefaction. DIN concentrations were 7x lower at urban sites affected by heavy liquefaction (597µgN/L) than urban sites unaffected by liquefaction (3,492µgN/L). SRP levels were slightly higher in heavy liquefaction sites (20µgP/L) than urban sites (15µgP/L), along with NH_4^+ in heavy (171 µgN/L) and unaffected sites (134 µgN/L), although these differences are not large (see Figure 3.23). As a result, DIN:SRP ratios were 6 times lower in heavy liquefaction sites (145:1) compared to urban sites with no liquefaction (953:1).

RR_N was negative at all urban sites independent of their liquefaction status. Sites with heavy liquefaction had significantly lower N responses than sites with no liquefaction (ANOVA, $P = 0.002$), heavy liquefaction (ANOVA, $P = 0.010$), or reference sites (ANOVA, $P = 0.036$). The pattern of N suppression in Christchurch is consistent with what was found from water chemistry and response ratio analysis, where N was suppressed in all but two sites (Figure 2.10). The categories light, none, and reference all showed similar positive responses to +P and +NP enrichment, with no significant difference in responses between categories (ANOVA, $P > 0.05$).

Impacts of liquefaction were less significant on inorganic substrates, with more variation in the data (Figure 2.14 B). RR_P was negative responses in all categories apart from reference. Heavy liquefaction sites showed positive response to +NP, whilst the other categories showed negative responses to this treatment. Overall, inorganic substrates demonstrate few differences between liquefaction categories. The response patterns are however quite different to organic substrates. Suppression of N was common on organic substrata and P on inorganic substrata; similar to what bioassay results revealed (Table 2.6).

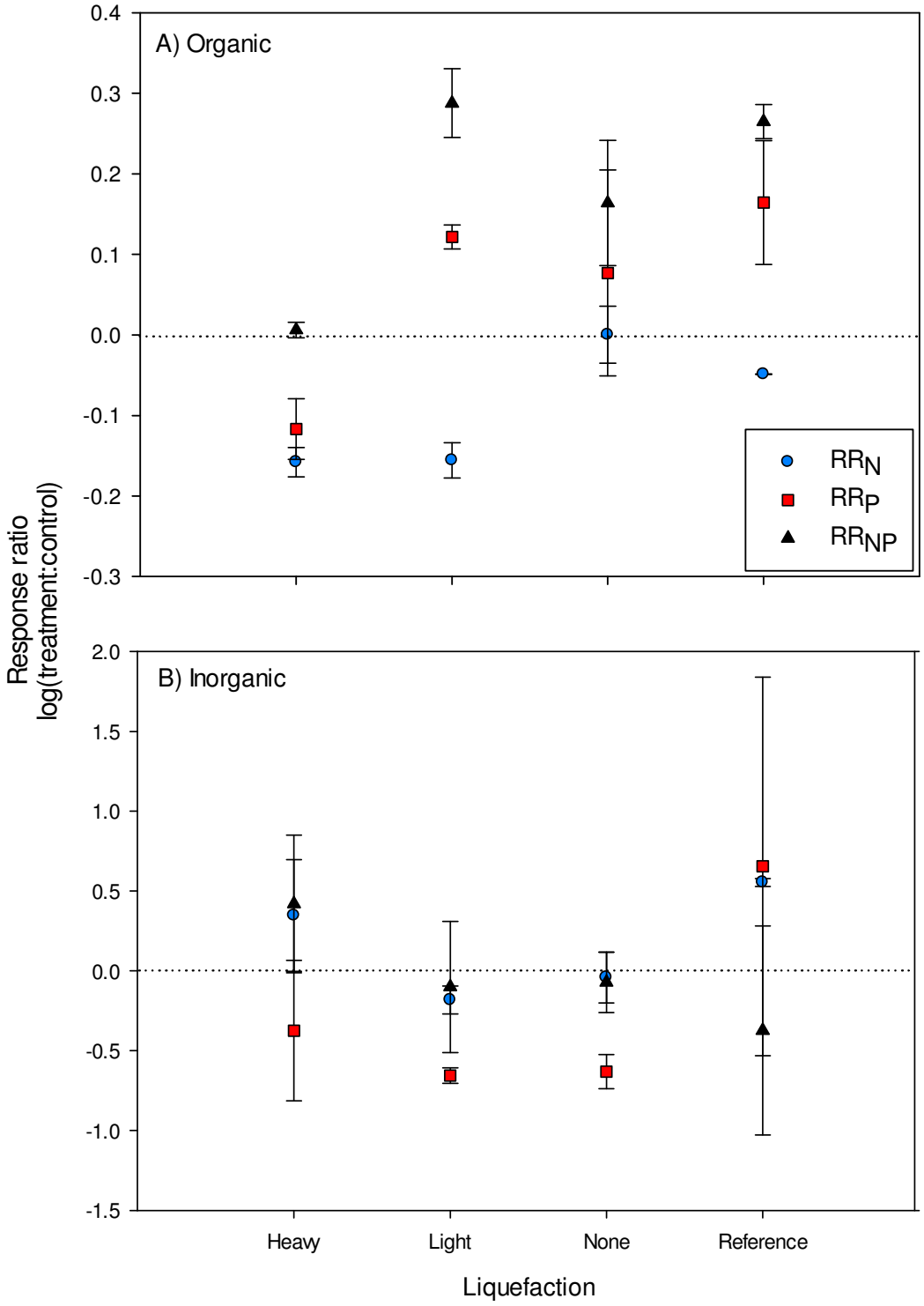


Figure 2. 14. Average (±SE) response ratios across liquefaction categories on A) organic (respiration) and B) inorganic (chlorophyll *a*) substrates in Christchurch urban streams. Dashed line at the response ratio of 0 indicates no response.

2.4. Discussion

The substantial effects of urbanisation are well recognised for some stream organisms such as macroinvertebrates and fish, but responses by microbial organisms are not well characterised (Allan, 2004; Wegner *et al.,* 2009). I found that urbanisation has a strong effect on microbial nutrient limitation, including alterations to the magnitude of nutrient limitation and identity of limiting nutrients. Consistent with other studies limitation patterns generally differed between autotrophic and heterotrophic biofilms, with a higher incidence of limitation observed for heterotrophs (Tank and Dodds, 2003; Hoellein *et al.*, 2011a). Nutrient limitation shifted from N to P limitation with increasing urbanisation with a clear threshold at a level of urbanisation below 30%. This phenomenon may increase the likelihood of downstream transport of nutrients and eutrophication. Heterotrophic biofilm responses demonstrated rough agreement with the benchmark Redfield ratio, something which to my knowledge has only been demonstrated on inorganic biofilms (Redfield, 1958). A novel finding leading from this research was the effect of earthquake damage (liquefaction) on stream nutrient processing, with significant differences in water chemistry and microbial nutrient suppression.

2.4.1. Nutrient limitation as indicated by autotrophic (inorganic) biofilms

Autotrophic biofilms were not commonly limited by nitrogen or phosphorus, with nutrient limitation occurring in less than 30% of experiments. It should be noted that chlorophyll *a* levels in this study demonstrated substantial variation within sites; including some sites with readings close to the detection limit (<0.1mg/L). Infrequent nutrient limitation of autotrophic biofilms is common in the literature for both reference and human-influenced systems, with other studies reporting no limitation as a dominant response from chlorophyll *a* analysis (Tank and Dodds, 2003; Hoellein *et al.*, 2010; Hoellein *et al.*, 2011a). In a meta-analysis of 237 nutrient limitation experiments Francoeur (2001) noted that no limitation was the most common response from autotrophic limitation experiments, occurring in 42.6% of experiments. Subsequent studies have confirmed this with Johnson *et al* (2009a) reporting no limitation in 75% of streams in streams across the U.S. This lack of limitation noted in my study and in other studies has not been explained in the literature, this calls into question the suitability of chlorophyll *a* as a means of detecting nutrient limitation and should be explored in future research. Results show that where limitation did occur, streams were primarily limited by N or colimited by N and P, with only one instance of P limitation. Other studies have similarly found P limitation to be rare from inorganic NDS experiments (Francoeur, 2001; Johnson *et al.*, 2009a). Lack of P limitation of autotrophic biofilms may be related to the ability of algal cells to

store nutrients in internal vacuoles (luxury uptake) and draw upon this resource when required (Marcarelli and Wurstsbaugh, 2007; Schade *et al.*, 2011).

Nutrient limitation of autotrophic biofilms in urban streams was rare, occurring at two of the nine urban streams in Christchurch and never in Auckland. The two urban sites which did experience limitation (Shirely and Steamwharf Streams) have both been affected by earthquake damage which may be related to this response (see 2.4.6). Given the lack of canopy cover leading to increased light levels at urban sites it was expected that primary productivity would increase as any light limitation would be relieved (Taulbee *et al.*, 2005). Autotrophic biofilms in urban streams showed little response to any of the nutrient enrichments, whereas responses from reference sites were more pronounced, suggesting a saturation of nutrient demand by biological organisms at urban sites. Other studies have also found higher incidences of no limitation at urban sites and associated this with elevated nutrient concentrations and light levels (Cheeseman *et al.,* 1992; Johnson *et al.,* 2009a; Hoellein *et al.,* 2011). Urban sites in my study had elevated nutrient concentrations and light levels when compared to reference sties; it is therefore unsurprising such few instances of limitation were found. However, the influence of other environmental factors cannot be ruled out and may have contributed to some of the variation in the data, for example increased turbidity in urban streams could reduce primary production potential (Marcarelli *et al.*, 2009). Taken together the consistent lack of nutrient limitation at urban sites in this study and others suggests that microbial biofilms on inorganic substrate in urban streams are often nutrient saturated.

In contrast to urban streams nutrient limitation at reference sites was slightly more common, occurring 50% of the time. Nutrient limitation was expected to be more prominent at reference sites given their low ambient nutrient levels, where nutrients would be more likely to limit growth (Tank and Dodds, 2003). Where Auckland reference streams did demonstrate nutrient limitation, they were always N limited. This result follows findings from other studies which have also found N limitation common in reference streams (or less impacted sites) throughout the U.S. and New Zealand (Cheeseman *et al.*, 1992; Biggs *et al.*, 1998; Tank and Dodds, 2003; Marcarelli *et al.*, 2009; Johnson *et al.*, 2009a). Cascades stream, in the Waitakere Ranges Regional Park, was the only reference site in Auckland to demonstrate limitation in both seasons. This was likely related to the large width of this stream (4-5m), providing increased ambient light levels which prevented any light limitation (Johnson *et al.*, 2009a). The prominence of N limitation may be related to the reduced N loading in reference sites which was noted in my study and in others (Hoellein *et al.,* 2010) (Table 2.5). Theoretically, N-fixing cyanobacteria should prevent long-term N limitation due to their ability to fix atmospheric N_2

into bioavailable forms (Schindler, 1977; Francoeur, 2001). The dominance of N limitation in NDS studies may therefore indicate that N-fixation is limited or N-fixers have not sufficiently established on experimental substrata. Maracelli and Wurtsbaugh (2006) concluded that N-fixation rates are limited by stream temperature and P availability; making N limitation common where these requirements are not met. The cooler temperatures of native sites may therefore limit N fixation rates and microbial organisms may not fix N if P is also limiting, as fixation is an energetically costly process (Maracelli and Wurtbaugh, 2007). Reference sites in Christchurch did not show the same pattern of N limitation, and one site demonstrated P limitation. This is likely to do with reference sites in Christchurch being in a least least-disturbed condition with relatively high nutrient concentrations, more in-line with Auckland's urban/ agricultural sites.

Other factors which can influence biofilms responses include flow rate, macronutrient concentrations, grazing pressure, trace metal levels, and light (Marcarelli and Wurstsbaugh, 2007; Von Schiller *et al.*, 2007). These factors may have contributed to the lack of N and P limitation at urban sites. Macronutrients and flow rate can have little effect on nutrient diffusing substrates (Cockrum, 1996; Bernhardt and Likens, 2004); making these unlikely to significantly influence responses. Grazers also may have masked biofilm response to nutrient amendment by preventing biofilm accrual (Winterbourn, 1990; Johnson *et al.*, 2009a). Grazers were not commonly observed on inorganic substrates and were absent in some sites during site visits, but observations were limited in scope. Light limits primary production, however many of the sites showing no nutrient limitation in this study had high light levels, suggesting that this is unlikely (Tank and Dodds, 2003; Johnson *et al.*, 2009a; Hoellein *et al.*, 2011b). However, interactive effects from any of these mechanisms may have influenced biofilm development; thus these factors cannot be entirely ruled out.

2.4.2. Nutrient limitation as indicated by heterotrophic (organic) biofilms

Biofilms on organic surfaces were more sensitive to nutrient limitation; with more than 70% of sites affected by nutrient limitation. Heterotrophic nutrient limitation switched from N to P limitation with increasing urbanisation. I found some form of N limitation (N or N+P) at 87% of reference sites and P limitation (P or N+P) at 67% of urban sites in Auckland and Christchurch. This pattern was also noted in a study by Johnson *et al* (2009a) where community respiration on organic substrata was predominantly P limited in 65% of urban sites and N limited in 94% of reference sites. Similar patterns were also found in a study using a wood veneer organic substrate, with N limitation in 60% of oligotrophic (reference) streams (Tank and Dodds, 2003). Wood veneer is a less labile carbon source and produces lower microbial responses than cellulose cloth, likely causing the lower limitation percentage (Tank and Winterbourn, 1996).

The causal mechanism behind this shift in limitation between land-uses is thought to be due to an increase in nitrate in streams draining urban and agricultural land-uses; intensive land-use generally exports more N relative to P (McDowell *et al.*, 2009). While both nitrate and phosphate increased with increasing land-use intensity in my study, nitrate increased faster, leading to an increase in DIN:SRP ratios (Tables 2.3 & 2.4). This explanation is also supported by strong correlations between land-use and nitrate concentrations evident in Table 2.5; with elevated nitrate concentrations related to increasing urbanisation. Furthermore, temporal trend analysis of nutrient data by Scarsbrook (2006) revealed that nitrate concentrations were increasing in streams over time across New Zealand suggesting that this trend may be widespread. This influx of N into urban and agricultural landscapes is likely alleviating any N limitation, causing P limitation to become more common.

Co-limitation or secondary limitation by biofilms is often reported in studies using nutrient diffusing substrates (Tank and Dodds, 2003; Marcarelli and Wurtsbaugh, 2007). The dominant paradigm that one nutrient limits productivity does not stand for multi-species communities such as biofilms (Marcarelli *et al.*, 2009). Liebig's Law of the minimum describes the theory that only one nutrient can be in demand and at time; this theory has been demonstrated for single species cultures, but is less viable when applied to multi-species communities which are recognised to have different nutrient requirements (Francoeur, 2001). In his review Francoeur (2001) concluded that stimulation of both N and P is common in NDS studies, but is often unreported due to the low statistical power of experiments or variation in the data (e.g. chlorophyll *a*). I found a synergistic response of heterotrophs to the addition of both N and P across land-uses (highest response ratios, see Figures 2.4 & 2.5) and co- or secondary limitation was common in bioassay results. This suggests that when one nutrient demand is satisfied growth is still constrained by the other nutrient (Francoeur, 2001; Allgeier *et al.*, 2011).

Grazers were ubiquitous on organic cellulose sponge in my study; potentially influencing limitation patterns. The most commonly observed invertebrates were molluscs (*Potamopyrgus*), and larval Chironomidae (Orthocladiinae), mayflies (Ephemeroptera), and caddisflies (Trichoptera). This contrasts with overseas studies which have noted that grazers were uncommon on cellulose substrates, occur occurring in only 9% of some experiments, and thus thought not to be a problem (Johnson *et al.,* 2009; Tank and Dodds, 2003; Von Schiller *et al.,* 2007). However, macroinvertebrates tend to be more commonly noted in New Zealand NDS studies (Winterbourn, 1990; Cockrum, 1996; Tank and Winterbourn, 1996); and have been found to counteract the effect of enrichment in one instance (Biggs *et al.*, 1998). Winterbourn (1990) included insecticide in nutrient diffusers and found that this reduced grazing activity

sufficiently to cause an increase in algal biomass. Cellulose sponge cloth provides an ideal substrate for bacteria and fungi, which consequently provides an ideal food source and secure habitat for Chironomidae larvae (Tank and Winterbourn, 1996). While invertebrates likely consumed the sponges in my experiment (evident as holes in sponges), I compensated for this by quantifying sponge area in respiration assays. Future studies would benefit from a more detailed analysis of consumers on sponges in NDS experiments.

2.4.3. Can nutrient limitation be predicted from water DIN:SRP ratios?

The Redfield ratio indicates a threshold of nutrient limitation; although not originally purposed for streams (based on marine algae) this ratio has been widely utilised in freshwater ecology for predicting nutrient limitation (Redfield, 1958; McDowell *et al.*, 2009; Keck and Lepori, 2012). Water DIN:SRP ratios were a reliable indicator of nutrient limitation of heterotrophic biofilms, but not autotrophic biofilms, in Auckland and Christchurch. Using the Redfield ratio (16N:1P) heterotrophic biofilms in streams could be broadly divided by N and P limitation; however bioassays are necessary to distinguish more complex patterns such as no limitation, colimitation, or secondary limitation (Redfield, 1958). Biofilms demonstrated a shift in identity of limiting nutrients between the molar DIN:SRP ratios of < 19:1 (indicating N limitation) > 15:1 (indicating P limitation) (Figure 2.12). Ratios also coincide with a switch in land-use; with N limitation indicated at native sites (<5N:1P) and P limitation at urban and agricultural sites (>16N:1P). This shift in identity of limiting nutrients is generally consistent with the Redfield ratio (Redfield, 1958). Although ratios do not exactly follow the Redfield ratio this was somewhat expected as biofilms are multi-species communities whereas the Redfield ratio was created for a single algal species (Redfield, 1958; Borchat, 1996). There were few sites that did not follow predictable patterns; these may have been influenced by nutrient suppression, which biofilm assays indicated as no-limitation (see 2.4.5 for discussion). Other studies have had mixed results using nutrient ratios to predict nutrient limitation; with some demonstrating support for their use (Grimm and Fisher, 1986; Lohman *et al.*, 1991; Cheeseman *et al.*, 1992; Peterson *et al.*, 1993), whilst others found no meaningful relationships (Francoeur *et al.*, 1999; Tank and Dodds, 2003; Von Schiller *et al.*, 2007). Francouer *et al* (1999) found that N limitation could occur from a DIN:SRP ratio of 4:1 to 400:1, supporting the argument that water ratios are weak predictors of microbial nutrient limitation. Furthermore, ratios have also been criticised for use under high or low ratio environments; where quantities of nutrients are thought to be more meaningful (Borchat, 1996; Tank and Dodds, 2003; Von Schiller *et al.*, 2007). It should be noted that many of these studies have primarily looked at autotrophic biofilms, with few looking at heterotrophic biofilms. As previously mentioned these two microbial communities have different

limitation patterns and elemental composition of their biomass; thus only looking at one component does not provide a good estimate of nutrient limitation which may explain why so many studies have found no relationship between biofilm and water limitation.

Studies which have described the relationship between water dissolved inorganic nutrients (DIN:SRP) and nutrient limitation as predicted by heterotrophic biofilms have found no relationship between these variables (Tank and Dodds, 2003; Hoellein *et al.,* 2010). Note that not all studies which have looked at heterotrophic biofilms have reported on the success of using water nutrient ratios to predict limitation, there is therefore a paucity of data on this topic. Hoellein *et al* (2010) explained that the lack of correlation between water and biofilm limitation was likely due to the ability of heterotrophic biofilms to take up nutrients from in stream organic matter (facultative exploitation). However his study only included relatively pristine sites all located in primarily forested catchments, it is therefore unsurprising that the findings are different to those found in this study. As the sponge cloth used in my study was composed entirely of cellulose, which contains no N or P, microbial biofilms would have been forced to rely exclusively on N and P in the water column. This may explain why microbial biofilms in my study were tightly linked to water column nutrient ratios. Tank and Dodds (2003) also found that water column nutrient ratios were weakly related to fungal biomass (a measure of the heterotrophic community). The different methods used to measure heterotrophic communities (fungal biomass and community respiration) may explain why results are dissimilar. Studies have however found relationships between whole stream respiration and water chemistry in small oligotrophic streams supporting results from this study (Hill *et al.,* 2001; Stelzer, *et al.,* 2003; Greenwood *et al.*, 2007). My results demonstrate that heterotrophic biofilms remain sensitive to nutrient enrichment, until their limiting nutrient is supplied in excess at which stage it is no longer taken up by the biofilm increasing the likelihood of downstream export. The lack of literature around this topic highlights the need for more studies to incorporate cellulose sponge substrates into their experimental designs, as these responded to water column nutrient ratios in a predictable manner, and thus may be a better tool for consistently gauging microbial response to land-use change compared to glass frits.

2.4.4. Non-linearity in biofilm response to urbanisation

High N concentrations associated with urban land-use consistently reduces the response to N enrichment by heterotrophic microbial biofilms, as confirmed in my study and others (Meyer *et al.*, 2005; Johnson *et al.*, 2009a; Hoellein *et al.*, 2011a). Autotrophic biofilms demonstrated no clear shift in the identity of limiting nutrients between land-uses. In contrast, heterotrophic biofilms demonstrated clear responses that differed between urban and reference

sites with little distinction between urban sites in Auckland and Christchurch. In streams with catchment urbanisation greater than 30% heterotrophic biofilms were apparently N-saturated (Figure 2.6). Similarly, streams with agricultural land-use stopped responding to N subsidy beyond 25% land-use. Thresholds in responses of other stream compartments (invertebrates, fish, etc.) are common and typically sit between 10 – 20% impervious area (Walsh, 2000; Beach, 2001; Paul and Meyer, 2001; Walsh *et al.*, 2005). Percentages suggested in this study are higher than what these studies have found; however there was a lack of sites with urbanisation intensities between 10-20%. Therefore, the estimate of 30% is conservative and is it likely that the urbanisation has strong impacts on microbial biofilms at lower intensities. Autotrophic biofilms demonstrated no consistent relationship with urban or agricultural land-uses; however response ratios to N increased on both autotrophic and heterotrophic biofilms with increasing native land-use. This demonstrates that N limitation in native sites is consistent across substrates; but other factors are likely influencing autotrophic biofilms at sites with anthropogenic impacts.

Threshold values for Christchurch could not be established due to the lack of land-use gradients in the city, with most land on the Canterbury plains intensity developed for either urban or agricultural land-uses. Interestingly, Auckland and Christchurch demonstrated the same trends in relation to increasing urbanisation on autotrophic and heterotrophic biofilms (Figure 2.13). This is significant as these cities differ considerably in their geology, climate, and vegetation types but yet showed the same responses to urbanisation. Results could therefore be relevant to other urban centres in New Zealand, potentially informing nutrient management in urban waterways. In contrast to my findings, a study in the U.S. found that the effect of land-use on primary productivity and respiration was not consistent between eco-regions and varies with land-use intensity (Meyer *et al.*, 2005; Johnson *et al.*, 2009a). The differences between that study my study are not surprising given that the U.S. is much larger, and therefore eco-regions are likely to experience more environmental variation. Variations in urban responses overseas have also been attributed to the extent of piped networks draining into urban streams (described as effective imperviousness); which may be a better predictor of stream ecological condition than catchment imperviousness (Wenger *et al.*, 2009). Land-use quantification in this study did not take underground piped networks into account, due to a lack of access to data, however the similarity of responses between the two study cities suggests that this was not necessary.

Assemblages of microbes vary with urbanisation impact, with species tolerant of eutrophication becoming dominant due to the multiple chemical stressors related with urbanisation (Fore and Grafe, 2002; Potapova and Charles, 2003; Newall and Walsh, 2005; Johnson *et al.*, 2009a). Increases in imperviousness may be linked to increasing nutrient

concentrations, heavy metals, and other chemicals (Walsh *et al.,* 2005). Excess heavy metals in storm water runoff, particularly zinc, copper and lead may impact biofilm structure and function (Bibby and Webster-Brown, 2005; Ancion *et al.*, 2010; Davis *et al.*, 2010). Heavy metal exposure can affect biofilm communities after only three days with rapid uptake during this period (Ancion *et al.,* 2010). In this study a build-up of what might have been heavy metals was visible of nutrient diffusers upon collection (Figure 2.15). Bacteria can oxidise metals in-streams leading to deposition of these on stream surfaces; which is a likely explanation for the difference in colour between nutrients incubated in urban and native streams, note that this was only observed in highly urbanised streams (Barlett and Leff, 2010). Differences between urban regions can therefore not be ruled out due to the multitude of stressors associated with urban land-use which may influence biofilm responses; this could be another potential avenue for future research (Johnson *et al.,* 2009a).

Figure 2. 15. Nutrient diffusers after 21 days of incubation, the diffusers on the left were incubated in a stream surrounded by native forest (Wairoa Tributary) and the diffusers on the right were from an urban stream (Pakuranga creek), both were removed on the same day within an hour of one another, substrates (sponge and glass) were removed for analysis.

Seasonality also modified nutrient limitation patterns in heterotrophic communities within urban streams. In Auckland, urbanisation was always negatively correlated with the degree of N limitation; but only had a positive correlation with P limitation in Spring. The lack of relationship between P and urbanisation in Summer is likely related to an increase in SRP concentrations in Summer (on average 64% higher). This translated to differences in limitation between seasons on heterotrophic biofilms; with colimitation becoming more common (from 1˚P, 2˚ N limitation) at anthropogenically impacted sites due to increase in P concentrations which relieved P limitation to some extent. Differences in biofilm nutrient demand were therefore related to water column nutrient concentrations which varied across seasons. Previous studies that have investigated seasonal differences focusing on autotrophic biofilms found large differences between seasons due to increased light levels over Summer which has increased

productivity, rather than being driven by nutrient concentrations (Biggs *et al.*, 1998; Francoeur *et al.*, 1999; Hoellein *et al.*, 2010). In contrast, studies looking at seasonal effects on heterotrophic biofilms show agreement with results of this study; Spring has the highest nutrient demand (Tank and Dodds, 2003; Hoellein *et al.*, 2010). This is as expected given as Spring is generally associated with an increase in inorganic nutrients due to the decomposition of terrestrial and aquatic of organic matter, further accompanied by an increase in temperature and light which drives productivity, increasing nutrient demand (Tank and Webster, 1998; Bernhardt and Likens, 2004; Hoellein *et al.*, 2010; Kirchman, 2012).

2.4.5. Nutrient suppression

Suppression of microbes by nutrient amendment is often noted in NDS studies but rarely explained (Francoeur, 2001; Tank and Dodds, 2003; Bernhardt and Likens, 2004; Danger *et al.*, 2007; Von Schiller *et al.*, 2007; Hoellein *et al.*, 2010). In my study, suppression was more common for respiration on organic biofilms (25%) than chlorophyll *a* on inorganic biofilms (14%). It also only occurred at heavily impacted sites, although not all suppressed sites had high nutrient concentrations; suggesting that excess water column nutrients were not suppressing responses. Where suppression occurred, respiration on organic biofilms generally demonstrated N suppression and chlorophyll *a* on inorganic biofilms generally demonstrated P suppression (see Table 2.6). Although there were instances of both nutrients causing suppression on organic and inorganic substrates, N and P simultaneously suppressed biofilms on four occasions. Unexpectedly, across all Christchurch sites N suppression became less pronounced as water column nutrients increased, with only two sites not demonstrating suppression (urban sites Okeover Stream and Upper Avon River) (Figure 2.10 B1). Suppression occurred at the urban sites Otaki and agricultural site Kaukapakapa in Auckland across both seasons, and in five urban sites in Christchurch, four of these impacted by heavy liquefaction (see 2.4.6).

Early lab work using chemostat cultures demonstrated that bacteria outcompete algae for P in P limited systems, leading to P suppression of primary producers (Currie and Kalff, 1984; Bratbak, 1987; Brussard and Riegman, 1998). NDS studies which found P suppression from chlorophyll *a* analysis have based their explanation around this theory of competition between autotrophs and heterotrophs (Bernhardt and Likens, 2004; Danger *et al.*, 2007). Previous studies have also observed N suppression on inorganic biofilms; this suppression has been linked to heavily impacted sites (Maracelli *et al.,* 2009), consistent with my study. In a meta-analysis Francoeur (2001) found that 1.7% of experiments showed N suppression, and 3.8% P suppression. Bernhardt and Likens (2004) hypothesised that N suppression may be linked to an increase in labile carbon, as N simulated periphyton growth when diffusers were topped with an

inorganic surface (glass fibre filters) but suppressed by all nutrients when diffusers were topped with wooden veneers. Thus, when given a labile carbon source heterotrophs could out-compete algae, again leading to a decrease in primary productivity. The competition hypothesis does not fit with findings from my study, which found incidences of suppression from community respiration and primary production. Community respiration is a functional indicator of productivity for biofilms and as such should be positive (Hoellein *et al.,* 2010).

Alternative explanations for suppression include: 1) a depletion of the nutrients in diffusers over the course of incubation, 2) selective feeding on substrates by invertebrates, 3) nutrient additions may be toxic to microbial organisms, or 4) influences from environmental factors (e.g. sediment scouring) (Bernhardt and Likens, 2004). First, a depletion of nutrients should results in a response similar to the control (no nutrients) rather than a statistically lower response, this hypothesis therefore does not hold. Additionally the incubation duration of 21 days has been proven in prior studies (Tank *et al.,* 2006; Capps *et al.,* 2011). Bernhardt and Likens (2004) discussed that the exclusion of grazers did not alter limitation status and toxicity was not a problem in subsequent experiments. However, it is possible that the ratio of nutrient salts used in the agar and their rate if diffusion through substrates may have led to suppression under certain environmental conditions. For example, N suppression may have been due to rapid diffusion of N salts through substrata, such that concentrations became toxic to microbes. This would explain why respiration remained high on diffusers which were not infused with nutrients. All sites generally had high nutrient concentrations, sandy substrates, and in Auckland were slow flowing perhaps indicating that these are common environmental conditions in which suppression occurs. If toxicity from salts is a causal mechanism the ratios at which these are used in future NDS experiments should be adjusted accordingly. The exact mechanisms behind nutrient suppression are not clear; however the frequency of this in the literature suggests that more research needs to be done into the causal mechanisms behind this response.

2.4.6. Earthquake damage

Earthquake damage in the form of liquefaction (groundwater, sand, and silt) had noticeable effects on stream biofilms (particularly heterotrophic) and water column nutrient concentrations. All sites which were affected by heavy liquefaction also experienced nutrient suppression (N and P) of heterotrophic biofilms (Figure 2.14). In comparison autotrophic biofilms showed a trend of increasing biomass on P enriched biofilms; although this difference was not statistically significant. Sites heavily impacted by the earthquakes received tonnes of liquefaction; leading to chronic sediment loading which can negatively affect ecosystem functioning through reductions in primary productivity, respiration rates, and species diversity

(Wood and Armitage, 1997; Atkinson *et al.*, 2008). In addition, streams received raw sewage for months after the earthquakes, e.g. 4,000m^3 a day into the Heathcote River, leading to chronic dissolved oxygen depletion with concentrations <1 mg/L (Hudson and Rutherford, 2011; Wells *et al.*, 2013). Analysis of uncontaminated liquefaction from Christchurch found it to be sterile (>95% silica), and was thought to have little impact on ecosystem functioning (Black, 2012). It may thus be concluded that the main impacts of the earthquake were from inputs of sewage; which decreased species richness (microbial and macroinvertebrates), impacts were however thought to be short-term with no lasting effects beyond six months (Rutherford and Hudson, 2011; ESR, 2012a; Wells *et al.*, 2013). Additionally, microbial organisms are considered to be robust against environmental disasters leading from their life histories of widespread dispersal, rapid growth rates, and large abundances (Allison and Martiny, 2008). They would therefore be expected to quickly recover from disturbance events.

My data suggest that liquefaction may have had a lasting effect on nutrient limitation of stream biofilms. At sites impacted by heavy liquefaction all three nutrient treatments on organic substrata produced a negative or a very small response (Figure 2.14). Thus, although microbial populations have been observed to recover following earthquake disturbance, on-going continued disturbance from wastewater and sediment scouring may alter microbial function and recovery (Christchurch District Health Board, 2013; Christchurch City Council, 2013; Wells *et al.*, 2013). In reaches where thick layers of liquefaction remain, nutrient uptake capacities of biofilms have been reduced possibly due to continued disturbance and/or suppression mechanisms. Sand is a non-cohesive and unstable substrate which is susceptible to constant shifting, especially under high flow conditions, which can inhibit biofilm formation (Atkinson *et al.*, 2008). For example, "tide" marks have been described on stream banks from liquefaction movement (Gorman, 2011). This constant shifting of sediments, especially in sites which to do not naturally have a sandy substrate may lead to a constant state of disturbance; affecting the ability of biofilms to establish (Wood and Armitage, 1997). Hoellein *et al.,* (2009) also noted that nutrient uptake and metabolism was lower in sandy reaches, related to the ability of sand to bury or scour substrata. Heavy liquefaction sites experienced suppression by N and P; P was only suppressed in the heavy liquefaction category, whereas N was suppressed across all sites regardless of liquefaction status. It may therefore be a combination of environmental factors (liquefaction/sewage) and suppression mechanisms (possibly salt toxicity) which are driving P suppression at sites with heavy liquefaction. There is a paucity of information regarding the effects of liquefaction on stream ecosystems, thus comparisons are hard to make. However, initial reports of no lasting ecosystem effects of earthquake damage may be inaccurate. Future

work should be done to look into the effects of liquefaction of stream nutrient cycling; liquefaction is expected to remain in Christchurch streams for decades, providing an opportunity for researchers to understand the consequences of liquefaction on stream ecosystems (Gorman, 2011).

Water chemistry also varied in areas affected by heavy liquefaction with decreased levels of NO_X, increased NH_4^+, and increases in SRP relative to urban areas unaffected by the earthquake (Figure 3.23). Wells *et al.,* (2013) found similar trends immediately following the Christchurch earthquake, and also noted an increase in dissolved organic carbon. If we assume that nutrient levels should be similar to urban sites unaffected by the earthquake, this suggests that impacts of the earthquake are still influencing stream nutrient levels. Immediately following the earthquake rates of denitrification (conversation of N species to N_2 gas) in the Heathcote River increased due to the influx of raw sewage (up to 80% attenuation), leading to lower levels of NO_X (Wells *et al.,* 2013). Levels of NH_4^+ may be explained by the lack of sewage treatment; nitrifying bacteria are used at wastewater treatment plants to oxidise NH_4^+ to NO_3^- before discharge (Paul and Meyer, 2001). Sewage leaks and overflows have occurred as recently as January 2013, two months prior to the bioassays in earthquake damaged areas; it is therefore unsurprising that trends in nutrient concentrations are similar to those immediately following the earthquakes (Canterbury District Health Board, 2013; Cairns, 2013; Christchurch City Council, 2013).

2.5. Conclusion

Urbanisation in New Zealand is linked to altered water chemistry which has significant implications for biofilm nutrient limitation. Specifically, human land-use, especially urbanisation, tended to switch microbial nutrient limitation from N to P or completely alleviate nutrient limitation. Differences were observed between autotrophic and heterotrophic nutrient limitation patterns, with heterotrophic biofilms providing more consistent results which suggest that these may be a useful tool for consistently gauging nutrient limitation. Heterotrophic biofilm responses to nutrient enrichment were strongly linked to the ratio of inorganic nutrients (DIN:SRP) in the water column, demonstrating rough agreement with the ratio suggested by Redfield (1958). Ratios also coincide with a switch in land-use; with N limitation indicated at native sites (<5N:1P) and P limitation at urban and agricultural sites (>16N:1P). Biofilms in urban sites were no longer N limited beyond urbanisation intensities of 30% increasing downstream nutrient transport and eutrophication potential. Lack of limitation was common at sites affected by heavy liquefaction in Christchurch, in addition to Otaki (urban) and

Kaukapakapa (pastoral) in Auckland. Interestingly, these sites also demonstrated nutrient suppression (usually N); potential mechanisms behind this should be explored in future research. Liquefaction and occasional inputs raw sewage are having continued effects of Christchurch's streams through suppression of P on biofilms and changes in water column nutrient concentrations, highlighting the long-lasting effects of disasters on stream ecosystems. Results from this study indicate that New Zealand's urban streams have become negatively impacted by urbanisation resulting in a change to the magnitude and identity of limiting nutrients which has consequences for higher ecosystem functionality.

Chapter 3

Assessing Nutrient Limitation using Microbial Extracellular Enzyme Activity in Aquatic Sediments

3.1. Introduction

Microbial organisms play an essential role in the degradation of organic matter and in-stream nutrient cycling (Mulholland, 1996). During decomposition microbes can obtain nutrients directly from inorganic sources in the overlying water column or from organic molecules using extracellular enzymes (Sinsabaugh *et al.,* 2010). Bioavailable nutrients are not always available for uptake in the water column, and when limiting nutrients become available there may be competition for their uptake (Romani *et al.,* 2012). Therefore, microbial production of enzymes is a major pathway for nutrient acquisition by microbes in streams (Chrost, 1991; Hill *et al.,* 2010a). Extracellular enzymes released by primarily bacterial cells remain bound in biofilms (ecoenzymes) until their target substrate becomes available at which stage enzymes can deconstruct cell walls and depolymerize macromolecules, producing a soluble substrate which can be easily assimilated (Sinsabaugh *et al.,* 2009). The use of organic matter through enzymatic hydrolysis prevents a build-up of organic detrital matter and allows bacterial populations to use nutrients which are then made available for autotrophs and higher organisms (Sinsabaugh and Shah, 2011; Romani *et al.,* 2012).

There are several classes of enzymes involved in the degradation of organic matter (Sinsabaugh *et al.,* 2009). However, ecological studies generally measure the activities of enzymes which generate the terminal products from the major C, N, and P sources. These include glycosidases which are linked to carbon processing, peptidases which are linked to N cycling and protein/chitin degradation, and phosphatases which are linked to phosphorus acquisition (Lehto and Hill, 2013). Cells within the biofilm release these enzymes relative to nutrient and carbon shortages and environmental availability (Romani *et al.,* 2004). Enzyme production is energetically expensive; if resources are used for the acquisition of nutrients (N and P) then there should be a consequent reduction of enzymes toward C acquisition. As supplies of inorganic nutrients increase, expression of the enzyme targeting that nutrient in organic substrates should decline and C-acquiring enzyme activity should increase. For example, as inorganic P becomes scarce production of phosphatase should increase and expression of C-acquiring enzymes should be suppressed (Figure 3.1). Thus, to maintain optimal growth microbes shift their enzyme production in response to nutrient or carbon deficiencies (Lehto and

Hill, 2011). Extracellular enzyme activity (EEA) can therefore inform us about microbial nutrient limitation through shifts in enzyme expression (Hill *et al.,* 2010a). Ecological stoichiometry theory describes the balance multiple elements and the regulation of these under different environmental conditions (Sterner and Elser, 2002). Alongside this the concept of threshold elemental ratios describes the switch between carbon and nutrient limitation associated with critical C:N and C:P thresholds (Frost *et al.,* 2006). EEA is thought to represent a combination of both theories, with shifts in bioavailable nutrients accompanied by shifts in EEA stoichiometry. As such, EEA may also yield insight into anthropogenic impacts through deviations in observed EEA stoichiometry (Hill *et al.,* 2012).

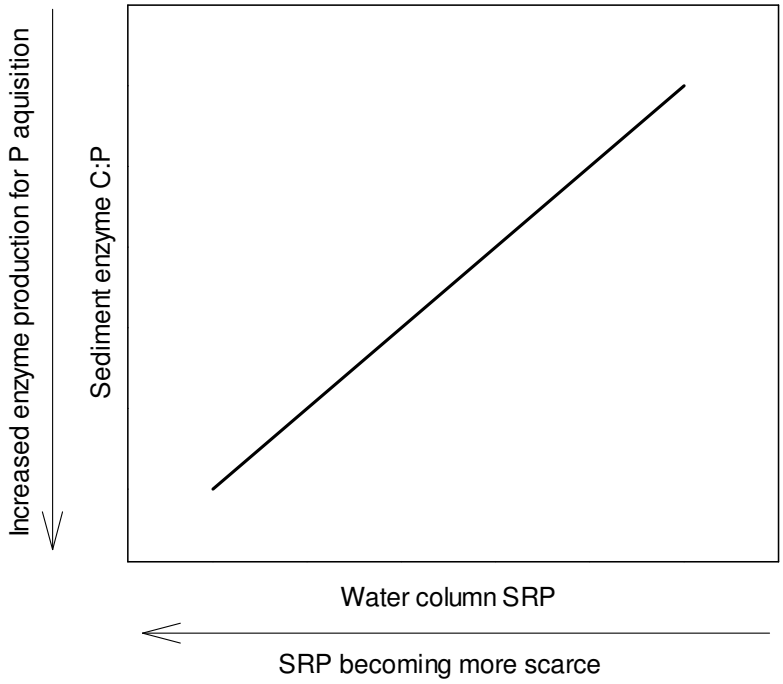


Figure 3. 1. Theoretical relationship between water column soluble reactive phosphorus (SRP) concentration and the production of enzymes for C:P (glycosidase: phosphatase) acquisition by microbial organisms.

Sediment EEA has been suggested a tool for measuring nutrient limitation in streams and informing us about wider ecosystem functioning (Hill *et al.,* 2006; Hill *et al.,* 2010a; Hill *et al.,* 2012b). The benefit of using sediment comes from the relative ease at which sediment can be sampled, for instance regional monitoring programmes could collect sediment at the same time as water samples with little extra effort. Furthermore, biotic metrics such as microbial communities may be better predictor of stream nutrient limitation status than water samples alone; providing more robust information of stream ecological functioning (Hill *et al.,* 2012). Studies to assess the viability of using sediment to predict nutrient limitation and assess ecosystem health have generally been successful, implying that nutrient acquisition by microbes

is tightly governed by C:N:P ratios such that enzyme production will be directly controlled by water column nutrient availability (Williams *et al.,* 2012; Hill *et al.,* 2012; Lehto and Hill, 2013).

The purpose of study was to assess the viability of using EEA on streambed sediments for bio-assessment in New Zealand streams. Specifically, to explore the relationship between EEA, nutrient limitation, and land-use impacts with a focus on urbanisation. Therefore, the main aims of this research are to:

a. Assess the viability of using stream benthic sediment to predict nutrient limitation,
b. Understand if urbanisation affects EEA and if this effect is consistent across regions,
c. Understand if earthquake damage impacted EEA.

My hypothesis was that stream sediments will be a viable monitoring tool for the assessment of nutrient limitation, based on a theoretically tight coupling of nutrients between water chemistry and sediment nutrient requirements environments. Microbial EEA should change predictably according to in stream nutrient concentrations (e.g. Figure 3.1), and this change should be consistent across both Auckland and Christchurch. Urbanisation should cause a shift in observed EEA stoichiometry related to variations in nutrient loads associated with urban land-use. Moreover, the effect of earthquake damage will affect EEA due to changes in sediment composition and wastewater inputs.

3.2. Methodology

3.2.1. Study design

A total of 56 sites were chosen to examine nutrient limitation through microbial enzyme assays, with 30 sites in Auckland and 26 from Christchurch (Figure 3.2). Sites build on those already described in chapter 2. In order to assess the effects of land-use on nutrient limitation, sites across a gradient of urban, pastoral, and native land-uses were chosen in order to attribute changes to the intensity of land-use. Sampling was carried out in both cities in Spring and Summer to incorporate seasonal fluctuations in nutrient concentrations. Sample collection was carried out mid-way through deployment of nutrient diffusing substrates, allowing for comparison between the experiments (chapter 4). Natural ecosystems and human influence both vary spatially across both cities; this was incorporated into sampling design by including a large number of sites across both cities which included variations in land-cover, geology, and land-use intensity (see chapter 2). Using historical data provided by Auckland Council, all Auckland sites were analysed for monthly trends in nutrient levels, this information was used to guide sample collection periods. This analysis revealed a spike in nutrient levels in rivers over January to

February and lower nutrient levels present September to December across most sites (Figure 2.2). These two seasons, Spring and Summer, were therefore used for analysis in this study.

3.2.2. Site selection

Auckland sites were chosen based on analysis of historical data from Auckland Council in addition to land-use information. Land cover data were derived from the Land Cover Database 3 (LCDB3) and is based on Land Use and Carbon Analysis System (LUCAS) satellite imagery from 2008 (Ministry for the Environment, 2013). Sites were ranked by their average nutrient concentrations (TN and TP) and ratios (DIN:SRP) based on monthly sampling from 2002 to 2012 and as well as their degree of urbanisation. Final sites had differing land-use intensities and included a range of water column nutrient levels.

Sites were visited in Auckland prior to sampling to assess the suitability of sites for this project. Sites were excluded if they were unsuitable for sampling due to water depth, if the site was in a difficult location to sample, or if the site was only accessible through private property and the landowner's permission had not been sought. A further five sites were chosen for analysis that are not monitored regularly by the Auckland Council. These sites were included to balance out the number of sites under each land- use category. The final thirty sites can be divided into five categories; urban, suburban, rural, rural-native, and native (Appendix B.1).

Sites in Christchurch covering a similar range of land-uses were chosen in addition to sites that had been impacted by the Christchurch earthquakes. Earthquake impact was categorised from the degree of liquefaction affecting the stream as either: none, light liquefaction, or heavy liquefaction. Light liquefaction streams describe streams with approximately ≤ 2cm of sediment covering the benthos, and heavy liquefaction is anything over this. Christchurch sites can be divided into four land-use categories: rural-suburban, urban-wetland, urban-industrial, and urban. Due to the lack of reference sites in Christchurch sites with the least disturbed conditions were used, in this instance the rural-suburban sites (Stoddard *et al.*, 2006). All Christchurch sites were assessed using the Freshwater Environments of New Zealand (FWENZ) and River Environment Classification (REC) databases on ArcView GIS 3.3 to gather basic catchment characteristics, including land cover data, catchment area, and height above sea level (Harding *et al.,* 2009) (Appendix B.2). Detailed site descriptions are available in chapter 2 and in appendices B.1 and B.2.

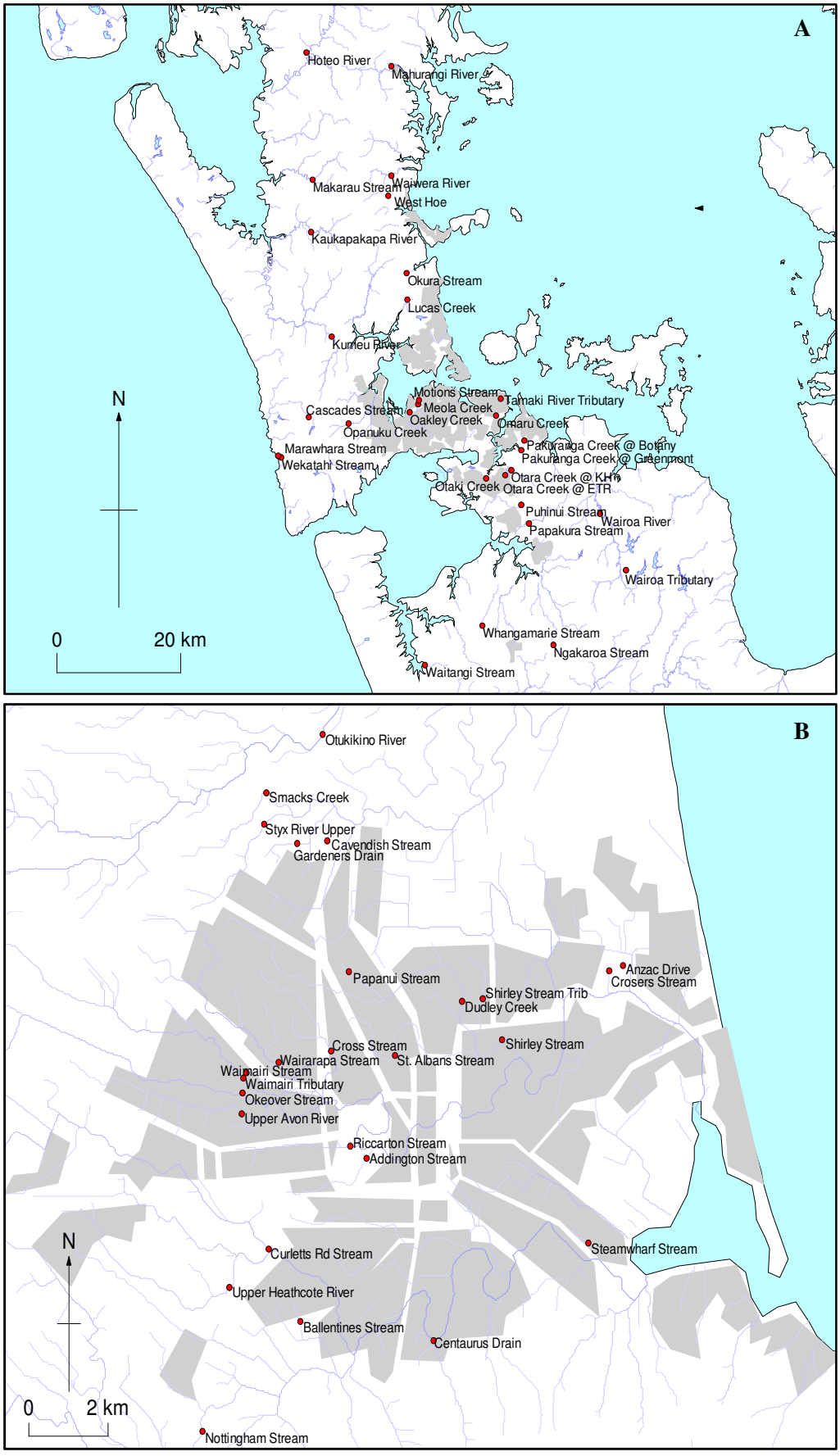


Figure 3. 2. Location of study sites in A) Auckland and B) Christchurch. Grey areas represent urban development; blue lines are freshwater systems (Adapted from the New Zealand Freshwater Fish Database, 2009).

3.2.3. Sample collection

Spring samples were collected in Auckland between the 16th October 2012 and the 24th October 2012, and Summer samples were collected from the 23rd to the 30th of January 2013. Christchurch Spring samples were collected between the 14th November 2012 and the 17th November 2012, and Summer samples between the 14th March and the 20th March 2013.

Two 100mL water samples were collected at each site, one unfiltered sample and one filtered (Whatman® GF/F glass microfibre filter). All sample bottles and syringe were rinsed with stream water at least five times before sample collection. At the same time sediment was also collected from each site. Five or more sediment samples were collected from different areas of a 100 metre stretch of river and sorted onsite using a 2mm (-1Φ) sediment sieve to standardise sediment size fractions across sites, material greater than 2mm was discarded. The remaining sediment was mixed in the sorting pan and a random sample was placed into a 30mL sample container. All samples were placed on ice and frozen within 12 hours for later analysis.

3.2.4. Physicochemical variables

Physiochemical variables were recorded at each site. Temperature, pH, and dissolved oxygen were measured using a handheld Hach 40D multi meter with a LDO dissolved oxygen probe and a pH probe. Specific conductance was measured using an EDT Instruments GP383 conductivity meter**.** All instruments were calibrated daily with standards to ensure accuracy.

3.2.5. Sample analysis

Water chemistry

Filtered water samples were analysed for nitrate (NO_3^-) and nitrite (NO_2^-) as NO_X-N, ammonium (NH_4^+), and phosphate (PO_4^{3-}) using a Lachat QuikChem® 8500 Series 2 Flow Injection Analysis System. Standards were run at the beginning of every run and at random points throughout sample processing for quality assurance. Some sites had NO_X concentrations that exceeded its limit of quantification of the linear range, 1000 µgN/L, of the machine. Those samples were diluted by tenfold and re-run. Detection limits were 5µg N or P/L and any samples with measured values below these were adjusted to one half the detection limit (2.5µg/L) for subsequent analysis.

Extracellular enzyme activity

Four extracellular enzymes, produced for C, N, and P acquisition were measured using from sediment samples (Table 3.1). Enzymes were measured using fluorescent linked substrates, 4-methylumbelliferone (MUB) or 7-amino-4-methylcoumarin (AMC). There are numerous different enzymes involved in the degradation of biopolymers (e.g. facilitate in the cleavage of bonds), however those used in this study are commonly measured enzymes in ecological studies as they catalyse the terminal reactions of enzyme hydrolysis (Sinsabaugh *et al.,* 2009; Romani, 2000; Hill *et al.,* 2006; Hill *et al.,* 2010a). Sediment samples were prepared for analysis using a modification of two microplate protocols (Sinsabaugh and Findlay, 1995; Sinsabaugh, 2009).

Table 3. 1. Description of the enzyme substrates used in this study (Romani *et al.,* 2009; Sinsabaugh, 2009).

Enzyme	Substrate	Code	Linked Substrate	Function
Leucine-aminopeptidase	L-Leucine 7 amino-4-methylcoumarin	LAP	AMC	Peptide decomposition (protein)
β-N-acetylglycosamindase	4-MUB-N-acetyl-β-glucosaminide	NAG	MUB	Chitin decomposition
Phosphatase	4-MUB-phosphate	PHOS	MUB	Hydrolyses phosphate
$B\text{-}_{D}$-glycosidase	4-MUB-β-glucoside	βGLUC	MUB	Organic carbon processing (cellulose)

Prior to running assays enzyme substrate and standard concentration optimization test runs were carried out over a number of different time periods as noted as a key gap in enzyme studies (German *et al.*, 2011; Burns *et al.*, 2013). Spare samples from two sites, one impact site with intensive urban land-use (Otaki Stream – 100% urban) and the other pristine with native forest (Cascades Stream – 100 % native), were run using enzyme substrates for two enzymes βGLUC and PHOS, the substrates were run at a number of different concentrations:1000, 500, 250, 125, and 65.2mM. After the additions of the two substrates at various concentrations the plates were run at a number of time intervals to gauge rate of reaction, these time intervals were 5, 15, and 30 minutes, then hourly for six hours and again after 24 hours. Data were plotted and analysed using Michaelis–Menten enzyme kinetics, by relating the substrate concentration to fluorescence at a time point which showed a linear increase in enzyme fluorescence. The Michaelis–Menten constant (K_d) which describes half of the maximum reaction rate (V_{max}) was used to determine appropriate substrate concentration to saturate enzyme kinetics (Appendix B.3).

Microplates were prepared for analysis by weighing out approximately 3g of sediment from a site and adding this to a centrifuge tube along with 35ml of 5µM sodium bicarbonate buffer ($NaHCO_3$). The buffer solution was adjusted to a pH of 8, the average stream pH across

all study sites. Each sample was vortexed for 10 seconds before being decanted into a glass dish on a stir plate, and a further 40mL of bicarbonate buffer added. Vortexing disrupts the biofilms, dislodging the extracellular enzymes into the buffer giving a better measure of potential EEA (Romani *et al.*, 2012). The sample was then mixed until homogenised and pipetted into the appropriate wells of a 96 well microplate (Tables 3.2 and 3.3). Each microplate was set-up to hold samples from five sites, with replicate plates made for each of the enzymes tested for. Multiple controls are included in the set-up of the microplate (Table 3.2); these ensure that any fluorescence readings seen are from enzymes and not due to the standards, substrates, samples, or due to quenching (Simon *et al.*, 2009).

Sediment subsamples were kept for ash free dry mass (AFDM) analysis. Samples were dried for 24 hours at 50˚c and weighed for dry mass; these were then ashed in a muffle furnace at 500˚c for four hours before reweighing the sample. Ash free dry mass is calculated as the difference between the dried and ashed weights.

Table 3. 2. Controls used and quantities to be loaded to microplate

Sample	Code	Load 1 (µl)	Load 2 (µl)
Reference Control	RC	200 Buffer	50 standard
Substrate Control	SuC	200 Buffer	50 substrate
Sample Control	SaC	200 Sample	50 buffer
Quench Control	QC	200 Sample	50 standard
Assay Sample	AS	200 Sample	50 substrate

Table 3. 3.Loading of micro plate, note that five samples were loaded per plate (2 rows each).

	1	2	3	4	5	6	7	8	9	10	11	12
A	RC	SuC	SaC1	SaC1	SaC2	SaC2	SaC3	SaC3	SaC4	SaC4	SaC5	SaC5
B	RC	SuC	QC1	QC1	QC2	QC2	QC3	QC3	QC4	QC4	QC5	QC5
C	RC	SuC	QC1	QC1	QC2	QC2	QC3	QC3	QC4	QC4	QC5	QC5
D	RC	SuC	QC1	QC1	QC2	QC2	QC3	QC3	QC4	QC4	QC5	QC5
E	RC	SuC	AS1	AS1	AS2	AS2	AS3	AS3	AS4	AS4	AS5	AS5
F	RC	SuC	AS1	AS1	AS2	AS2	AS3	AS3	AS4	AS4	AS5	AS5
G	RC	SuC	AS1	AS1	AS2	AS2	AS3	AS3	AS4	AS4	AS5	AS5
H	RC	SuC	AS1	AS1	AS2	AS2	AS3	AS3	AS4	AS4	AS5	AS5

Once prepared plates were frozen until the day of analysis, at which stage the appropriate reference standard were added (Table 3.1). Substrates were added at concentrations of 1000µM for LAP and PHOS and at 2000µM for β-GLUC and PHOS. Substrates and standards were made the morning of running the plates with Milli-Q water. All plates were read on a PerkinElmer EnSpire^{TM} 2300 microplate reader at excitation (EX) and emission (EM) wavelengths dependant on the standard used: MUB was read at 365_{EX}/ 450_{EM} and AMC was read at 380_{EX}/440_{EM}, giving

relative fluoresce units for each of the wells. Enzyme activity was expressed as nmol.[g AFDM]$^{-1}$ h^{-1}, by relating fluorescence to the time since substrate addition and AFDM of the sample.

3.2.6. Statistical analysis

All statistical analysis was completed using SigmaPlot or SPSS statistical software packages. Prior to analysis, nutrients NO_X and NH_4^+ were added together to be analysed as dissolved inorganic nitrogen (DIN) in addition to being analysed separately. The enzymes LAP and NAG both play a role in N cycling (peptidases) and their activities were summed for analysis. Other enzymes are discussed as phosphatase for P cycling (PHOS) and glycosidase for C cycling (βGLUC). Enzymes were also analysed as ratios to normalise for differences in microbial biomass between sites.

Physiochemical parameters were averaged across seasons and exceedances of ANZECC water quality guidelines were noted (ANZECC & ARMCANZ, 2000). Seasonality in enzyme production and physiochemical variables was tested for using a paired *t*-test, with significance indicated at $\alpha = 0.05$ level.

Spearman rank correlations were used to assess the relationship between land-use percentage data and chemistry and enzyme variables for Auckland and Christchurch in Spring and Summer. The strength and direction of the relationship was noted from Spearman's rank correlation coefficient (r_s) and a *P*-value. Spearman correlations were also used to test for a relationship between enzyme ratios, enzyme activity, and physiochemical variables.

Differences in nutrient concentrations and enzyme production between land-use categories were analysed using a one-way analysis of variance (ANOVA) followed by a *post-hoc* LSM where significant interaction terms were obtained ($\alpha < 0.05$). Liquefaction impact on Christchurch streams was also assessed using one-way ANOVA. Urban sites with either heavy, light, or no liquefaction were used as categories within which enzyme activity, and enzyme ratios, and water column nutrients were assessed. Reference sites were included in this analysis to provide a base-line.

Differences in urbanisation between cities were assessed by taking the most urban sites from both Auckland and Christchurch and comparing enzyme activities and ratios. Urban sites affected by liquefaction in Christchurch were excluded from this analysis. Urban sites from both Auckland and Christchurch were assessed for any significant differences in enzyme activity using an Independent samples *t*-test. The equality of variances assumption was tested using a Levene's Test; if the resulting *P* value was less than 0.05 samples were assumed to have non equal variances. Paired *t*-tests were also used to determine if enzyme activity was significantly different between seasons within Auckland or Christchurch.

Linear regression was used to determine the relationships between enzyme activity/enzyme ratios and gradients of land-use (urban, pastoral, and native) and water chemistry. Regression equations, including the slope and intercept, as well as r^2 and P values are reported on graphs. Data were log-transformed where necessary to meet the assumptions of linear regression. Data were split by season and region for analysis.

Nutrient limitation was identified where appropriate using two stoichiometric classifications. The Redfield ratio was used to estimate the relative N or P limitation from inorganic water column nutrient concentrations as 16N:1P (Redfield, 1958). The second classification is based on the ideal ratio of extracellular enzyme activity, 7N:1P, which represents the threshold between N and P limitation (Cleveland and Liptzin, 2007; Hill *et al.,* 2010). Deviations from these ratios were used to describe the nature of N or P limitation.

3.3. Results

3.3.1. Physiochemical summary

In Auckland concentrations of DIN were on average 25% higher in Spring (1462 ± 532µgN/L) compared to Summer (1089 ± 485µgN/L) (*t*-test, $P = 0.014$). Whangamarie Stream, an agricultural/ horticultural site, had the highest DIN concentration in Spring (13,953µgN/L) and Summer (11,753µgN/L). Concentrations of NH_4^+ were on average 2 times was higher in Summer (61 ± 37µgN/L) compared to Spring (26 ± 10µgN/L); this difference was however not significant (*t*-test, $P = 0.218$). Meola Creek had the highest NH_4^+ concentrations in Spring (280µgN/L) and Summer (1119µgN/L). SRP was on average 2 times higher in Summer (26 ± 10µgP/L) compared to Spring (14 ± 4µgP/L); although this difference was not significant (*t*-test, $P = 0.118$). Meola Creek, an urban stream, had the highest SRP concentrations in Summer (317 µgP/L) and Motions Creek in Spring (96µgP/L). Ratios of DIN:SRP tended to be higher in Spring (448:1) than Summer (306:1), but differences were not statistically significant (*t*-test, $P = 0.062$).

Conductivity was 15% higher in Auckland Spring (213 ± 18µS/cm) compared to Summer (182 ± 24µS/cm) (*t*-test, $P = 0.046$). Dissolved Oxygen was also 30% higher in Spring (9.8 ± 0.3 mg/L) than in Summer (6.9 ± 0.5mg/L) (*t*-test, $P < 0.0001$). pH was more basic in Spring (7.69 ± 0.05) compared to Summer (7.34 ± 0.07) (*t*-test, $P < 0.0001$). Temperature increased from an average of 14.5˚C (±0.4) in Spring to 19.9˚C (±0.4) in Summer (*t*-test, $P < 0.0001$).

Nutrient concentrations in Christchurch generally peaked in Summer. DIN concentrations were similar across seasons, with no significant differences between Summer (1738 ± 356µgN/L) and Spring (1616 ± 342µgN/L) (*t*-test, $P = 0.695$). The Upper Heathcote River, an

urban stream, had the highest DIN concentrations in Spring (7633µgN/L) and Ballentines Stream, a semi-urban stream, had the highest concentrations of DIN in Summer (6937µgN/L). Concentrations of NH_4^+ were 2.5 times higher in Summer (80 ± 23µgN/L) compared to Spring (32 ± 9µgN/L) (*t*-test, $P = 0.020$). Reaching a maximum of 363µgN/L in St. Albans Stream (urban) in Summer and 165µgN/L in Shirley Stream (urban) in Spring. Concentrations of SRP were on average 4 times higher in Summer (43 ± 16µgP/L) compared to Spring (10 ± 2µgP/L) (*t*-test, $P < 0.0001$). SRP reached a maximum concentration of 334µgP/L at Anzac Drive Stream (urban) in Summer and 37µP/L at Centaurus Drain (urban) in Spring.

Like Auckland, stream temperature in Christchurch was warmer in Summer (16 ± 0.2˚C) than in Spring (14 ± 0.3˚C) (*t*-test, $P < 0.0001$); and pH was more basic in Spring (7.79 ± 0.11) than in Summer (7.30 ± 0.07) (*t*-test, $P = 0.001$). Conductivity was on average higher in Spring (394 ± 148 µS/cm) than in Summer (320 ± 311 µS/cm); similarly with dissolved oxygen which was higher in Spring (7.9 ± 0.5 mg/L) than Summer (7.0 ± 0.4 mg/L); neither of these differences were however statistically significant (ANOVA, $P > 0.05$).

Auckland native land-use streams had the lowest DIN concentrations in Spring (15µgN/L) and Summer (18µgN/L), with concentrations 141 times lower than urban streams in Spring (2152µgN/L) and 56 times lower in Summer (1460µgN/L) (Figure 3.3A). DIN concentrations in native, native/pastoral, and suburban land-uses were consistently under the ANZECC trigger value, whereas urban and pastoral land-uses generally exceeded trigger values. In Spring, concentrations of DIN were significantly higher in pastoral land-use than in native (ANOVA, $P = 0.041$) and native/pastoral (ANOVA, $P = 0.039$); differences between urban and native land-use were however not significant (ANOVA, $P = 0.186$). In Summer, differences between DIN concentrations across all land-uses were non-significant. In Spring NH_4^+ concentrations in urban sites were statistically higher than any other land-use category, the largest difference was between urban (81µgN/L) and native (2.5µgN/L) land-uses (ANOVA, $P = 0.005$). Concentrations were however not statistically different from one another in Summer.

All land-use categories in Auckland exceeded the trigger values for SRP in Summer with urban land-use demonstrating the highest average concentrations in Spring (28µgP/L) and Summer (61µgP/L); differences between land-uses in either season were however not statistically different (ANOVA, $P > 0.05$) (Figure 3.3). Pastoral land-use had the highest DIN:SRP ratios in Spring (1443:1) and Summer (1209:1), followed by urban land-use (Spring: 351:1, Summer: 68:1). Lowest DIN:SRP ratios were from native sites in Spring (5:1) and Summer (2:1). Ratios were statistically different between pastoral land-use and all other land-use

categories in both seasons (ANOVA, $P < 0.05$). DIN:SRP ratios in urban-land were however not statistically higher than the other land-use categories (ANOVA, $P > 0.05$).

Nutrients in Christchurch demonstrated less variation among land-use categories when compared to Auckland (Figure 3.3B). All land-use categories exceeded guideline values for DIN in both seasons apart from urban-wetland. In Summer, concentrations of DIN were significantly higher in semi-urban than in rural-suburban (ANOVA, $P = 0.023$) and urban-wetland categories (ANOVA, $P = 0.040$), there were however no statistical differences in DIN in Spring. SRP is higher in Christchurch in Summer, with all sites exceeding guidelines in this season, with the highest concentration in the urban-wetland (176µgP/L) land-use category. This concentration was significantly higher than rural/suburban (ANOVA, $P = 0.013$), semi-urban (ANOVA, $P = 0.041$), and urban (ANOVA, $P = 0.011$), but not urban-industrial (ANOVA, $P = 0.336$). In Spring only urban (12µgP/L) and urban-wetland (20µgP/L) exceeded SRP guideline values and concentrations between sites were not statistically different from one another (ANOVA, $P > 0.05$). Average water column ratios for all categories in Spring and Summer were above 16N:1P in Christchurch. Results show that only three sites from Christchurch had ratios less than 16:1, these were Centaurus Drain in Spring, Anzac Drive Stream in Summer, and Cavendish Stream in Summer. DIN:SRP was highest in semi-urban sites in Spring (2143:1) and Summer (1372:1), and lowest in urban-wetland sites in Spring (34:1) and Summer (17:1). Differences between semi-urban and urban-industrial (ANOVA, $P = 0.036$) and rural suburban (ANOVA, $P = 0.050$) land-uses were statistically different in Spring. In general, Christchurch rural-suburban (reference) sites demonstrated similar trends to Auckland suburban sites rather than Auckland's reference native sites.

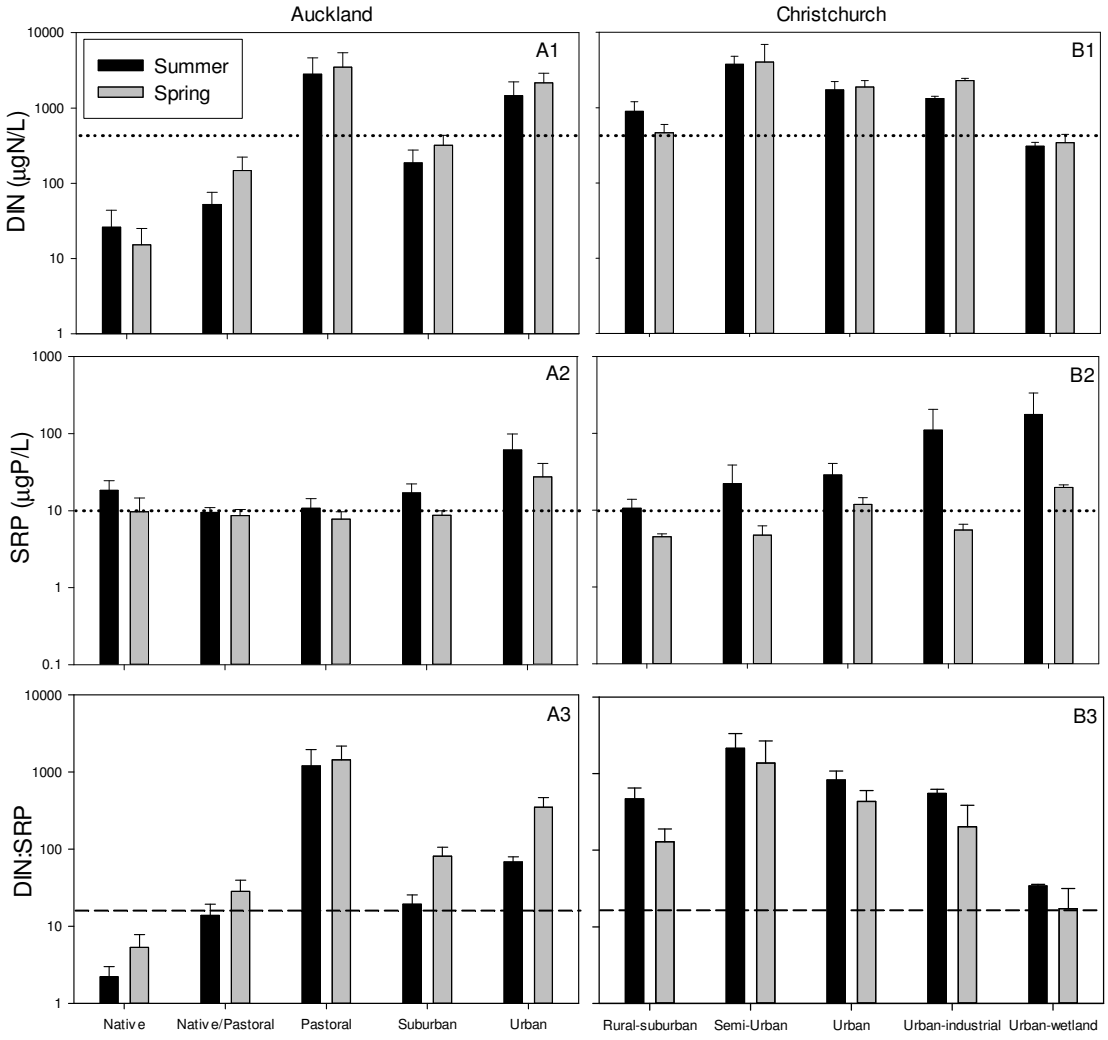


Figure 3. 3. Average (±SE) water chemistry values grouped by land-use category for A) Auckland and B) Christchurch showing 1) DIN, 2) SRP, and 3) molar DIN:SRP ratios. Trigger values are indicated for DIN (444µgN/L) and SRP (10µgP/L) with a dotted line. The Redfield ratio of 16N:1P is marked with a dashed line on graphs A3 and C3.

3.3.2. Correlations

Native and urban land-uses in Auckland had strong effects on stream nutrient concentrations and demonstrated opposing trends in nutrient and physiochemical parameters (Table 3.4). In contrast, pastoral land-use was weakly linked to nutrient concentrations and physiochemical parameters. Data confirm a significant positive association between urban land-use and DIN:SRP (Spring: $r_s = 0.547$, $P = 0.002$; Summer: $r_s = 0.498$, $P = 0.005$), and a negative association between native land-use and DIN:SRP in both seasons (Spring: $r_s = -0.855$, $P < 0.0001$; Summer: $r_s = -0.779$, $P < 0.0001$). DIN concentrations had the strongest correlations with urban and native land-uses, whereas SRP was not correlated with either of these but was negatively correlated with pastoral land-use over Summer. Urban land-use was positively correlated with increases in NH_4^+, conductivity, and temperature. Whereas native land-use was negatively correlated with these variables in addition to being positively correlated to dissolved

oxygen. These findings are consistent with expectations of native and urban sites. Note that no significant interactions existed between land-use and measured variables in Christchurch.

Table 3. 4. Spearman correlations (r_s) between land-use (%) and physiochemical variables in Auckland, significance level is indicated as, $P < 0.05$=*, $P < 0.005$=, $P < 0.0005$***, or ns = non-significant.**

Variable	Season	Land use %		
		Native	Urban	Pastoral
DIN (µgN/L)	Spring	-0.840***	0.545**	ns
	Summer	-0.786***	0.559**	ns
SRP (µgP/L)	Spring	ns	ns	ns
	Summer	ns	ns	-0.437*
NH_4^+ (µgN/L)	Spring	-0.477**	0.600***	ns
	Summer	-0.528**	0.710***	ns
DIN:SRP	Spring	-0.855***	0.547**	ns
	Summer	-0.779***	0.498*	ns
DO (mg/L)	Spring	ns	ns	ns
	Summer	0.397*	ns	-0.408*
Temperature (˚C)	Spring	-0.627***	0.619***	ns
	Summer	ns	0.493*	ns
Conductivity (µS/cm)	Spring	-0.586**	0.569**	ns
	Summer	-0.632***	0.600***	ns

In Auckland, water column DIN and SRP were weakly related to enzyme activity and enzyme ratios in Spring and Summer (Table 3.5). Concentration of NH_4^+ was positively related to activity of phosphatase and peptidase. Urban land-use in Auckland was positively correlated with increases in peptidase and phosphatase in Spring and Summer, and glycosidase in Summer. In contrast, native land-use in Summer was negatively correlated with all measured enzyme activities. Urban sites had low C:N and C:P enzyme activity ratios suggesting C was not limiting. In contrast, pastoral land-use was positively correlated with C:P enzyme ratios. Increased dissolved oxygen levels were related to increases C:N enzyme activity ratios in both Spring and Summer in Auckland, and negatively related to the production of N acquiring enzymes in Spring. Activity of phosphatase was positively related to increased temperatures in both seasons.

Table 3. 5. Spearman correlations (r_s) in Auckland of physiochemical variables and catchment characteristics sediment enzyme activity and enzyme ratios. Significance is noted as, $P < 0.05$=*, $P < 0.005$=, $P < 0.0005$=***, or ns = not significant.**

Season	Variable	Enzyme activity			Enzyme activity ratios		
		Peptidase (N)	Phosphatase (P)	Glycosidase (C)	N:P	C:N	C:P
Spring	DIN (µgN/L)	ns	ns	ns	ns	ns	ns
	NH_4^+ (µgN/L)	0.481*	0.556**	ns	0.373*	-0.491*	ns
	SRP (µgP/L)	ns	ns	ns	ns	ns	ns
	DIN:SRP	ns	ns	ns	ns	ns	ns
	Dissolved oxygen (mg/L)	-0.430*	ns	ns	-0.402*	0.391*	ns
	Temperature (˚C)	ns	0.477*	ns	ns	ns	ns
	Catchment area (ha)	0.432*	ns	ns	0.557**	ns	ns
	Elevation (m)	ns	ns	ns	ns	ns	ns
	% Urban	0.514**	0.664***	ns	ns	-0.492**	-0.381*
	% Native	ns	-0.452*	ns	ns	ns	ns
	% Pastoral	ns	ns	ns	ns	ns	0.522**
	% Horticultural	ns	ns	ns	ns	ns	0.420*
Summer	DIN(µgN/L)	ns	ns	0.458*	ns	ns	ns
	NH_4^+ (µgN/L)	0.603***	0.591**	0.679***	ns	ns	ns
	SRP (µgP/L)	ns	ns	ns	ns	ns	ns
	DIN:SRP	0.368*	ns	0.394*	ns	ns	ns
	Dissolved oxygen (mg/L)	ns	ns	ns	ns	ns	ns
	Temperature (˚C)	ns	0.382*	ns	ns	ns	ns
	Catchment area (ha)	ns	ns	ns	ns	-0.357*	ns
	Elevation (m)	ns	-0.544**	-0.488*	ns	ns	ns
	% Urban	0.523**	0.626***	0.607***	ns	ns	ns
	% Native	-0.422*	-0.477**	-0.528**	ns	ns	ns
	% Pastoral	ns	ns	ns	ns	ns	ns
	% Horticultural	ns	ns	ns	ns	ns	ns

Christchurch correlations reveal relationships between water column nutrients and enzyme activity Spring, but few significant correlations in Summer (Tables 3.6). In Spring, DIN concentrations were negatively correlated to activity of N acquiring enzymes, consistent with expectations. Inconsistent though is the positive relationship between SRP and phosphatase activity. Concentrations of SRP were also positively related to activity of N and C acquiring enzymes in Spring. Additionally, concentrations of NH_4^+ were positively correlated with peptidase in Spring, however in Summer DIN was negatively correlated with peptidase. Sites which were closer to the coast, had a lower elevation, higher temperatures, and increased NH_4^+ levels had higher N:P enzyme activity levels. In contrast, C:N enzyme activity ratios were

associated with an increased elevation and decreased NH_4^+ concentrations, similarly to Auckland Spring. In Summer urban land-use was positively correlated to enzyme P and C activity, in contrast these variables were negatively correlated with pastoral land-use.

Table 3. 6. Spearman correlations (r_s) in Christchurch of physiochemical variables and catchment characteristics sediment enzyme activity and enzyme ratios. Significance is noted as, $P < 0.05$=*, $P < 0.005$=, $P < 0.0005$=***, or ns = not significant.**

Season	Variable	Enzyme activity			Enzyme activity ratios		
		Peptidase (N)	Phosphatase (P)	Glycosidase (C)	N:P	C:N	C:P
Spring	NO_X (µgN/L)	-0.621**	-0.486*	ns	ns	ns	ns
	NH_4^+ (µgN/L)	0.696***	0.451*	ns	0.449*	-0.491*	ns
	DIN (µgN/L)	-0.597**	-0.503*	ns	ns	ns	ns
	SRP (µgP/L)	0.633**	0.448*	0.478*	ns	ns	ns
	DIN:SRP	-0.658**	-0.493*	ns	ns	ns	ns
	Temperature (˚C)	ns	ns	ns	0.403*	ns	ns
	Dissolved oxygen	ns	ns	ns	ns	ns	ns
	pH	ns	ns	ns	ns	ns	0.465*
	Conductivity	ns	ns	ns	ns	ns	ns
	Distance to coast (m)	-0.588**	-0.519*	ns	-0.418*	ns	ns
	Elevation (m)	-0.599**	ns	ns	-0.556**	0.389*	ns
	% Urban	ns	ns	ns	ns	ns	ns
	% Pastoral	ns	ns	ns	ns	ns	ns
Summer	NO_X (µgN/L)	ns	ns	ns	ns	ns	ns
	NH_4^+(µgN/L)	ns	ns	ns	ns	ns	0.508**
	DIN (µgN/L)	ns	ns	ns	ns	ns	ns
	SRP (µgP/L)	ns	ns	ns	ns	ns	ns
	DIN:SRP	ns	ns	ns	ns	ns	ns
	Temperature (˚C)	ns	ns	ns	ns	ns	ns
	Dissolved Oxygen (mg/L)	ns	ns	0.652***	ns	ns	ns
	pH	ns	ns	0.643**	ns	ns	ns
	Conductivity (µS/cm)	ns	ns	0.504*	ns	ns	ns
	% Urban	ns	0.506*	0.408*	ns	ns	ns
	% Pastoral	ns	-0.553**	-0.483*	ns	ns	ns

3.3.3. Extracellular enzyme activity across land-use categories

Native sites in Auckland had the lowest enzyme activity when compared to other land-use categories in both seasons (Figure 3.4). Peptidase activity was significantly higher in the urban land-use category than native in Spring (ANOVA, P = 0.018), with no significant differences between the other categories. Phosphatase activity was highest in urban sites compared to any other land-use, with significant differences between native and urban categories in Spring and Summer (ANOVA, P = 0.009, P = 0.006 respectively), and between urban and native/pastoral in Summer (ANOVA, P = 0.032). Phosphatase activity reached 61 nmol.[g AFDM]$^{-1}$ h^{-1} in urban sites in Summer compared to 11 nmol.[g AFDM]$^{-1}$ h^{-1} in Spring. Phosphatase activity in suburban land-use was also significantly higher than activity in native (ANOVA, P = 0.005), native/pastoral (ANOVA, P = 0.016), and pastoral (ANOVA, P = 0.034). Glycosidase activity was also significantly higher in urban than in native (ANOVA, P = 0.013) and native/pastoral (ANOVA, P = 0.019) in Spring, with no significant differences in glycosidase activity in Summer. Enzyme activity in Summer had more variation within the categories, particularly within pastoral and suburban land-uses.

Christchurch urban sites along with urban-industrial and urban-wetland demonstrated increased levels of enzyme activity compared to rural-suburban (reference) (Figure 3.5). This difference is however not a large as found in Auckland between urban and native (reference) land-uses. Patterns in the data are clearer over Summer due to lower variation within land-use categories. From the three urban land-use categories urban-wetland had the highest average enzyme activity levels. In Spring and Summer urban-wetland sites had significantly more phosphatase activity than rural-suburban (ANOVA, P = 0.009, P = 0.016 respectively), and in Spring urban phosphatase activity was significantly lower in urban than in urban-industrial (ANOVA, P = 0.028). Glycosidase levels were their highest at urban sites in Christchurch over Summer, with activity significantly higher in urban sites compared to rural-suburban in Summer (ANOVA, P = 0.010).

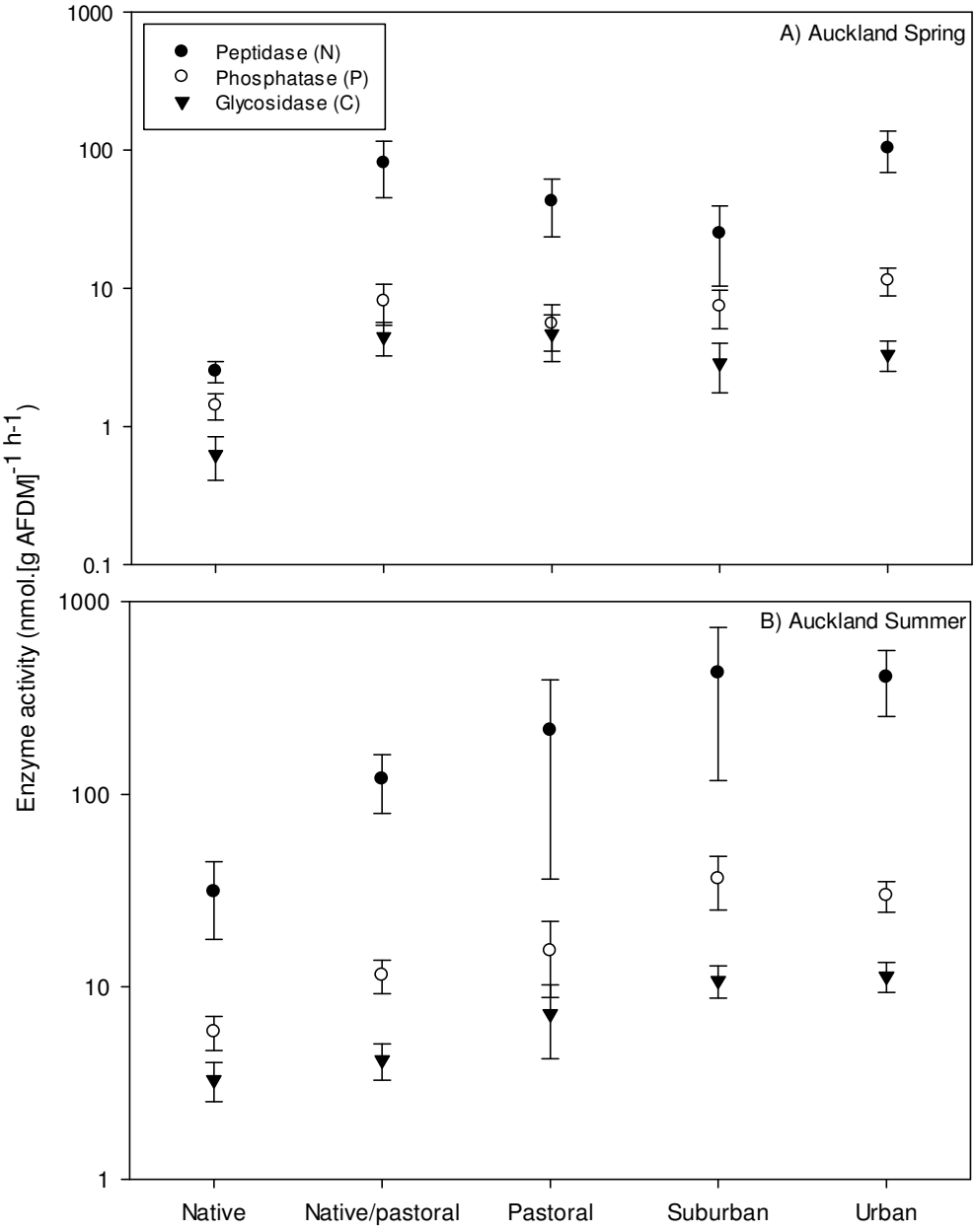


Figure 3. 4. Average (±SE) enzyme activity across land-use categories in Auckland A) Spring and B) Summer, note that activity is on a log scale.

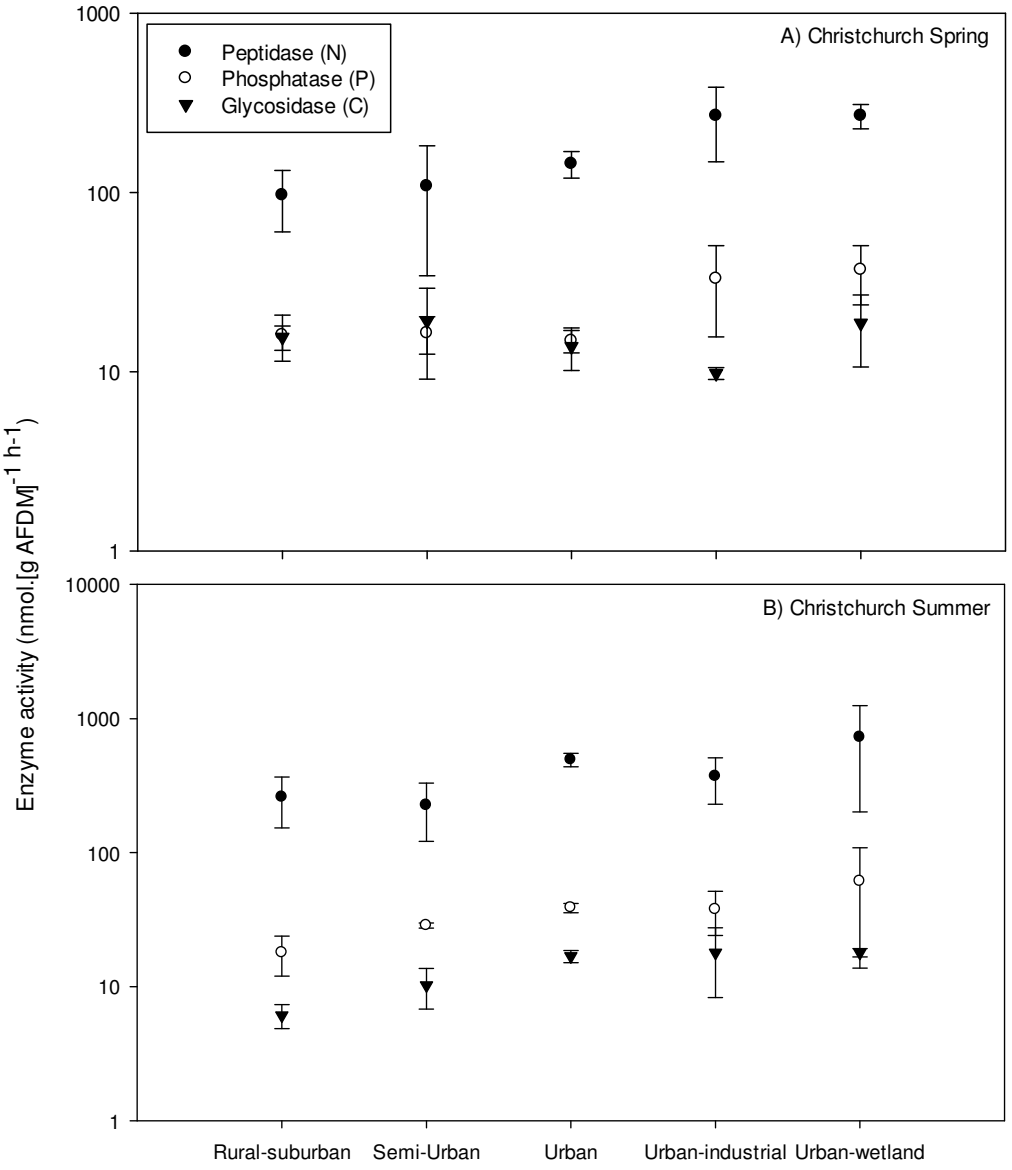


Figure 3. 5. Average (±SE) enzyme activity across land-use categories in Christchurch A) Spring and B) Summer, note that activity is on a log scale.

3.3.4. Extracellular enzyme activity across a gradient of land-use

Enzyme activity declined with loss of native land cover and increased with increasing urban land-use in Auckland (Figure 3.6). Spring and Summer regressions show the same trends, with Spring regressions consistently showing higher levels of enzyme activity when compared to Summer. Native land-use regressions show large amounts of variation at 0% native land-use, likely related to a mixture of other land-uses here such as urban and pastoral. Pastoral land-use was not significantly related (ANOVA, $P > 0.05$) to enzyme activity in Auckland Spring and Summer.

In Christchurch, enzyme activity of phosphatase and glycosidase was significantly related to urban and pastoral land-uses (Figure 3.7). Similar to Auckland, urban land-use was associated with increases in enzyme activity. Pastoral land-use (inclusive of reference sites) showed similar trends to Auckland native sites, with activity decreasing as pastoral land-use increased. Peptidase activity was consistently high across both land-use categories; showing no significant associations with land-use percentage. Enzyme activity in Christchurch Spring showed no significant relationships with land-use data.

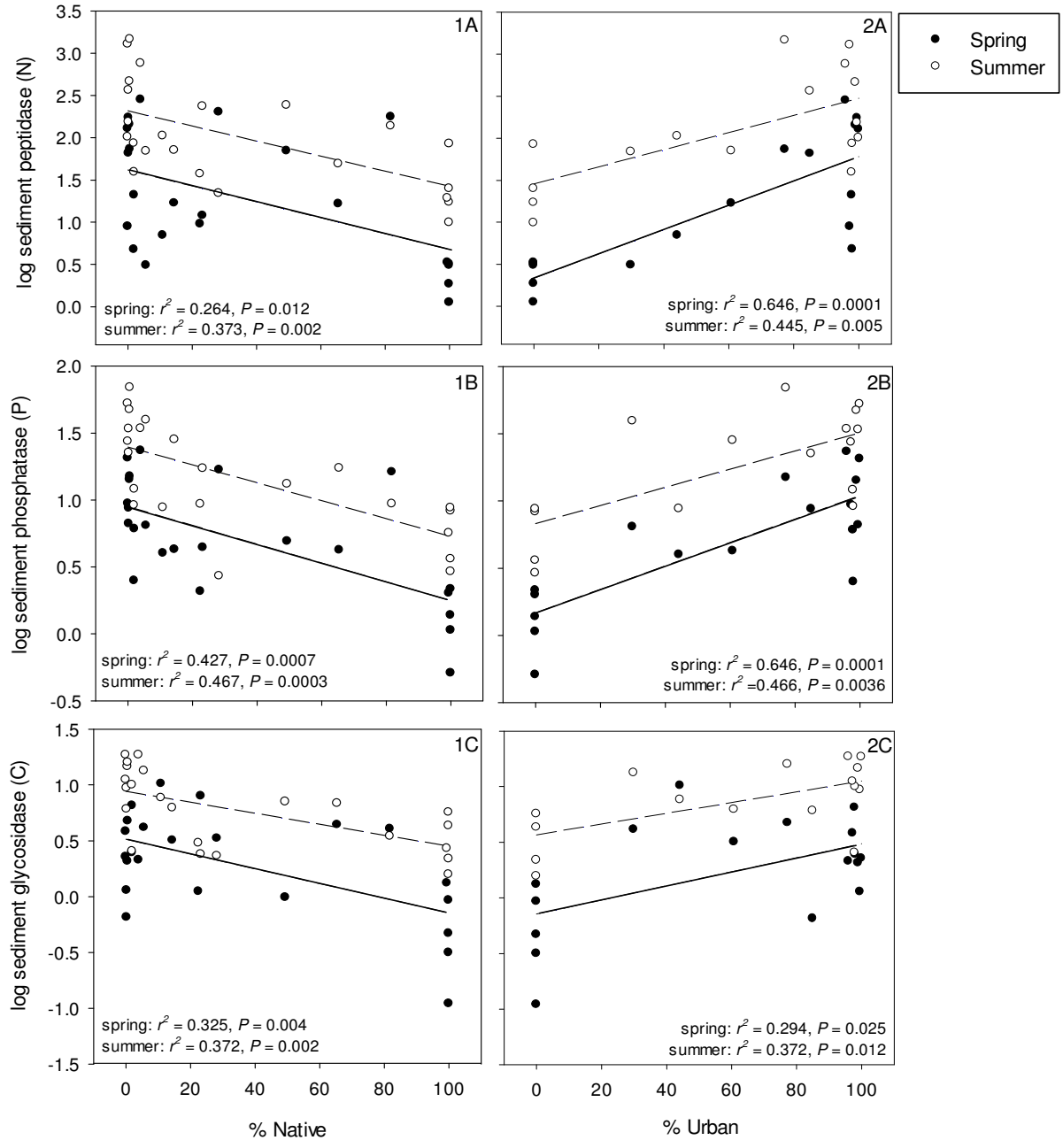


Figure 3. 6. Enzyme activity (nmol.[g AFDM]$^{-1}$ h^{-}1)across native and urban land-use gradients in Auckland Spring and Summer with linear regression models fitted, Spring regressions have a solid line and Summer regressions have a dashed line.

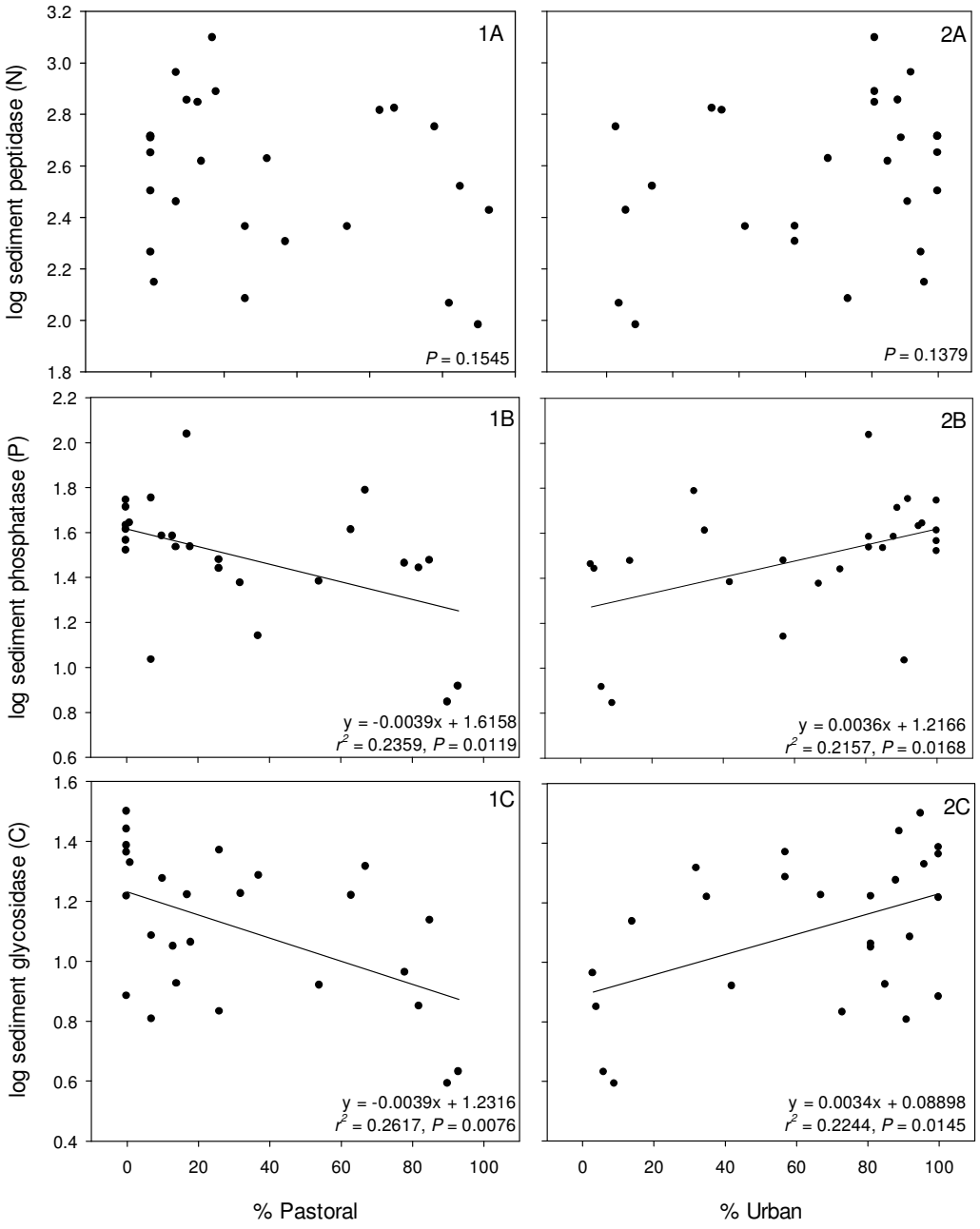


Figure 3. 7. Christchurch Summer sediment enzyme activity (nmol.[g AFDM]$^{-1}$ h^{-1}) presented as log values across land-use gradients, 1) pastoral and 2) urban with a linear regression models fitted.

3.3.5. Extracellular enzyme activity and water chemistry

Enzyme activity increased with water column N in Auckland over Spring and Summer (Figure 3.8). Sediment peptidase and water column N showed positive relationships; with more N acquiring enzymes produced as water column N increased. One large difference between seasons was the form of N related to enzyme activity; in general DIN was best related to enzyme activity in Auckland Spring and NH_4^+ in Auckland Summer (Figure 3.8). In all NH_4^+ regressions the data show large amounts of variation at lower concentrations. In both seasons SRP demonstrated positive trends between water P and enzyme P, relationships were not however significant (Spring: $r^2 = 0.08$, $P = 0.131$; Summer: $r^2 = 0.107$, $P = 0.083$).

Water column nutrients DIN and SRP were negatively associated with enzyme activity in Christchurch during Spring, but sparsely related in Summer. In Spring there was a negative relationship between enzyme activity from all three enzyme categories and water DIN (Figure 3.9-1). As would be expected, less N acquiring enzymes were produced with increased water column DIN concentrations. The continuation of this trend within the other enzyme categories suggests that the production of enzymes is linked through microbial biomass.

Also in Spring, concentrations of SRP increased with phosphatase, along with the production of peptidase and glycosidase (Figure 3.9-2). In these regressions two distinct groupings of data can easily be distinguished; with a smaller group of 7 data points showing constant high levels of SRP and enzyme activity. These data points all belong to heavy liquefaction sites, SRP concentrations here are more than double that of many sites.

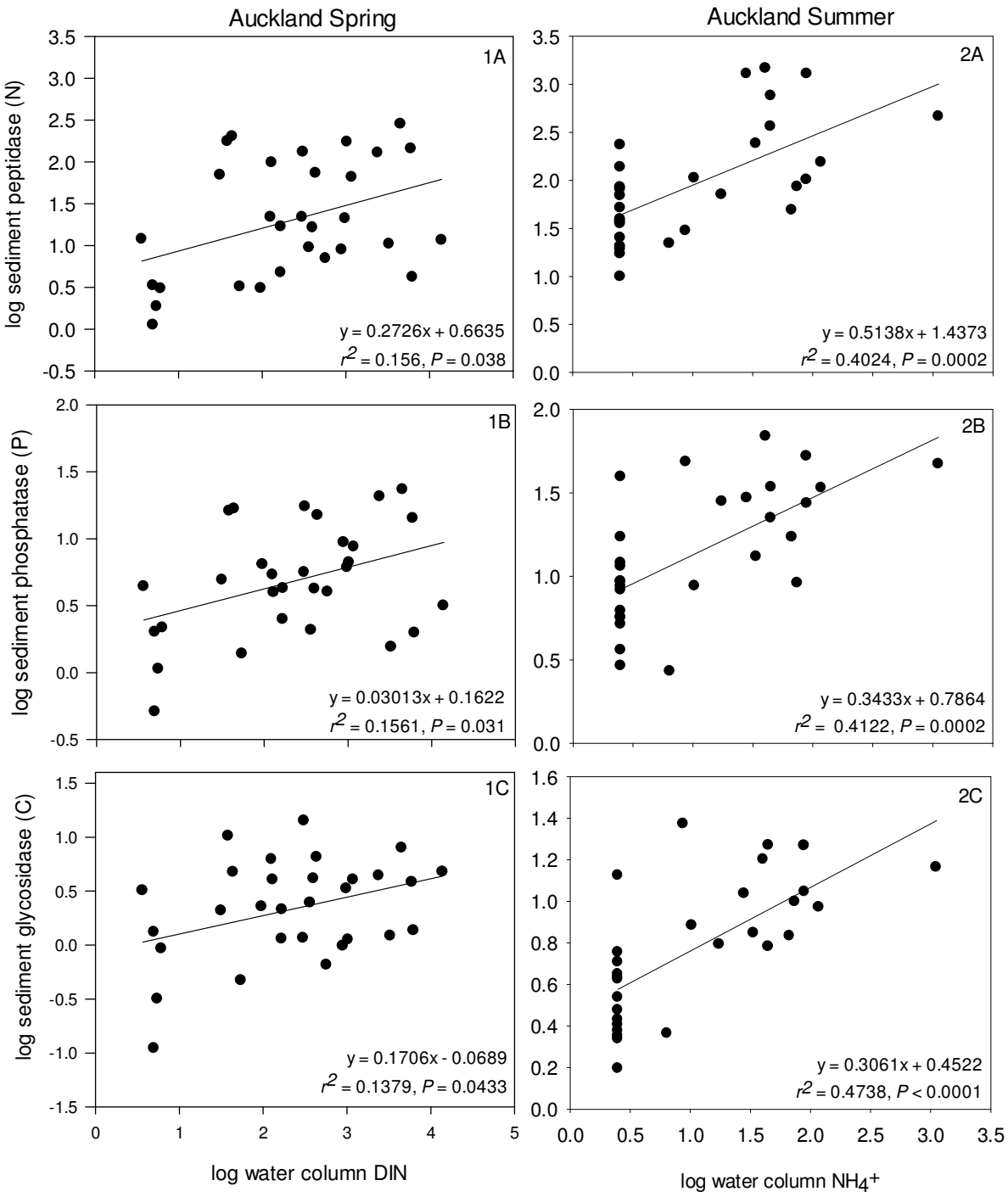


Figure 3. 8. Auckland Spring water column log DIN (µgN/L) and Auckland Summer water column log NH_4^+ (µgN/L) against enzyme activity (nmol.[g AFDM]$^{-1}$ h^{-1}) for A) peptidase, B) phosphatase, and C) glycosidase.

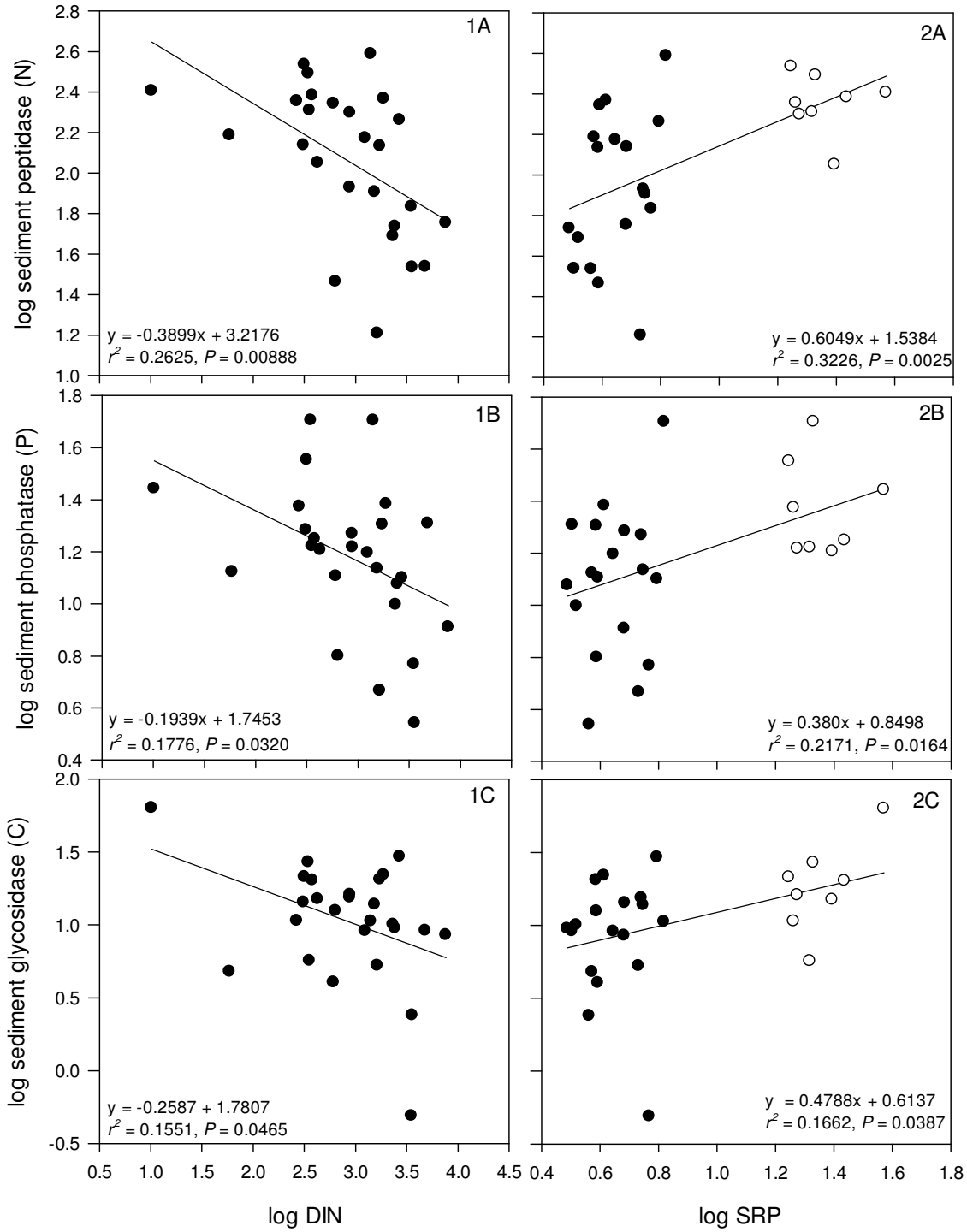


Figure 3. 9.Water column nutrients (µg/L) against enzyme activity (nmol.[g AFDM]$^{-1}$ h^{-1}) in Christchurch Spring with linear regression models fitted, data presented as log values . White circles indicate sites affected by liquefaction.

3.3.6. Extracellular enzyme stoichiometry and land-use

Microbial biomass may be higher in human-influenced sites causing an increase in absolute enzyme activity with land-use gradient. To normalise for this potential biomass effect it is also useful to analyse enzyme activity as ratios, as described below.

Ratios of enzyme activity were generally similar across land-use categories, but with few exceptions. In Auckland Spring N:P ratios were lowest in native sites, followed by suburban with the other three land-use categories showing ratios close to 7:1 – the ideal stoichiometric ratio (Figure 3.10). This suggests that at native sites microbial communities in stream sediments were P limited. Urban sites had a ratio of 8.5:1 (± 2.9), and native/pastoral had a ratio of 8:1(± 2.0), suggesting N limitation or co-limitation. Pastoral sites had a ratio of 7.5:1(± 2.96), indicating that these sites were on average co-limited, or limited by another element. Enzyme C:P ratios were significantly higher in pastoral land-use than in native (ANOVA, $P = 0.005$), suburban (ANOVA, $P = 0.008$), and urban (ANOVA, $P = 0.001$). Lowest C:P levels were found in urban sites, with C:P levels three-fold higher in pastoral sites compared to urban sites in Spring.

EEA ratios in Auckland generally increased across all land-uses from Spring to Summer (Figure 3.10). Notably the N:P ratio in native sites increased from an average ratio of 2:1(± 0.2) in Spring to 7:1(± 4.1) in Summer, along with this the C:P ratio which approximately doubles whilst the C:N ratio decreased. These changes suggest a change from P limitation to N limitation in native sites. In urban sites N:P ratio increased from 8.5:1(± 2.9) to 14:1(± 5.3), suggesting stronger N limitation over Summer. This is similarly the case for suburban sites which shifted from P to N limited between seasons with ratios increasing three fold from 3:1(± 0.9) to 9.4(± 4.1). The C:P ratio in pastoral sites decreased by half between Spring and Summer, whilst the C:N ratio stayed the same and the ratio of N:P increased.

Between land-uses in Christchurch enzyme C:N was significantly higher in rural-suburban than urban-wetland (ANOVA, $P = 0.05$) (Figure 3.11). There were no other statistically significant relationships between land-use categories. Christchurch EEA ratios also increased between Spring and Summer. Differences between seasons or categories were not however statistically significant ($P > 0.05$). Rural-suburban sites shifted to being N limited in Spring with a mean ratio of 6:1(± 1.0) to P limited in Summer with a mean of 17:1 (± 5.9). All other land-uses remained above the ideal N:P ratio of 7:1, suggesting a predominance of N limitation in Christchurch. Carbon and nutrient ratios (C:N and C:P) generally decreased

between Spring and Summer, with the exception of C:P ratios in urban-wetland land-use which increased in Summer, there is however a lot of variation in the Summer values.

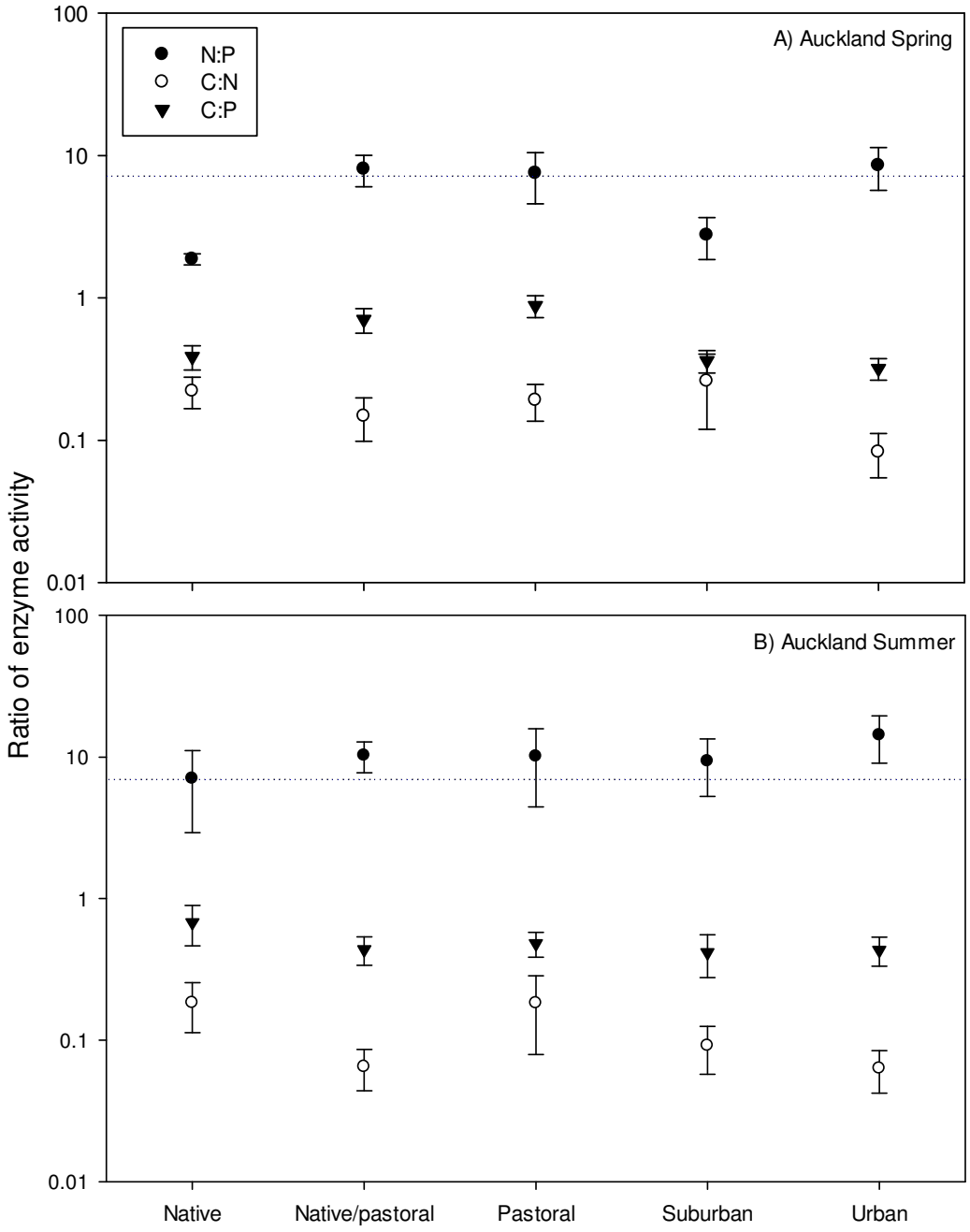


Figure 3. 10. Average (±SE) enzyme activity ratios in Auckland A) Spring and B) Summer across land-use categories. Line at 7:1 indicated the enzymatic N:P stoichiometric ratio.

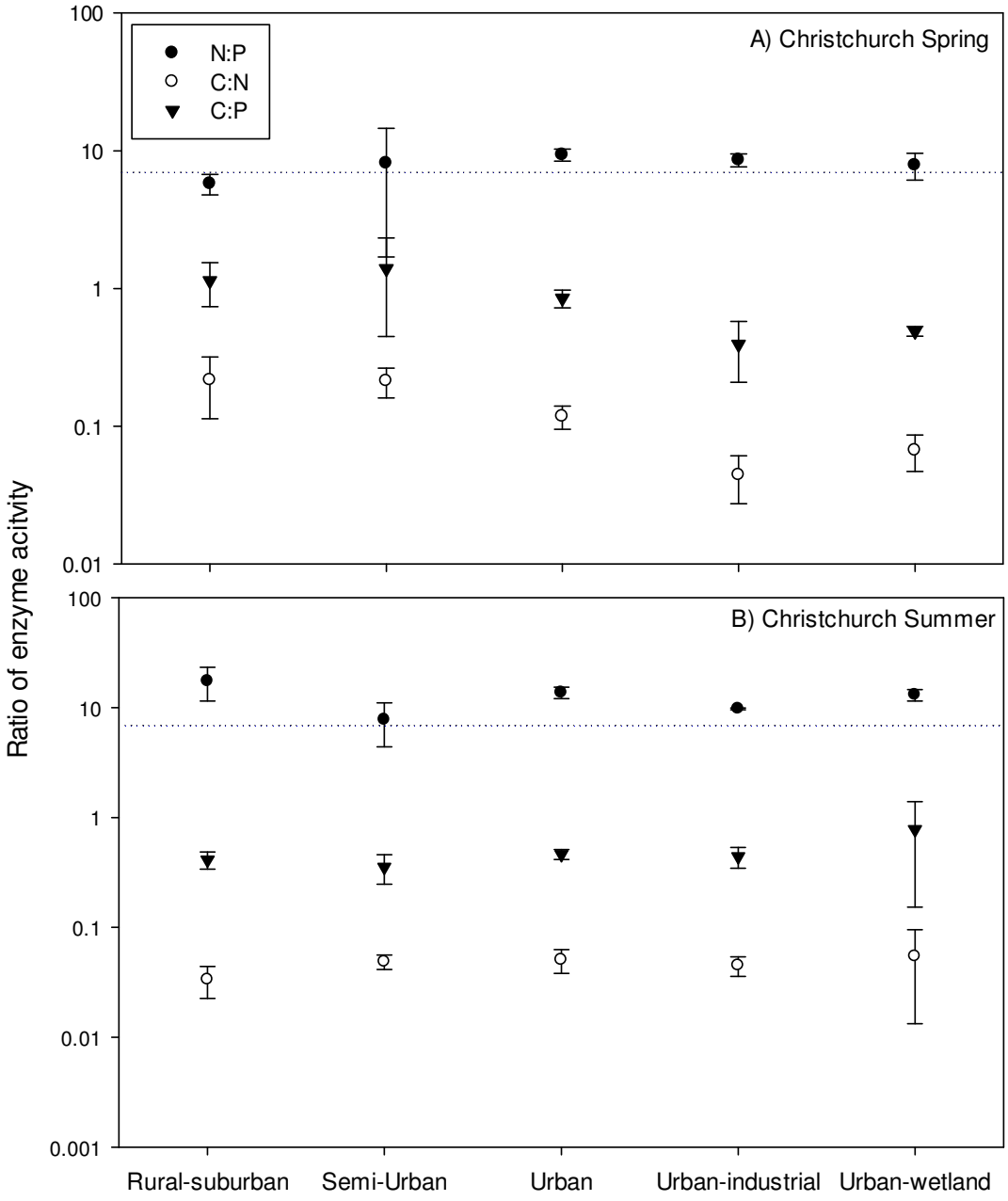


Figure 3. 11. Average (±SE) enzyme activity ratios in Christchurch A) Spring and B) Summer across land-use categories. Line at 7:1 indicated the N:P stoichiometric ratio.

Enzyme activity ratios were weakly linked to land-use percentage, with few relationships of significance in Auckland and Christchurch. Urban and pastoral land-uses in Auckland showed opposite trends in response to increasing C:P enzyme activity ratios (Figure 3.12A). Native land-use demonstrated no clear relationship with enzyme C:P ratios, the influence of native land-use is likely obscured by the opposite directionality of urban and pastoral land-uses; which sit at 0% native land-use (Figure 3.12A). C:P ratios suggest that sites which have increasing amounts of urban land-use were increasingly P limited and less C-limited (Figure 3.12B). Pastoral land-use shows the opposite trend with P-limitation decreasing as percentage pastoral land-use increases. Again, there is considerable variation in C:P ratios at the lower percentages of land-use,

especially for urban land-use. Urban land-use showed the same negative trend over Auckland Summer, this was not however significant (P = 0.393). Pastoral and native land-uses showed no significant relationships with land-use percentage in Auckland Summer.

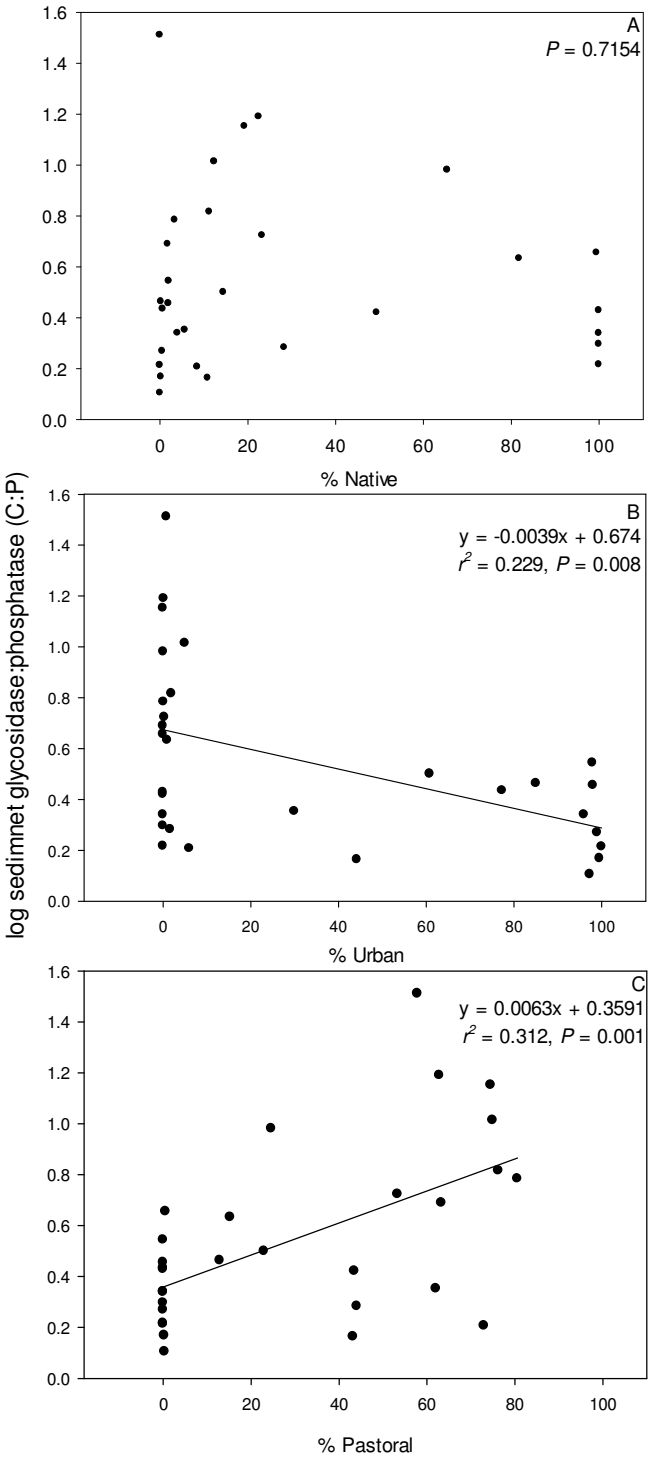


Figure 3. 12. Ratio of enzyme activity, C:P, across a land-use gradient Auckland in Spring against land-use with linear regression lines fitted for A) native B) urban and C) pastoral land-uses.

3.3.7. Extracellular enzyme activity stoichiometry and water chemistry

The plot of water column and enzyme N:P interactions shows that water chemistry and enzyme activity do not interact as predicted based on theoretical thresholds (Figure 3.13). According to thresholds, data points should fall into the grey shaded quadrants (upper left, and lower right). Thus, these theoretical thresholds show little agreement in nutrient limitation predictions. For example, water column ratios for most Christchurch sites are over the Redfield ratio (>1.2 on the x-axis) which suggests P limitation; and sediment enzyme ratios are over their ideal ratio (>0.84 on the y-axis) which suggests N limitation. There is a noticeable divergence between Auckland and Christchurch water column and enzyme ratios. Christchurch sites were generally P limited according to water chemistry and N limited according to enzyme activity. Auckland sites were more variable across categories, however most sites fell into categories which demonstrated opposite trends in limitation between water and enzymes (e.g. into the white quadrants).

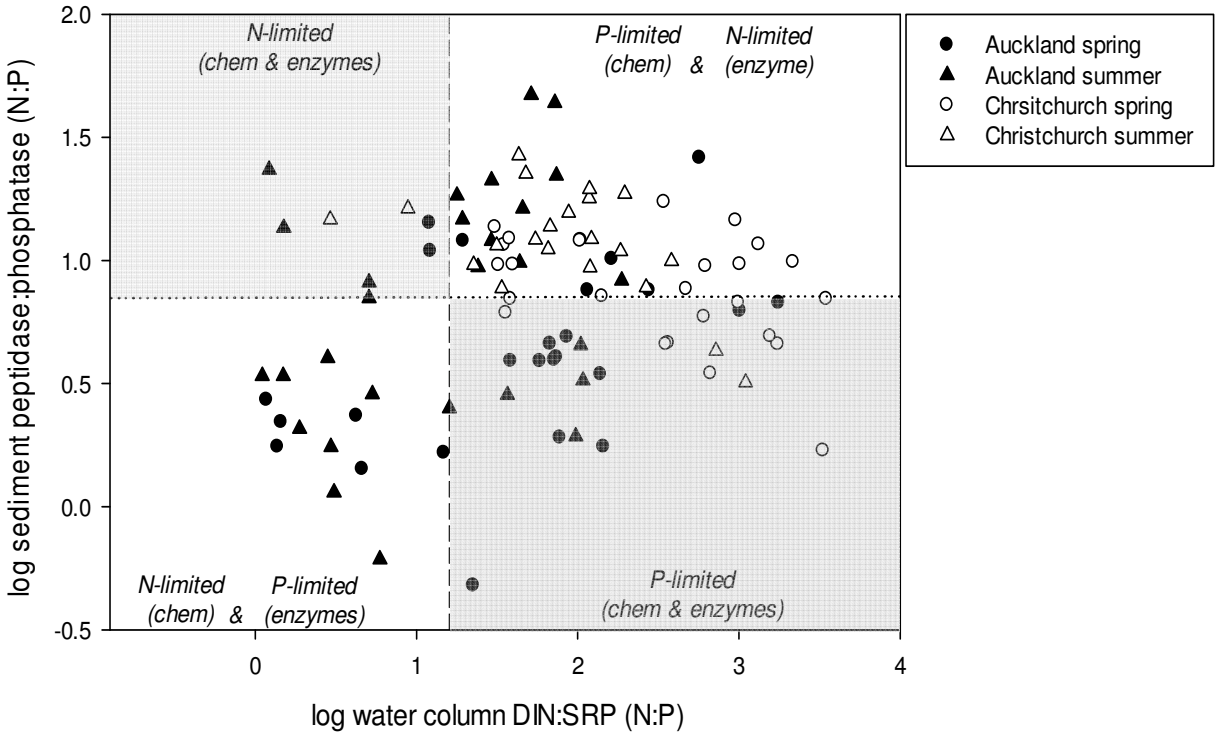


Figure 3. 13. Scatterplot showing the relationship between nitrogen: phosphorus ratios between enzymes and water chemistry on a log scale (linear regression: r^2 = 0.0358, P = 0.060), lines indicate the ideal nutrient ratios as the Redfield ratio (log[16:1]= 1.2:1) for water chemistry and a theorised enzyme production ratio of stream sediment (log[7:1]= 0.84:1). Grey shaded quadrants show where data should fall according to the theoretical thresholds.

Water and enzyme ratios in Auckland and Christchurch demonstrated opposite trends (Figure 3.14). Auckland sites showed a positive and counterintuitive relationship between the two variables; as water demonstrated stronger P limitation enzymes demonstrated stronger N limitation. Note that Auckland Summer regression did not meet significance (P = 0.09).

Auckland sites showed a mixture of N and P limitation according to water and enzyme ratios, these were however rarely in agreement for the same sites.

In Christchurch, the relationship between enzyme and water N:P ratios was negative, consistent with theoretical predictions. This relationship shows enzyme ratios decreasing as the ratio of water column nutrient increases (Figure 3.14). Over the course of this study there were only two sites in Christchurch that had DIN:SRP ratios suggestive of N limitation yet enzyme ratios at the majority of sites suggested N limitation. There is a large difference between inferences made from the two ratios with water chemistry showing P limitation and enzyme activity showing N limitation. Despite the inverse trend as theoretically predicted, limitations assumed by the two ratios in Christchurch do not show agreement with one another.

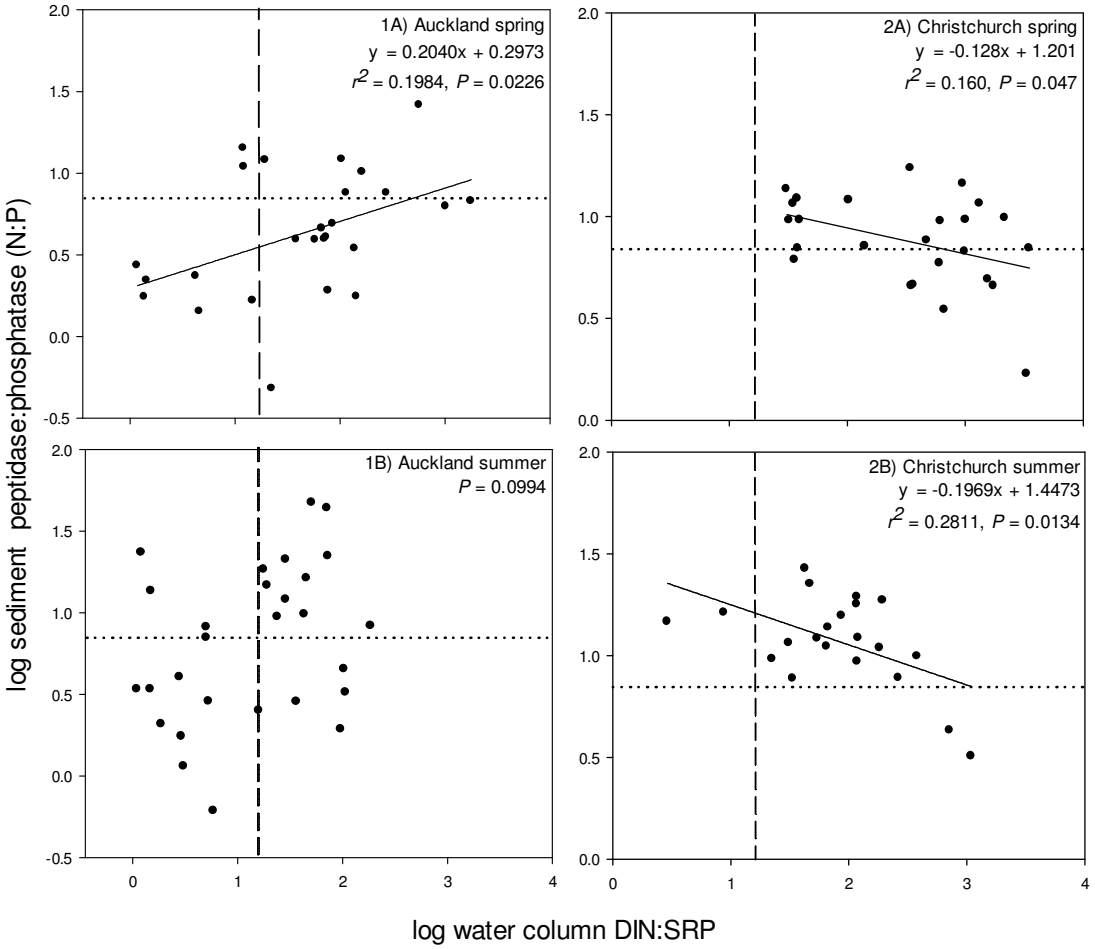


Figure 3. 14. Relationship between the stoichiometric ratios of water column DIN:SRP and sediment enzymes peptidase: phosphatase (N:P), data presented as log values. Note the difference in scale on the Y-axis between Auckland and Christchurch. Dashed vertical lines indicate the Redfield ratio (log[16:1] = 1.2:1).

Water column DIN was negatively related to enzyme C:N ratios in Auckland Spring, thus enzyme were demonstrated stronger N limitation when water DIN was elevated (Figure 3.15). All enzyme C:N ratios were negative, demonstrating weaker C and stronger N limitation. There was a weak relationship between the enzyme C:N ratio and the water N:P ratio (r^2 = 0.143, P =

0.05). Indicating that more N acquiring enzymes were produced when less N was available in the water column, consistent with expectations. These relationships did not exist in Auckland Summer. Along with this there were no other significant relationships between water column nutrient concentrations and enzyme ratios in Auckland.

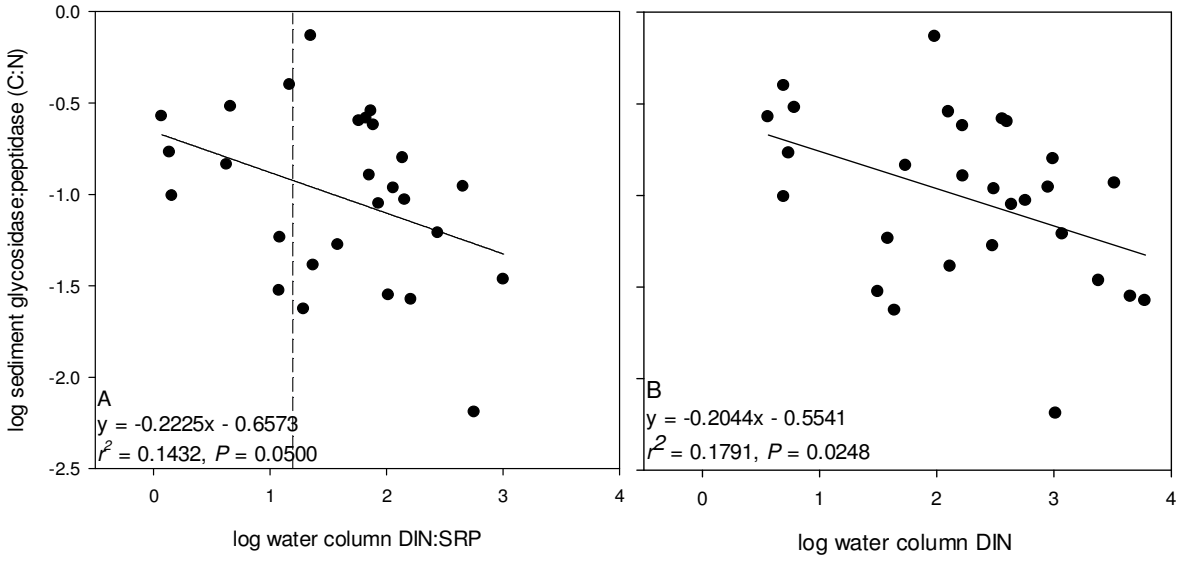


Figure 3. 15. Auckland Spring water column A) DIN:SRP and B) DIN (µgN/L) both against the ratio of glycosidase: peptidase (C:N). Data presented as log values. Note, there was also a relationship between NH_4^+ and enzyme C:N, r^2 = 0.3783, P = 0.0042. Dashed vertical lines indicate the Redfield ratio (log[16:1] = 1.2:1).

Like in Auckland, water column DIN in Christchurch Summer best predicted enzyme activity (Figures 3.16). As DIN increased C:N increased and N:P decreased, suggesting the relative investment in peptidase goes down as DIN goes up, which is consistent with expectations. The ratio of C:P enzymes also increased with increasing water NH_4^+, indicating increased production of enzymes for C acquisition when water NH_4^+ levels were higher (Figure 3.17).

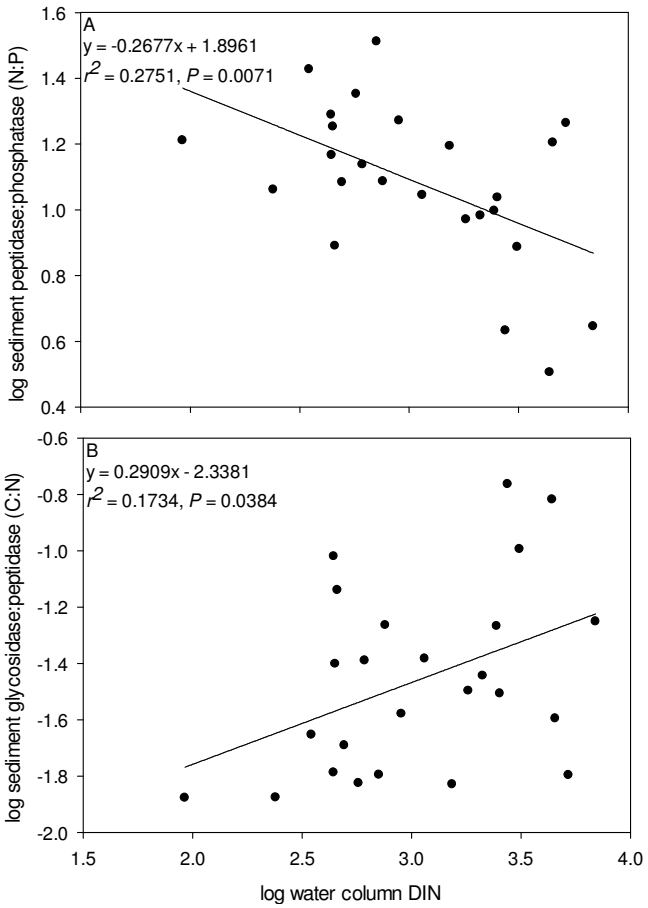


Figure 3. 16. Christchurch Summer log water column DIN (µgN/L) against log A) peptidase: phosphatase (N:P), and glycosidase: peptidase (C:N) activity.

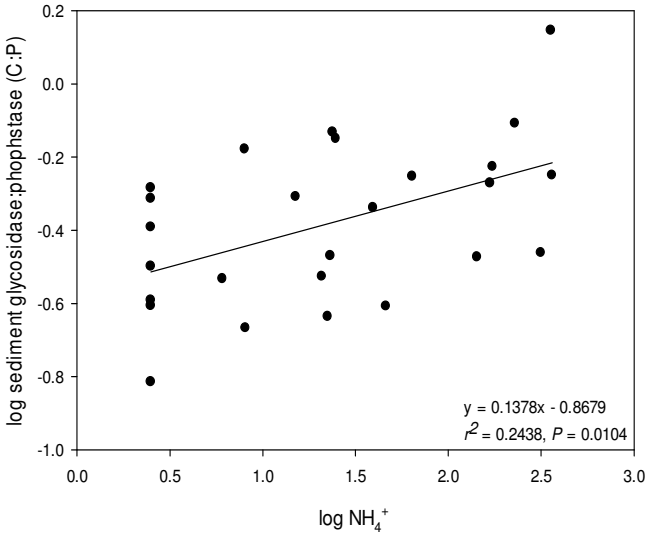


Figure 3. 17. Christchurch Summer log water column NH_4^+ (µgN/L) against log glycosidase: phosphatase (C:P) activity.

3.3.8. Nutrient limitation

The relative limitation of C, N, and P is shown through the distribution of enzyme N:P and C:N ratios relative to theoretical thresholds in C, N, and P limitation (Figure 3.18). There was a predominance of P limitation in Auckland sites and N limitation at Christchurch sites; with no obvious seasonal pattern in enzyme limitation. According to thresholds C was not limiting at any of the sites included in this study. In general, C:N ratios were lower in Christchurch than in Auckland.

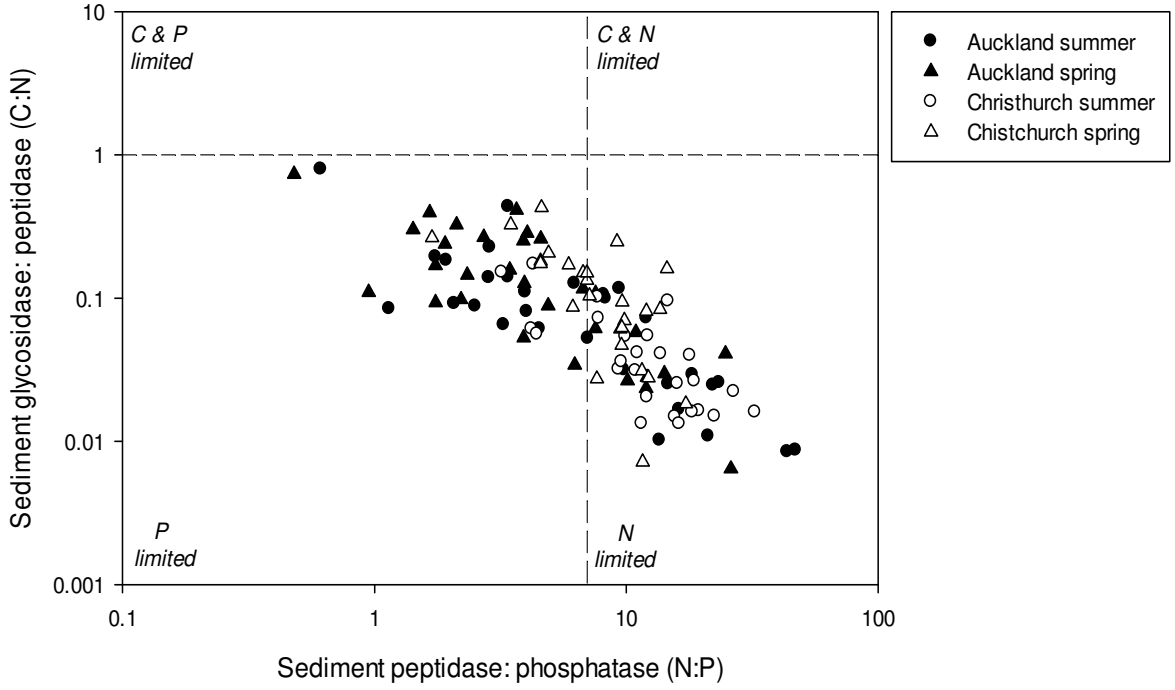


Figure 3. 18. Enzyme N:P:C stoichiometry for stream sediements over Spring and Summer in Auckland and Christchurch. Dashed lines are proposed limitation thresholds from Hill *et al* (2012).

Expectations of nutrient limitation based on water column N:P and enzyme N:P did not align (Figure 3.19). In Auckland Spring and Summer enzymes demonstrated N limitation across most sites, with the exception of native and suburban sites in Spring which were P limited. In contrast ratios of water DIN:SRP suggest microbes should be N limited at native sites in Spring and Summer with P limitation common urban and pastoral sites. Limitation by P was stronger according to water chemistry than enzyme stoichiometry. Christchurch similarly demonstrated a predominance of N limitation according to enzyme allocation and P limitation according to water DIN:SRP (Figure 3.20). Despite trends often being in opposition, water enzyme stoichiometry do vary accordingly with water DIN:SRP. N limitation became stronger in Summer in both Auckland and Christchurch according to both parameters, with water DIN:SRP decreasing and enzyme N:P increasing.

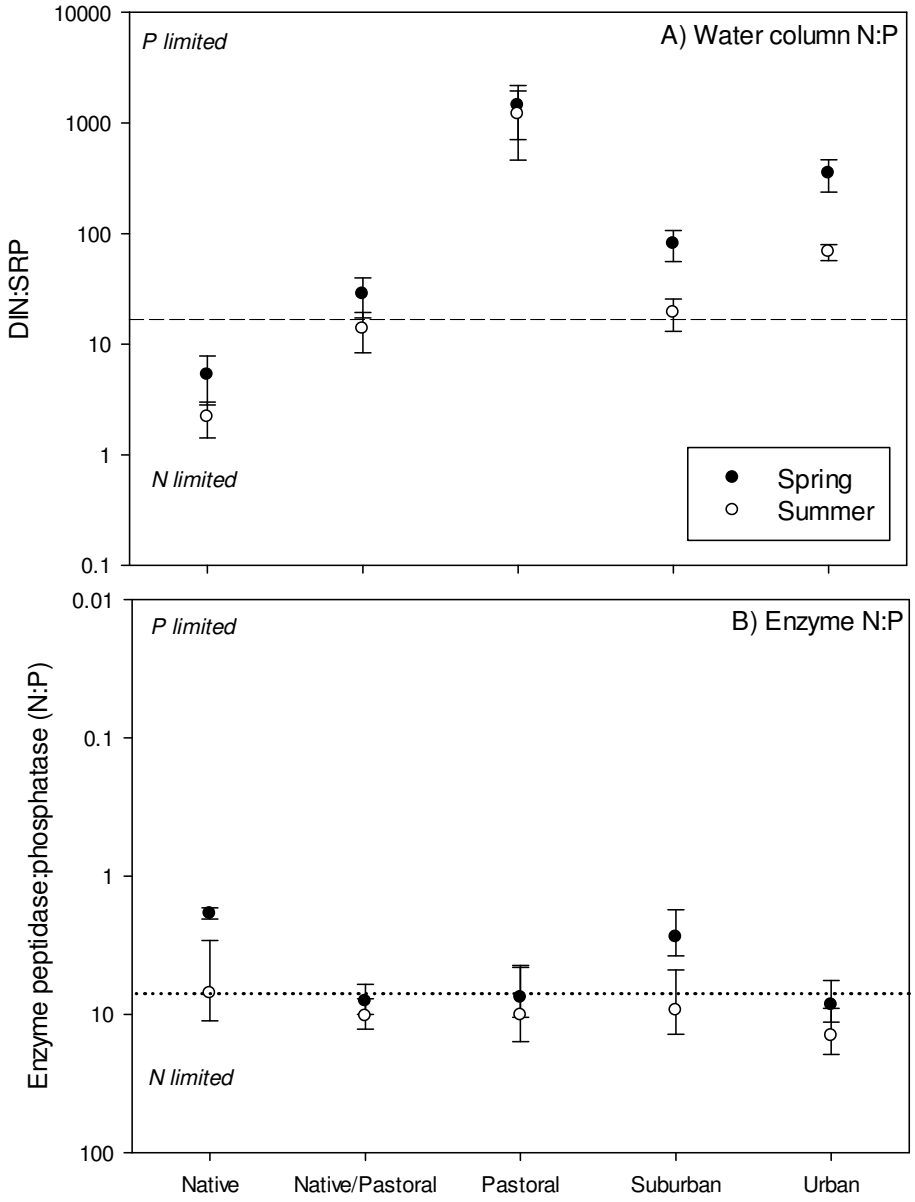


Figure 3. 19. Average (±SE) water column and enzyme N:P ratios in Auckland. Panel A indicates the stoichiometric ratio of inorganic water column nutrients and the proposed N and P limitation based on the Redfield Ratio (dashed line). Panel B shows the ratio of N and P acquiring enzymes and proposed N and P limitation based on theoretical thresholds in Hill *et al* (2012). Note the log scales on the y-axes, and the inverted scale on graph B for comparison on limitation trends between ratios.

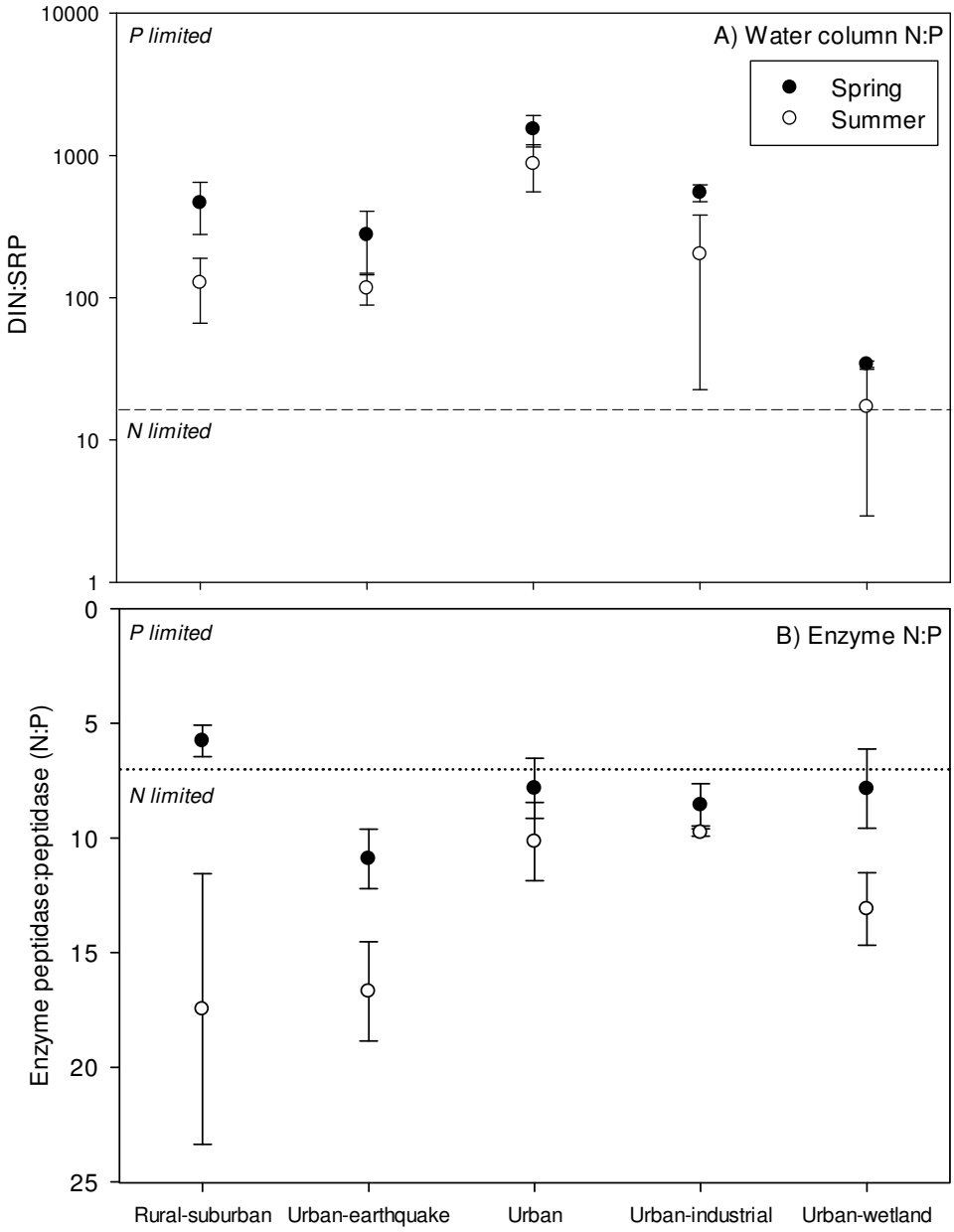


Figure 3. 20. Average (±SE) water column and enzyme N:P ratios in Christchurch. Panel A indicates the stoichiometric ratio of inorganic water column nutrients and the proposed N and P limitation based on the Redfield Ratio (dashed line). Panel B shows the ratio of N and P acquiring enzymes and proposed N and P limitation based on theoretical thresholds in Hill *et al* (2012). Note the log scales on the y-axes, and the inverted scale on graph B for comparison on limitation trends between ratios.

3.3.9. Coherence in enzyme response to urbanisation across cities and seasons

Enzyme activity was more consistent between urban sites in Summer than in Spring (Figure 3.21). In Spring peptidase and phosphatase activities showed similar trends in Auckland and Christchurch, with overlap in error bars between the regions. Glycosidase activity was lower in Auckland urban sites, although this difference was not significant. Phosphatase and glycosidase activity in Christchurch Summer was significantly higher than in Auckland Summer (*t*-test, $P = 0.028$; $P = 0.024$ respectively). Peptidase activity tripled in Summer in both Auckland and Christchurch, there was also consistent variation surrounding peptidase activity demonstrating that the activity of this enzyme is variable across urban sites.

Between seasons phosphatase activity was significantly higher in Summer than in Spring in both Auckland and Christchurch (*t*-test, $P = 0.005$; $P = 0.002$ respectively). Peptidase was also higher in Christchurch Summer than in Spring (*t*-test, $P = 0.003$); peptidase activity in Auckland showed no significant differences between seasons. Glycosidase activity was significantly higher in Auckland Summer than Auckland Spring (*t*-test, $P = 0.002$).

Enzyme ratios show very similar trends between regions and seasons, with data points often overlapping (Figure 3.22). Ratios of N:P were the highest followed by C:P and finally C:N. The difference of significance between regions was the higher ratio of C:P in Christchurch Spring compared to Auckland Spring (*t*-test, $P = 0.025$). C:P ratios were also higher in Christchurch Spring than in Christchurch Summer (*t*-test, $P = 0.04$). Overall, urban sites in Auckland and Christchurch showed similar trends in enzyme activity, which was consistent between seasons.

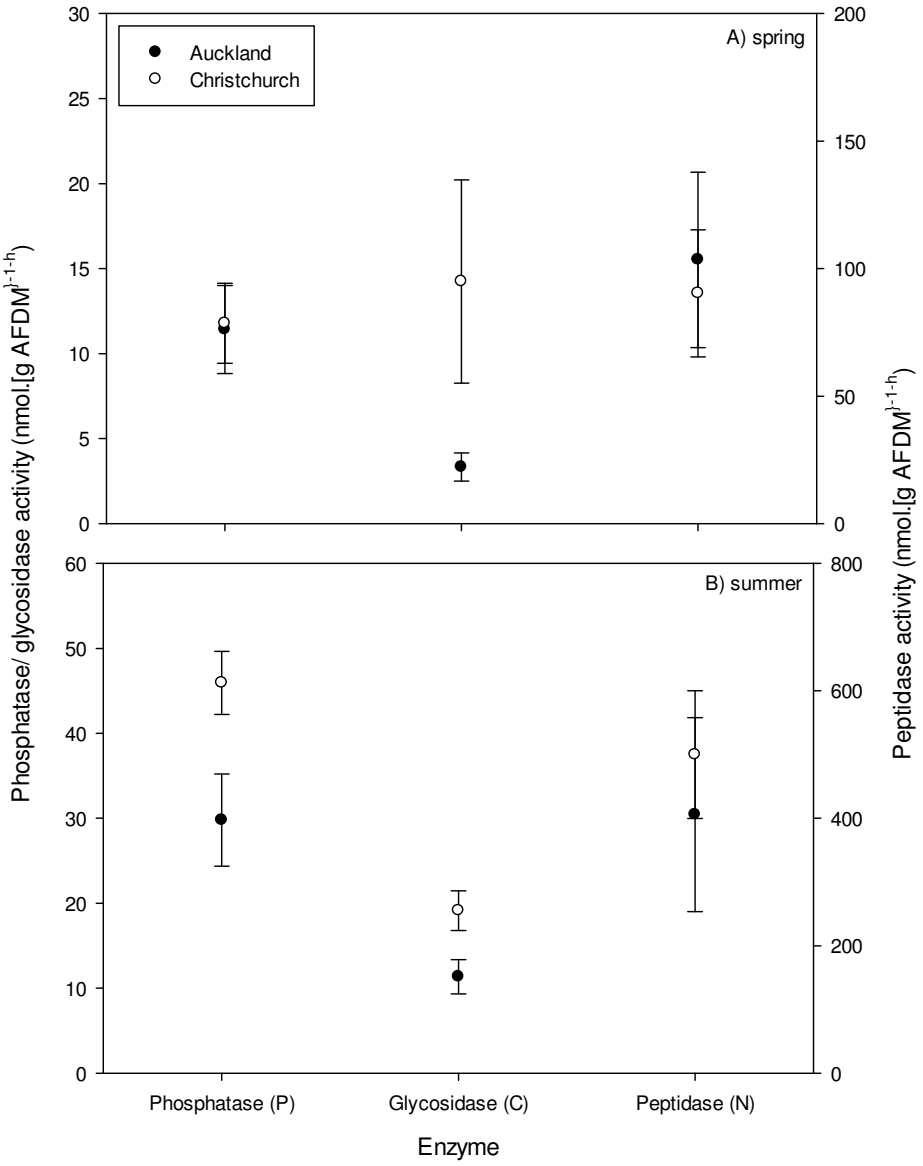


Figure 3. 21. Average (±SE) enzyme activity at urban sites in Auckland and Christchurch in A) Spring and B) Summer. Note peptidase is on the secondary y-axis due to the difference in scale between the activity of the three enzymes.

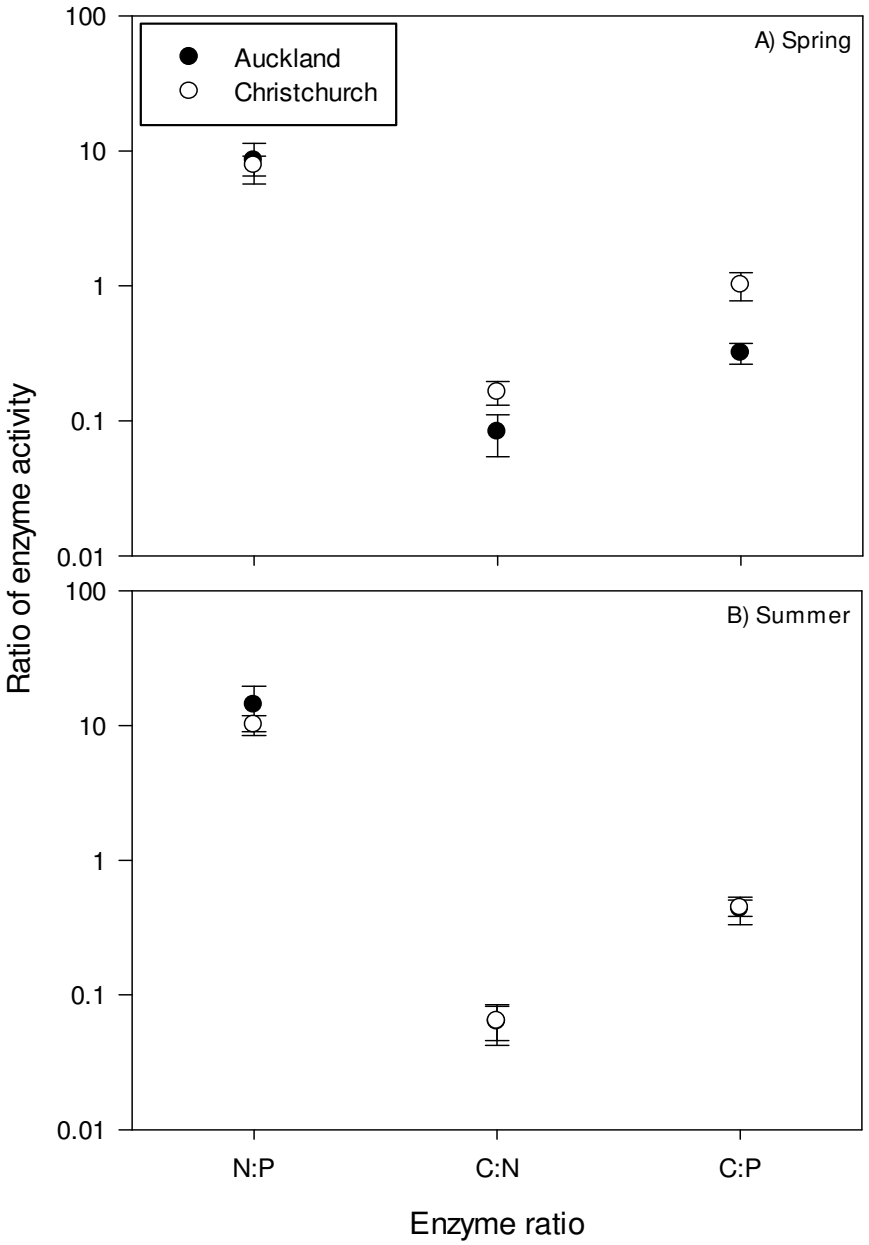


Figure 3. 22. Average (±SE) ratios of enzyme activity at urban sites in Auckland and Christchurch in A) Spring and B) Summer.Note the log scale on the y-axis.

3.3.10. Effects of earthquake damage

Liquefaction categories showed marked differences in water column nutrient concentrations (Figure 3.23). In Summer, urban sites with no liquefaction had the higher NO_X concentrations than reference (ANOVA, $P = 0.001$), heavy liquefaction (ANOVA, $P = 0.0002$), and light liquefaction sites (ANOVA, $P = 0.006$). In Spring a significant difference existed between no liquefaction and heavy liquefaction (ANOVA, $P = 0.008$). In both seasons there was a seven-fold difference NO_X between urban sites unaffected by liquefaction and heavy liquefaction sites. In Summer, for example, NO_X had an average concentration of 474(±62) µg/NL at heavy liquefaction sites and 3272(±565) µgN/L at urban sites with no earthquake impact. In both seasons NO_X was lower in heavy liquefaction sites than in references sites, this was especially noticeable in Summer where concentrations in reference sites (894 ± 305 µgN/L) were double that of heavy liquefaction sites (426 ± 79 µgN/L).

While NO_X levels were lower at heavy liquefaction sites, NH_4^+ concentrations were at least four-fold higher in heavy liquefaction sites than in any other category. In Spring, heavy liquefaction sites had significantly higher NH_4^+ concentrations than all other categories (ANOVA, $P > 0.05$), with an average concentration of 95(±26)µgN/L, compared to 4(±1) µgN/L in urban sites unaffected by liquefaction. There was more variation in the data in Summer, with no significant differences; despite this the data does show similar trends.

Another clear difference in the data is the elevated SRP concentrations at sites affected by heavy liquefaction in Spring (Figure 3.23). SRP was significantly higher in heavy liquefaction sites than in light liquefaction (ANOVA, $P = 0.001$), no liquefaction (ANOVA, $P = 0.0004$), or reference sites (ANOVA, $P = 0.0009$). In contrast SRP were similarly elevated across all sites in Summer.

Due to the low NO_X and higher SRP levels, heavy liquefaction sites had the lowest DIN:SRP ratios. In both seasons DIN:SRP ratios were highest in the category none, followed by light and finally references sites. The difference in ratios between heavy liquefaction and no liquefaction was significant in both Spring (ANOVA, $P = 0.003$) and Summer (ANOVA, $P = 0.029$). Ratios were always over the Redfield ratio of 16N:1P, with Spring heavy liquefaction sites having the lowest average ratio with a ratio of 46:1(±10).

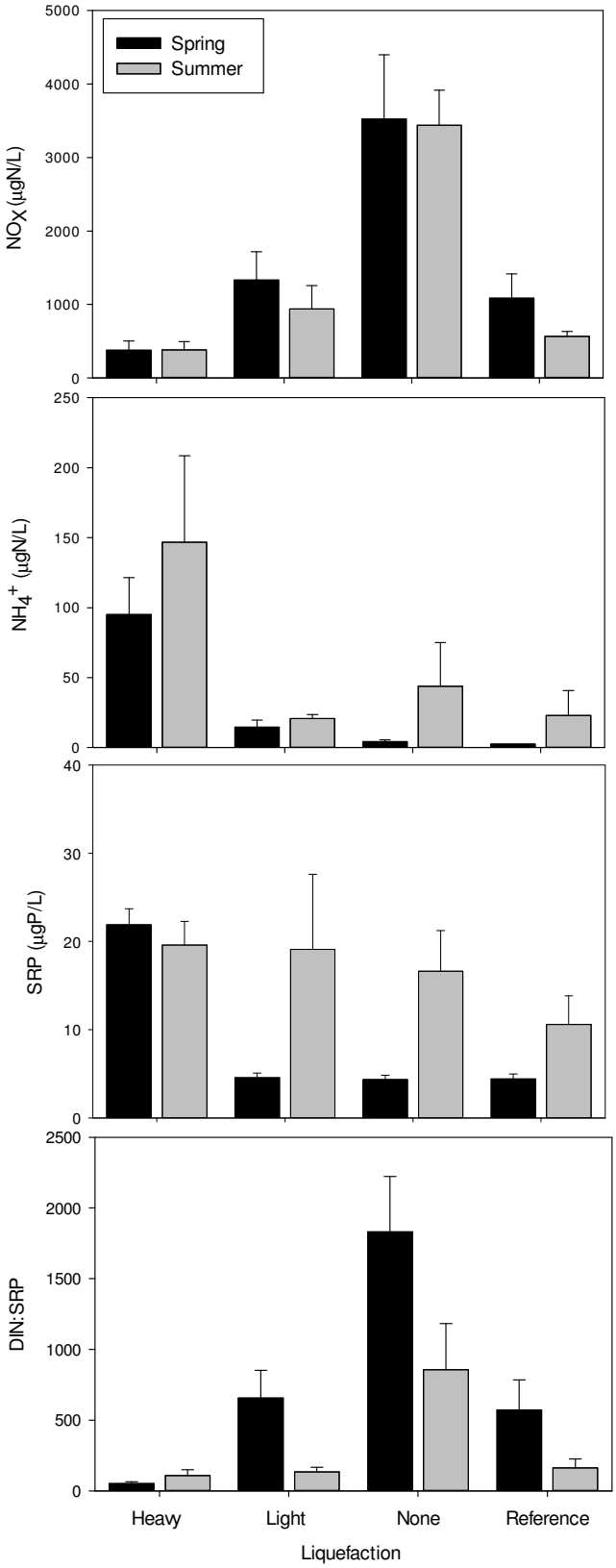


Figure 3. 23. Average (±SE) nutrient concentrations and molar ratios against liquefaction categories, all liquefaction categories are urban sites, reference sites are also included for comparison.

Enzyme activity in Spring was highest at urban sites with heavy liquefaction and lowest at urban sites unaffected by liquefaction (Figure 3.24). Peptidase activity was significantly higher in heavy liquefaction sites than in sites with no liquefaction (ANOVA, $P = 0.014$) and reference sites (ANOVA, $P = 0.028$) in Spring. Phosphatase and glycosidase showed the same trends at peptidase, there was however no significant difference between liquefaction categories. In Summer all urban sites, regardless of liquefaction status, showed similar trends in enzyme activity. Enzyme activity was lower in reference sites than any of the urban sites, with glycosidase activity significantly lower in reference sites compared to heavy liquefaction (ANOVA, $P = 0.036$) and no liquefaction (ANOVA, $P = 0.007$) categories.

The ratio of N:P enzyme activity was highest in sites affected by liquefaction in Spring, with these sites over the ideal ratio suggestive of N limitation (Figure 3.25). Heavy and light liquefaction had similar effects of enzyme stoichiometry between seasons, with no significant differences between these in Spring or Summer (ANOVA, $P > 0.05$). In Spring enzyme N:P ratios were highest in light 12:1 (±3) and heavy liquefaction sites 10:1 (±1), urban sites with no liquefaction had a ratio of 7:1 (±1). Sites with light liquefaction has significant higher enzyme N:P than unaffected urban (ANOVA, $P = 0.030$) and reference (ANOVA, $P = 0.016$) sites. Whilst heavy liquefaction sites had higher N:P ratios than reference sites only (ANOVA, $P = 0.050$). In Spring the ratio of C:N decreased with earthquake impact, with a significant difference between reference and heavy liquefaction sites (ANOVA, $P = 0.030$). C:P ratios were similar across all categories in Spring and Summer with no significant differences; in Summer there were also no significant differences between N:P and C:P ratios.

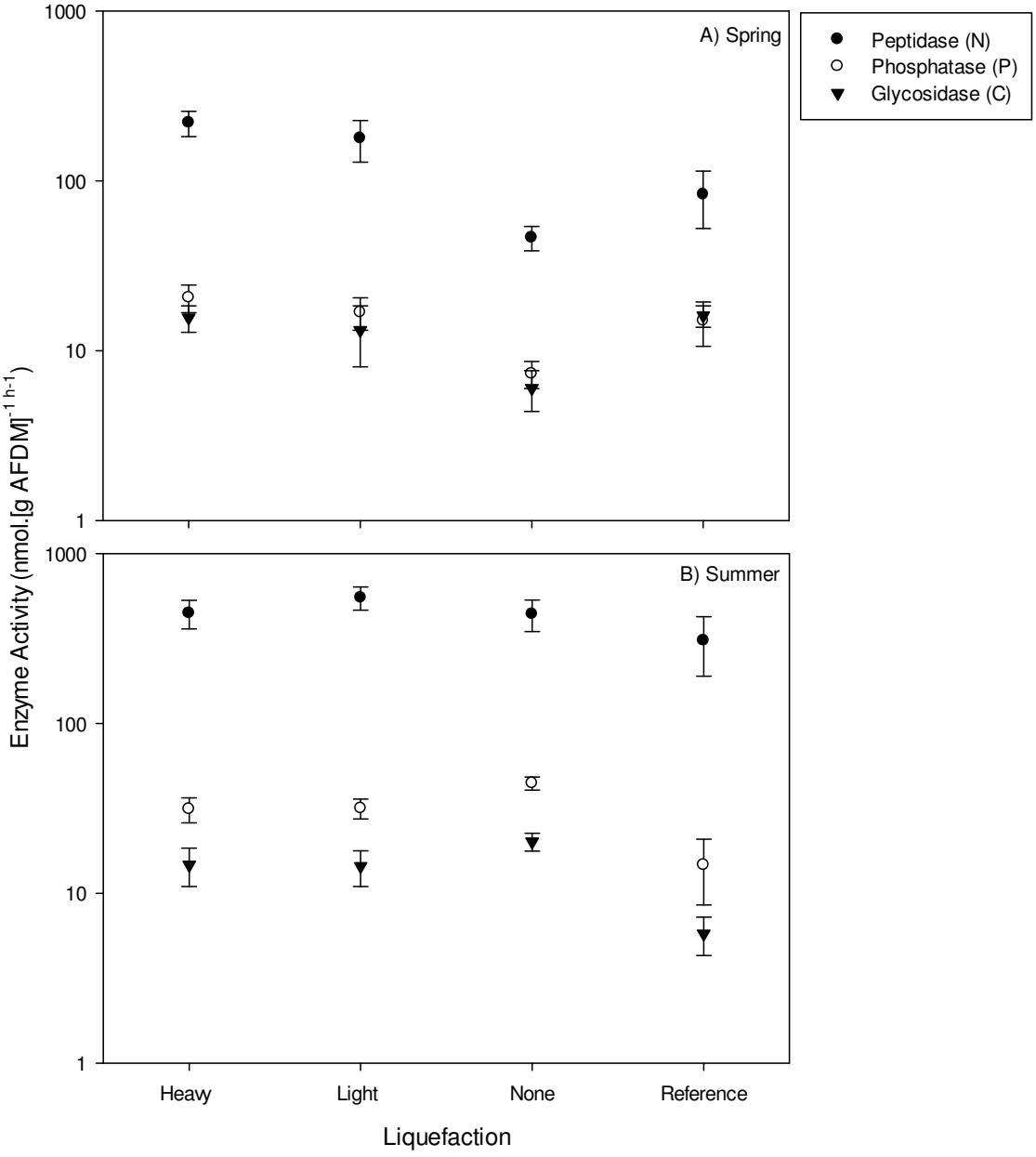


Figure 3. 24. Average (±SE) enzyme activity at urban sites affected by heavy, light, or no liquefaction and activity at reference sites (rural-suburban).

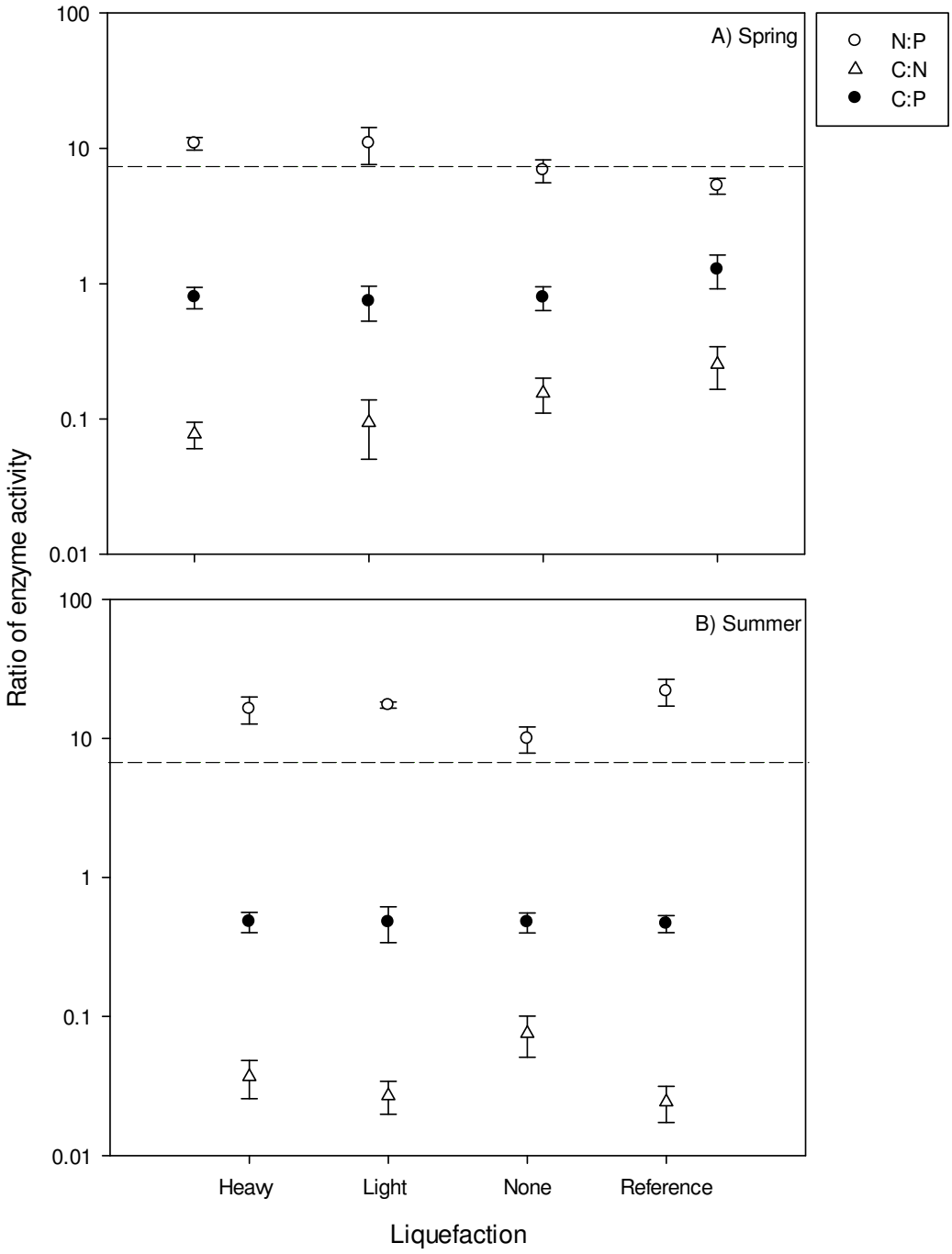


Figure 3. 25. Average (±SE) ratios of enzyme activity at urban sites affected by heavy, light, or no liquefaction and activity at reference sites (rural-suburban). Dashed line at 7:1 on the y-axis indicates the ideal ratio of enzyme activity for N:P.

3.4. Discussion

The ability to use sediment to predict nutrient limitation and inform us about in-stream nutrient cycling is a relatively new concept (Hill *et al.*, 2006; Hill *et al.*, 2010a; Hill *et al.*, 2010b). I found that patterns of enzyme activity on sediments did not neatly align with water column nutrients. Observed nutrient limitation patterns differed considerably based on water or sediment analysis suggesting that organisms living in these two environments differ in terms of their nutrient sources and thus nutrient requirements. Urbanisation demonstrated noticeable differences in enzyme stoichiometry and activity, particularly in Spring, with less enzymatic allocation towards carbon acquisition and increased overall enzyme activity, suggesting that EEA could be used to detect anthropogenic impacts. Earthquake damage also caused changes to EEA and water column nutrient concentrations associated with wastewater inputs.

3.4.1. Land-use and extracellular enzyme activity

Enzyme activity was elevated in urban and lower in native and pastoral catchments across Auckland and Christchurch (Figures 3.6 & 3.7). This may be due to higher microbial biomass in human-influenced streams. Increased carbon (both autochthonous and allochthonous) and nutrient inputs associated with urbanisation stimulates bacterial production increasing microbial biomass (Williams *et al.*, 2010). Auckland and Christchurch urban and agricultural sites had elevated DIN concentrations compared to reference sites which surpassed guideline values and urban sites had much higher levels of SRP and NH_4^+ than the other land-uses. Elevated nutrient levels at anthropogenically impacted sites stimulates microbial biomass which in turn increases enzymes associated with N, P, and C acquisition (Rier *et al.*, 2011). For example, increasing nutrient concentrations by 15-20% can cause a 200 fold increase in biomass production (Van Horn *et al.*, 2011). Raised enzyme activity at urban sites has been observed in many other studies and is generally associated with this biomass response (Harbott and Grace, 2005; Williams *et al.*, 2010; Tiquia, 2011; Williams *et al.*, 2012). When microbial biomass is taken into consideration; urban sites generally have lower extracellular enzyme activities due to nutrient rich run-off (Hill *et al.*, 2012; Lehto and Hill, 2013). For this reason the stoichiometry of enzyme allocation may be more useful when looking at the ability of microbial organisms to respond to urbanisation impacts because is permits detection of shifts in relative allocation toward C, N and P acquisition (Hill *et al.*, 2010a). Ecological stoichiometry emphasises the ability of an organism to regulate its uptake of nutrients in response to environmental changes, and as such should be represented by changes in stoichiometric ratios.

Results from enzyme stoichiometry reveal a decrease in allocation in enzymes for the uptake of C, and increase in allocation towards N at urban sites (Figures 3.10, 3.11 & 3.12). Decreases in microbial allocation to C uptake may be related to influxes of labile carbon in urban areas. Urban runoff carries a range of carbon compounds which may increase dissolved organic carbon (DOC) concentrations and may also alter enzyme activity related to changes in carbon source (decrease in labile cellulose sources) (Harbott and Grace, 2005). DOC sources in urban streams are also susceptible to degradation by peptidases (non-specific enzymes), thus peptidases may provide important C sources for microbes in urban streams and may explain why peptidase activity was high in urban sites (Harbott and Grace, 2005; Tiquia, 2011). Alternatively, increased carbon inputs at urban sites may have caused activity of carbon acquiring enzymes to decrease; as DOC is often abundant in stream sediment interstices (Wilczek *et al.,* 2005). Enzymes for C and N acquisition are tightly coupled; with increases in DOC stimulating N uptake by microbial biofilms (Bernhardt and Likens, 2004; Mineau *et al.*, 2013a). Elevated carbon concentrations in urban streams may be driving the production of N acquiring enzymes, this stoichiometric imbalance in available C:N may therefore by limiting microbial growth. Although carbon concentrations were not measured in this study; there are several lines of evidence from other studies which support this conclusion (Romani *et al.*, 2004; Williams *et al.*, 2012; Mineau *et al.*, 2013a). In contrast to urban sites, relative activity of carbon acquiring enzymes was higher in native and agricultural sites suggesting that carbon from hemi-cellulose degradation and detritus is more important in these sites (Harbott and Grace, 2005). Extracellular enzyme activity does therefore respond to changes in urbanisation; indicating that microbial biofilm taxa are sensitive to catchment scale processes and may be useful for the assessment of anthropogenic impacts.

Both eco-regions demonstrated similar enzyme activity patterns, indicating that urbanisation produces a characteristics response in enzyme production in sediments (stronger N, weaker C limitation) (Figure 3.24). The only difference in enzyme activity allocation was an increase in carbon acquiring enzymes in Christchurch during Spring compared to Auckland; this difference was however not apparent in Summer suggesting seasonal controls on enzyme activity. Trends in enzyme allocation in both Auckland and Christchurch were more pronounced in Spring, possibly related to shifts in nutrient ratios (Figure 3.3). Although trends in nutrient concentrations were not complementary between regions, peaks in DIN and SRP in Auckland in Spring and Christchurch in Summer, which suggests that carbon or other environmental factors (e.g. discharge/temperature) may also be important. In other studies where enzymes have been measured across seasons results have been variable, with seasonal differences generally associated with stored of organic matter in the sediment (Romani *et al.*, 1998; Wilczek *et al.*,

2005). Temperature and discharge can also affect microbial population sizes, and rates of metabolism which include enzyme production rate (Clinton *et al.*, 2010). Discharge varies across a vertical gradient within streams, with max rates 40% from the benthos. On the streambed biofilms are exposed to lower shear forces as they are in a boundary layer meaning that are not at as susceptible to forces which may cause biofilm detachment or prevent organic matter build-up (Mulholland, 1996). Absolute increases in enzyme activity in Summer have also been associated with increases to temperature and DOC which stimulates enzyme activity (Figure 3.23) (Romani *et al.*, 1998; Wilczek *et al.*, 2005). More specifically, Wilczek *et al* (2005) observed an increase in sediment DOC over Summer; leading to changes in sediment enzyme stoichiometry. This build-up of DOC can have lasting effects on enzyme activity for up to 3 months by decreasing microbial reliance on the above water column for carbon sources (Wilczek *et al.*, 2005). This emphasises the variability between water column and sediment stream compartments and their resources which are available for microbial organisms.

3.4.2. Water chemistry and extracellular enzyme activity

Whilst water and sediment enzyme stoichiometry are conceptually coupled, results suggest that other factors are import in regulating nutrient demand in sediments. Water chemistry trends suggest that sites were dominantly P limited; whereas enzyme activity suggests N limitation was more common (particularly in Christchurch) (Figure 3.13). Relationships between water and sediment enzyme stoichiometry were not consistent and sometimes contradictory within or across regions; making relationships difficult to predict (Tables 3.5 & 3.6). Enzyme activity was tightly coupled with water chemistry in Christchurch Spring but no relationship existed in Summer; suggesting different environmental controls on enzyme activity (Figure 3.9). Many studies have found an inverse relationship between phosphatase and water column P, as theoretically predicted (Sinsabaugh and Moorhead, 1994; Sinsabaugh *et al.*, 1997; Wright and Reddy, 2001; Harbott and Grace, 2005; Hill *et al.*, 2012; Sinsabaugh and Shah, 2012; Mineau *et al.*, 2013b). Relationships between water inorganic N and enzyme N production are less common. N exists in many different forms in streams and therefore microbial organisms have different mechanisms for regulating their internal N concentrations which can be difficult to detect in enzymatic assays (e.g. utilisation of amino acids for C and N) (Sinsabaugh and Moorhead, 1994; Sinsabaugh *et al.*, 2008). Similarly, enzymes for C acquisition and DOC concentrations are often not predictable due to the diverse range of forms of which carbon is present in the environment (e.g. proteins, humic substances, nucleic acids, ect) (Sinsabaugh and Moorhead, 1994; Harbott and Grace, 2005). Despite this studies have found predictable relationship between microbial enzyme C and N enzyme allocation and water column N and

DOC concentrations (Ainsworth and Goulder, 2000; Williams *et al.*, 2012; Lehto and Hill, 2013). These studies however commonly use water enzyme activity rather than streambed sediment activity. It is therefore unsurprising that the results of these studies are not in-line with results presented here as these compartments are thought to differ considerably in terms of their nutrient requirements (Hill *et al.,* 2010b).

Microbial organisms on stream sediments differ in terms of their nutrient requirements when compared to water column organisms due to differences in nutrient and carbon resources (Mulholland, 1996) (Figure 3.26). Microbial activity in soils and stream sediments is often decoupled from ambient nutrients due to exogenous inputs and internal cycling of nutrients (Sinsabaugh and Moorhead, 1994; Wright and Reddy, 2001; Fischer *et al.*, 2002a; Romani *et al.*, 2004; Hill *et al.*, 2006). Klotz (1985) noted that P adsorbed onto stream sediments may be an important source of P for benthic algae; early lake experiments demonstrated that benthic microbes can 'actively mine' the substrate for nutrients in addition to receiving nutrients through enzymatic processes (Hansson, 1989; Mulholland, 1996). Hill *et al* (2012) also found difference in streambed and epilithic nutrient limitation trends thought to be due to P adsorption on sediments. This storage of organic matter on stream beds and adsorption of P is likely driving the observed variations between nutrient requirements according to water column nutrient concentrations and microbial EEA (Fischer *et al.*, 2002b; Romani *et al.*, 2004). By decreasing the reliance solely on the water column for energy and nutrient sources; suggesting the two compartments are not as tightly coupled as originally hypothesised. This may also explain why enzyme N production was elevated in stream sediments when DIN was abundant in the water column at most sites.

Despite limited relationships between water chemistry and enzyme activity, there were instances where enzyme activity or ratios demonstrated agreement (Figures 3.14, 3.15, 3.16, & 3.17). In general, patterns were more complementary in Christchurch than in Auckland, possibly relating to difference in organic matter deposition or nutrient sorption to sediments as described above. Water chemistry ratios demonstrated an inverse relationship with enzyme activity ratios in Christchurch as theoretically predicted (Figure 3.1). This same relationship was positive in Auckland, thus as water chemistry suggested stronger P-limitation sediment enzymes suggested demonstrated stronger N limitation. This counterintuitive response has been previously demonstrated for absolute enzyme activity, which was also observed in this study (Figure 3.9) (Rier *et al.*, 2011). And can be attributed to an overall increase in microbial metabolism and biomass at sites with elevated P concentrations (biomass response). However, significant but counterintuitive trends in stoichiometric ratios are either infrequently observed or unreported in

the literature; as there are no reports of this response that I am aware of. An inverse relationship between water and enzyme N:P, as in Christchurch, is more frequently reported (Romani *et al.*, 1998; Rier *et al.*, 2011). This was also accompanied by an inverse relationship between enzyme C:N activity and water DIN in Christchurch Summer and water DIN/SRP in Auckland Spring. These relationships suggest that microbial organisms expend less energy of nutrient acquisition when nutrients are available in the water column; and instead allocate more energy into the acquisition of carbon as predicted by the resource allocation theory (Mineau *et al.*, 2013b). This predicts a trade off in allocation of enzymes towards carbon or nutrient acquisition dependant on environmental resources and substrate supply (Sinsabaugh and Moorhead, 1994). Microbial communities living on stream sediments are therefore in the unique position of being able to utilise resources from the substrate or the water column to regulate their metabolic demands.

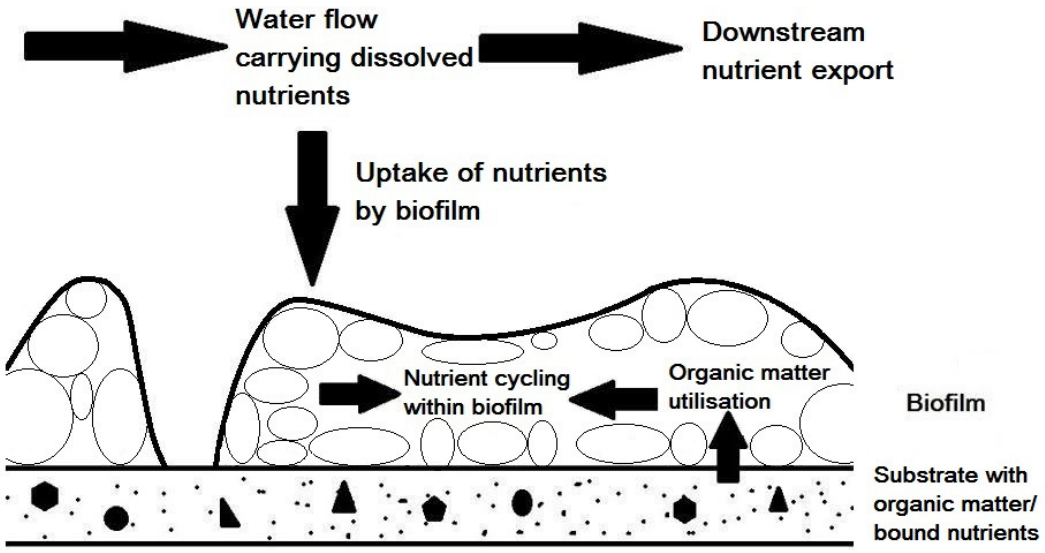


Figure 3. 26. Conceptual diagram of a stream biofilm attached to the benthos (e.g. sediment). Microbial organisms within the biofilm are able to obtain nutrients from the overlying water column in addition to utilising organic matter deposited within the interstices of the stream sediments and nutrients bound onto sediment. Within the biofilm heterotrophic organisms can utilise organic matter and nutrients from algal exudates. Arrows indicate fluxes of C, N, and P.

In addition to differences between water column and sediment environments other factors may also influence enzyme production and allocation. Water chemistry only provides information on nutrient conditions at a single point in time whereas microbes are integrative of nutrient conditions over 2-4 weeks (Hill *et al.,* 2012). Microbial communities are not sensitive to short-term perturbations in nutrient concentrations; taking up to a month of nutrient enrichment to alter enzyme allocation in one study (Olander and Vituosek, 2000; Bowen *et al.*, 2011). Thus, biotic communities are more reflective of in-stream nutrient histories than water samples, leading to discrepancies between the two measurements. Additionally, enzyme assays do not account for intra- and extra-cellular storage products which can influence microbial nutrient demands, luxury consumption of P may lower enzyme P production (Sinsabaugh *et al.*, 2009; Rier *et al.*, 2011;

Hill *et al.*, 2012). A surplus of nutrients can also result for low enzymatic turnover, once enzymes are secreted by microbes or enter the environment through cell lysis these remain attached to the biofilm until their demand is met, even if the cell no longer requires the nutrient (Sinsabaugh *et al.*, 2010). Additionally, physiochemical differences in temperatures and pH also affect enzyme activity (Wilczek *et al.*, 2005; Simon *et al.*, 2009). Enzyme stoichiometry stayed consistent between seasons despite an increase in temperature over Summer and pH was circumneutral across all sites; unlikely to affect microbial assemblages.

3.4.3. Can enzyme stoichiometry be used to predict nutrient limitation?

Microbial enzyme stoichiometry should not be independently used to predict in-stream nutrient limitation due to the distinctly different limitation patterns between the streambed and water column. Rather, nutrient limitation assessments should take into account that there is no single nutrient limitation for streams; different compartments within the stream may be differentially nutrient limited because they depend on water column N and P to different degrees. The theoretical model predicted an inverse relationship between water column nutrients and microbial enzyme allocation, this did not hold true in all instances. Furthermore, comparisons of microbial and water N:P nutrient limitation using the Redfield ratio (16N:1P) and the microbial equivalent (7N:1P), reveal that limitation trends are most frequently in opposition (Figure 3.13) (Redfield, 1958; Cleveland and Liptizin, 2007). However, this study did find evidence that water column nutrient concentrations do affect enzyme allocation by sediment microbial organisms. For example, in Auckland and Christchurch during Spring water chemistry demonstrated stronger P limitation, owing to an increase in water column DIN relative to SRP, which is reflected in enzyme activity through a decrease in N:P activity when compared to Summer trends (Figures 3.19 & 3.20). This suggests that microbial organisms are still reliant on the overlying water column for nutrients and change their allocation of enzyme correspondingly; but this does not lead to a coordinated switch in limitation.

This lack of agreement in limitation status but changes to enzyme allocation owing to anthropogenic impacts has been noted in other studies (Hill *et al.*, 2006; Hill *et al.*, 2010a; Hill *et al.*, 2012). These studies, all based in the U.S., described water chemistry and epilithic biofilms as P limited, but benthic sediments as N limited (Hill *et al.,* 2012; Hill *et al.,* 2010a; Romani *et al.,* 2004). This same trend was found in my study suggesting a divergence of limitation patterns between the water column and streambed compartments which holds true across eco-regions and countries where streams have been impacted by anthropogenic land-use. Differences may be simply be explained by an increase in N concentrations associated with human land-use causing P-limitation in the water column, and adsorption of P and deposition of organic matter (C) in the

streambed; leading to N limitation (McDowell *et al.*, 2009; Hill *et al.*, 2012; Mineau *et al.*, 2013b). Microbes have high N requirements (7N:1P); it follows therefore that when provided with sufficient C and P, N limitation should result (Allison, 2005). Native sites showed the opposite pattern, N limited based on water chemistry and P limited based on sediment enzymes, likely related to a lack of nutrient rich run off into these streams and less P adsorption to sediment. No sites demonstrated C limitation based on sediment enzyme allocation; suggesting that carbon sources are abundant across land-uses; or carbon is utilised from different sources (i.e. not cellulose degradation) (Figure 3.18). This could possibly be explored in further studies through an assessment of carbon content in sediment. Variations in EEA stoichiometry with changes in nutrient concentrations and land-use suggest that EEA is sensitive to anthropogenic impacts. Furthermore, it provides a biotic perspective on nutrient limitation which may be a useful tool in the assessment of land-use impacts on in-stream communities (Hill *et al.,* 2012).

3.4.4. Effects of earthquake damage on extracellular enzyme activity

Christchurch streams affected by earthquake damage experienced significant decreases in NO_X and increases in SRP and NH_4^+ in addition to receiving tonnes of silt/sand (Figures 3.9 & 3.23) (ESR, 2012a). As previously discussed wastewater inputs have continued into 2013, as infrastructure is still being repaired, causing these shifts in nutrient concentrations (chapter 2) (Environment Canterbury, 2013). The main effect of earthquake damage on sediment microbial assemblages for stronger N limitation; with absolute peptidase activity increasing along with an increase in the stoichiometric ratio of N:P acquiring enzymes (Figures 3.24 & 3.25). This trend was only observed in Spring, likely due to an increase in SRP across all sites in Summer causing an overall increase in peptidase activity. Influxes of organic matter and sewage have been noted to increase glycosidase and peptidase activities in sediments due to increased enzymatic hydrolysis of polymeric compounds, thus increasing the supply of low molecular weight compounds for microbial use (Montuelle and Volat, 1998; Hill *et al.*, 2006; Tiquia, 2011). From this it can be determined that inputs of raw sewage increased bioavailable SRP concentrations leading to an increase in sediment peptidase activity, and a slight but non-significant increase in glycosidase activity. This demonstrates that sites affected by the earthquakes changed their enzyme stoichiometry relative to influxes on nutrients. Moreover, this is another indicator that benthic microbial assemblages are sensitive to changes in environmental conditions.

Influxes of organic matter from wastewater may have affected enzyme production for carbon acquisition following the earthquakes (Montuelle and Volat, 1998). Sediments, particularly sand, are an important place for the storage and cycling of organic matter and can

efficiently process cellulose (Fischer *et al.*, 2002b; Romani *et al.*, 2004). Microbial populations can become adapted to living in these high C:N ratio habitats, such that increases in inputs of wastewater may have little effect (Montuelle and Volat, 1998). However in substrates where organic matter retention is not as high (larger substrates) microbial enzyme production has been consistently observed to increase following wastewater inputs (Montuelle and Volat, 1998; Harbott and Grace, 2005; O'Brien and Wehr, 2010; Tiquia, 2011). Christchurch sites did not have sand/silt substrates such as they have now until the earthquake caused an influx of liquefaction. An increase in enzyme production, as was the case for peptidase, is therefore expected. However, increases in fine sand/silt which contain higher amounts of organic matter following the earthquake may explain why activities of carbon acquiring enzymes did not increase as has been observed in other studies following sewage inputs (Chappell and Goulder, 1994; Montuelle and Volat, 1998; Romani *et al.*, 2004).

3.5. Conclusion

The relationship between enzyme N, P, and C stoichiometry yields insight into nutrient limitation patterns of microbial organisms, and demonstrates the complexity of limitation patterns within streams. Nutrient limitation patterns vary between water column and streambed habitats owing variation in nutrient and organic matter supply; leading to complex patterns of nutrient limitation within streams. EEA stoichiometry is of value as it allows biotic nutrient limitation patterns to be detected, and may help improve our understanding of in-stream nutrient processing by microbial communities. EEA stoichiometry was affected by nutrient concentrations and land-use impacts with anthropogenically impacted sites experiencing stronger N limitation and weaker C limitation. Earthquake impacts further demonstrated that benthic microbial assemblages are sensitive to water column nutrient inputs. Therefore, there is potential in using EEA for the assessment of in-stream nutrient processing and land-use impacts. However, validation from future studies is needed to better understand the inconsistencies and causal mechanisms behind some of the trends described here. Overall, results presented here suggest that the allocation of extracellular enzymes by microbial assemblages is a promising tool for the bio-assessment of streams but may be of limited use for assessing water column nutrient limitation. My research has demonstrated that limitation between the two environments is not as tightly coupled as hypothesised. Future assessments of nutrient limitation should therefore take into account that biological limitation trends are not exclusively related to water chemistry and that other factors influence in-stream nutrient processing by microbial communities.

Chapter 4

Coherence in nutrient limitation trends between assessment methods

4.1. Introduction

Microbial organisms produce enzymes to hydrolyse high-molecular weight organic matter in response to shortages in ambient nutrient conditions (Chrost, 1991). Enzymes are energetically costly to produce, with enzyme synthesis only occurring in response to limitation (Lehto and Hill, 2013). Consequently, microbial enzyme activity should better reflect biological nutrient limitation than other metrics (e.g. water chemistry) (Hill *et al.,* 2010a). Therefore, various measurements of microbial metabolism are expected to be coherent with one another; as these should theoretically be responding to the same environmental shortages in nutrient supply. Measurements of EEA represent a promising tool for the assessment of stream nutrient limitation, as collection of samples and analysis is relatively inexpensive and quick when compared to other biological metrics (Hill *et al.,* 2006; Hill *et al.,* 2012). However, little is known about the differences, if any, between nutrient limitation assessments conducted with different methodologies, or between assessments of EEA on different substrates. This information may enhance our understanding of nutrient limitation within streams. Differences in nutrient limitation patterns may suggest complexities in limitation between stream compartments, indicating that management needs to account for differential nutrient limitation. In addition, agreement or disagreement in nutrient limitation between methodologies would provide proof-of-concept evidence about the ability of EEA to gauge nutrient limitation.

Three methods are typically used to define nutrient limitation in streams: 1) experimental nutrient diffusers, 2) ratios of enzyme activity and 3) ratios of water column nutrients. Experimental diffusers directly measure nutrient limitation. Limitation is inferred in from enzyme and water column ratios relative to theoretical thresholds (Redfield, 1958; Tank *et al.,* 2006; Cleveland and Liptzin, 2007; Hill *et al.,* 2012). This study has so far detailed the relationships between *in situ* nutrient limitation of biofilms and water chemistry (chapter 2) and nutrient limitation inferred from EEA and water chemistry (chapter 3). The purpose of this

chapter is to link these approaches to understand if nutrient limitation trends are consistent between biofilms and EEA. The aims of this chapter therefore are to:

a. Describe nutrient limitation relationships between *in situ* biofilms and EEA,
b. Examine the hypothesis that EEA on organic biofilms follows nutrient limitation as indicated by nutrient diffuser experiments.

I hypothesised that nutrient limitation as described by nutrient diffusers and enzyme activity in sediment would demonstrate a coherent response. Microbial organisms measured in both analyses were exposed to the same environmental conditions which will theoretically lead to a similar nutrient limitation status. In particular, increasing nutrient limitation indicated by response ratios (RR's) in nutrient diffuser experiments should be accompanied by increased microbial allocation to enzymes targeting acquisition of the limiting nutrient. For example, increasing N limitation (indicated by increasing RR_N in diffuser experiments) should be associated with increasing microbial investment in the enzyme targeting the limiting nutrient (indicated by declining enzyme C:N ratio) (Figure 4.1A). Similarly, I expected that a relative increase in N limitation of organic biofilms (indicated by an increasing RR_N:RR_P ratio) would be accompanied by a relative increase in the production of enzymes for N acquisition (indicated by an increase in N:P ratio) (Figure 4.1B). Additionally, that enzyme allocation should match the predetermined limitation status from respiration assays, given that both metrics were determined from the same substrates. For example, a P limited site would respond to experimental enrichment of P by decreasing their relative investment in producing the enzymes for P acquisition (indicated by an increase in N:P ratio) (Figure 4.2).

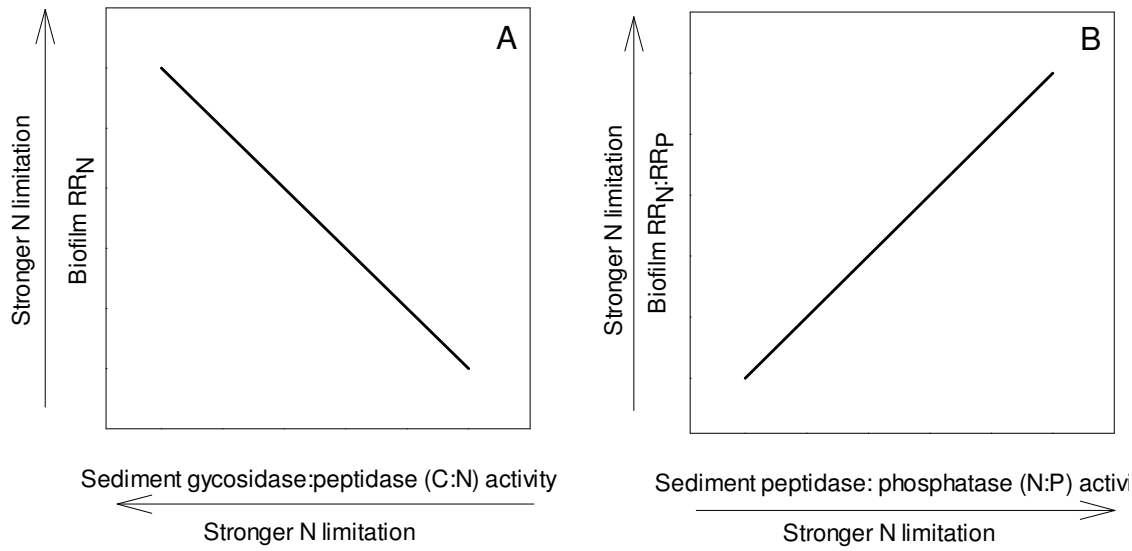


Figure 4. 1. Theoretical relationships between extracellular enzyme activity (EEA) on benthic sediments and nutrient response ratios (RR's) of *in situ* biofilms.

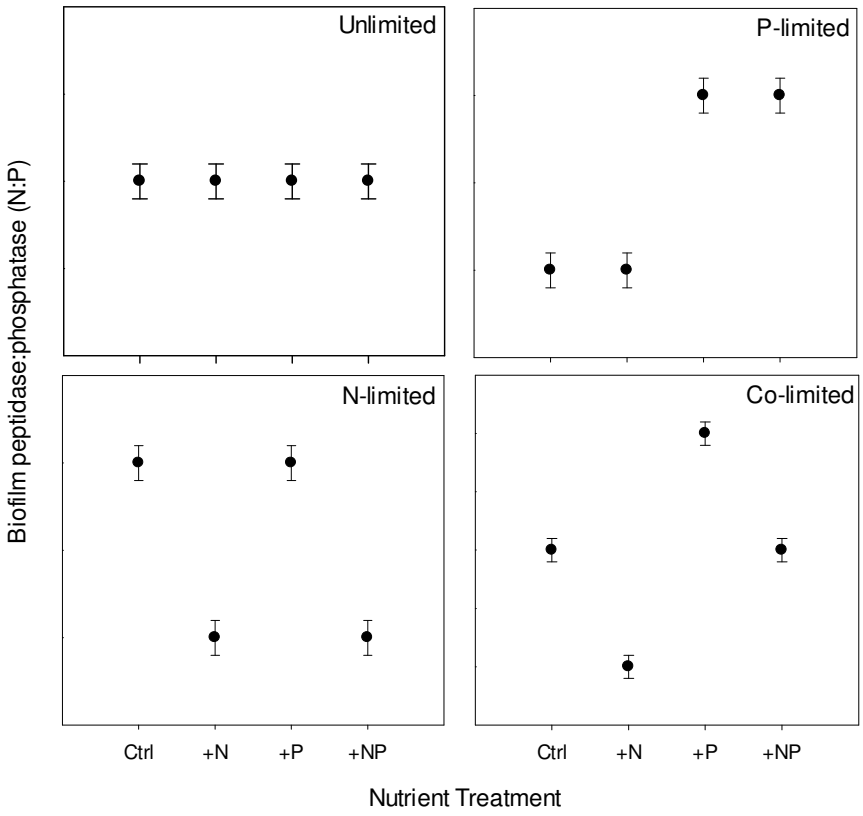


Figure 4. 2. Theoretical relationship of enzyme N:P stoichiometry of organic sponge experimentally manipulated with different nutrient treatments (control, nitrogen, phosphorus, nitrogen and phosphorus). The direction of the enzyme ratios demonstrates the strength of the enzyme activity, e.g. a higher response of enzyme N:P demonstrates stronger N limitation.

4.2. Methodology

Nutrient limitation in this chapter is compared using results from chapter two and three, in addition to carrying out enzymatic assays of organic sponge from nutrient diffusers. Two approaches used – 1) compared sediment enzymes to RR's derived from NDS across sites; 2) analysed enzyme activity on sponges used on NDS for a select set of sites.

4.2.1. Sample analysis

Organic biofilms from nutrient diffusers (described in chapter two) were analysed for extracellular enzyme activity. Assays were conducted on samples from a set of sites which were clearly N limited, P limited, co-limited, or not limited based on bioassays (Figure 4.3). Twelve sponge biofilms, three for each experimental treatment (+N, +P, +NP, and control), from four sites were analysed for extracellular enzymes (n= 48). These were prepared using the methodology outlined in chapter three except sponges were used in place of sediment. Each sponge sample was run in quadruplicate for each of the four enzymes measured. Sponges were later dried at 50ºC for 24 hours before being weighed for dry mass. Enzyme activity is expressed as nmol.[g dry mass]$^{-1}$ h^{-1}.

4.2.2. Statistical analysis

Responses between biofilms and sediment enzymes were compared using previously calculated RR's from sponges and enzyme activity on sediment (see chapters 2 and 3). As there are no clear independent or dependant variables, reduced major axis (RMA) regression was used to determine relationships between the variables. RMA regression ensures that sampling and measurement errors are taken into account for both regression variables, thus the regression line minimises error for both variables. Data were log-transformed prior to analysis where the homogeneity of variances assumption was not met.

Mean enzyme activity on sponges of the same treatment and from the same site and calculated as ratios, N:P, C:N, C:P for each of the sites (limitation categories). Significance testing was conducted using one-way ANOVA followed by *post-hoc* LSM where significant interaction terms ($\alpha < 0.5$) were obtained.

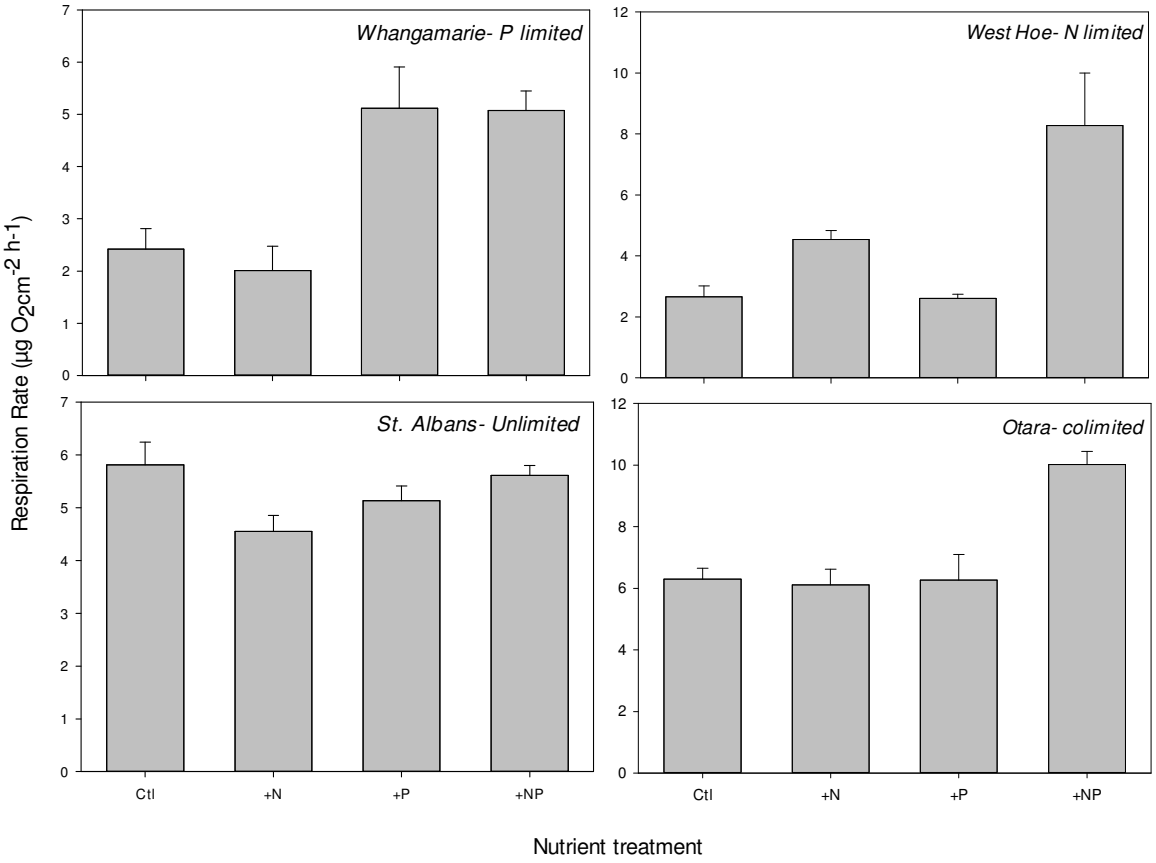


Figure 4. 3. Sites which extracellular enzyme assays were carried out on, each plot shows a different pattern of limitation as noted on the graph, for interpretation see chapter two.

4.3. Results

4.3.1. Nutrient limitation comparison

There is general agreement between respiration on organic biofilms and water chemistry but inconsistent responses from enzyme activity (Table 4.1). Organic biofilms and water chemistry confirm a switch from N to P limitation with human land-use influence, with DIN:SRP molar ratios >16 at urban and pastoral sites in Auckland. The only exception to this was Kaukapakapa, a rural site in North Auckland, which demonstrated consistent nutrient suppression through bioassays and had low DIN:SRP ratios. All Christchurch sites have been impacted by some form of human land-use (urban/agricultural) and demonstrate only P limitation or colimitation from bioassays and water chemistry. Enzyme activity ratios (N:P) are not consistent with findings from water chemistry or respiration data. Theoretically, the ratio of enzyme activity should be higher under N limited conditions, and lower under P limited conditions (Figure 3.1). Data in Table 4.1 shows that enzyme ratios are inconsistent with findings from water column nutrient concentrations and biofilm assays. If categorised by nutrient limitation as inferred from water and enzyme ratios, 38% of sites demonstrate the same type of

limitation (N or P). However, there were only 2 instances of respiration on organic biofilms and enzyme activity demonstrating agreement. These area Auckland streams, Oakley Creek (urban) and Whangamaire Stream (pastoral) in Summer.

Table 4. 1. Summary of nutrient limitation findings as indicated by respiration on organic biofilms, no nutrient limitation is indicated by a dashed line (-), enzyme peptidase: phosphatase (N:P) activity, and the ratio of water column dissolved inorganic nitrogen to soluble reactive phosphorus (DIN:SRP). N or P limitation for enzymes is based on Cleveland and Liptzin (2007) and DIN:SRP is based on the Redfield Ratio (1958). Limitation is marked as N (nitrogen), P (phosphorus), or CO (co-limited) according to thresholds proposed by these ratios. Sites are marked with their land-use as either: reference (REF), native (N), suburban (SU), urban (U), pastoral (P), rural-suburban (R-SU), urban-wetland (UW). Earthquake impact is also noted on Christchurch sites as: heavy-liquefaction (HL), or light-liquefaction (LL).

Season	Stream	Respiration on organic biofilms	Enzyme N:P	DIN:SRP
Spring	Cascades Stream (REF, N)	1˚N, 2˚ limited	2 (P)	1 (N)
	West Hoe (REF, N)	1˚N, 2˚P limited	1(P)	5 (N)
	Wairoa Trib (REF, N)	1˚N, 2˚P limited	2(P)	4 (N)
	Lucas Creek (SU)	Colimited	4(P)	72(P)
	Otara Creek (SU)	Colimited	0(P)	23(P)
	Puhinui Stream (SU)	1˚P, 2˚N limited	2(P)	145(P)
	Oakley Creek (U)	Colimited	3(P)	138(P)
	Otaki Stream (U)	-	6(P)	1016(P)
	Pakuranga Creek (U)	1˚P, 2˚N limited	26 (N)	571(P)
	Whangamarie Stream (P)	1˚P, 2˚N limited	4(P)	4906(P)
	Kaukapakapa River (P)	-	25(N)	24(P)
	Ngakaroa Stream (P)	1˚P, 2˚N limited	2(P)	3173(P)
Summer	Cascades Stream (REF, N)	Colimited	23 (N)	1(N)
	West Hoe (REF, N)	1˚N, 2˚P limited	2(P)	2(N)
	Wairoa Trib (REF, N)	1˚N, 2˚P limited	3(P)	5(N)
	Lucas Creek (SU)	Colimited	3(P)	16 (CO)
	Otara Creek (SU)	Colimited	2(P)	3(N)
	Puhinui Stream (SU)	Colimited	12(N)	29 (P)
	Oakley Creek (U)	P limited	3(P)	108(P)
	Otaki Stream (U)	-	2(P)	97(P)
	Pakuranga Creek (U)	-	5(P)	104(P)
	Whangamarie Stream (P)	P limited	6(P)	4129(P)
	Kaukapakapa River (P)	-	1(P)	3(N)
	Ngakaroa Stream (P)	Colimited	4(P)	4059(P)
Summer	Styx River Upper (REF, R-SU)	P limited	32 (N)	303(P)
	Smacks Creek (REF, R-SU)	Colimited	19(N)	118(P)
	Upper Avon River (U)	Colimited	18(N)	2409(P)
	Waimairi Stream (U)	P limited	16(N)	119(P)
	Okeover Stream (U)	Colimited	3(P)	1100(P)
	Upper Heathcote River (U)	-	11(N)	184(P)
	Wairapapa Stream (U, LL)	Colimited	19(N)	196(P)
	Papanui Stream (U, LL)	1˚P, 2˚N limited	18(N)	118(P)
	Steamwharf Stream (U, HL)	-	12(N)	121(P)
	Crosers Stream (UW, HL)	-	12(N)	31(P)
	Shirley Stream (U, HL)	-	27(N)	48(P)
	St. Albans Stream (U, HL)	-	8(N)	266(P)

4.3.2. Biofilm response ratios and extracellular enzyme activity

Sediment enzyme N:P was negatively correlated with response ratio N:P, contrary to expectations (Figures 4.1B and 4.4). This relationship is not strong as it is highly leveraged by a few points.

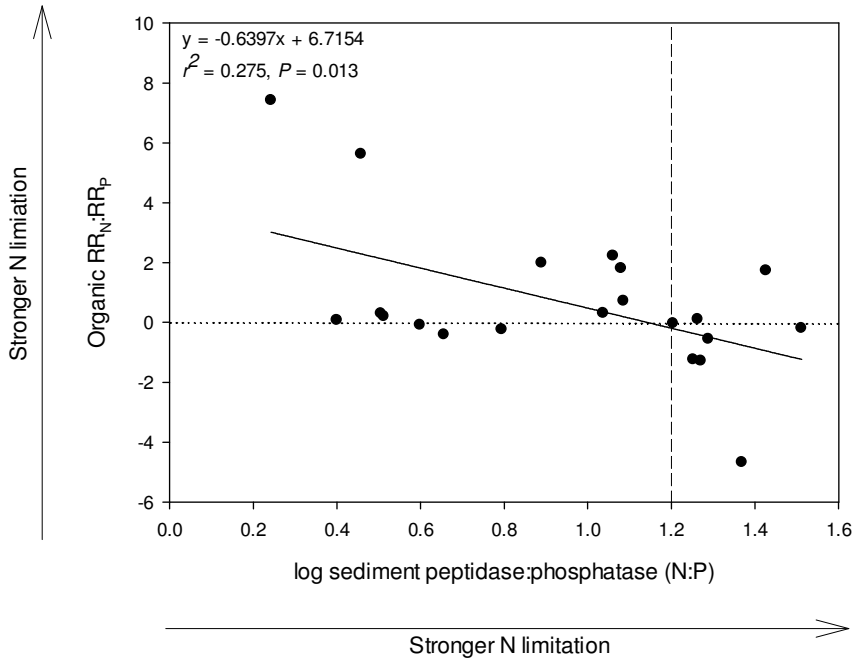


Figure 4. 4. The ratio of nitrogen and phosphorus biofilm responses against enzyme peptidase: phosphatase production in Auckland and Christchurch Summer, fitted with a log regression model. The dotted line at 0 on the y-axis indicates no response, and the dashed line at 0.84 on the x-axis indicates the ideal enzyme activity ratio (Cleveland &, Liptzin, 2007).

Data suggest microbial investment in the enzyme targeting nutrient acquisition declines or has no relationship with increasing nutrient limitation, counter to expectations (Figures 4.1A and 4.5). Sediment enzyme C:N ratios demonstrated a positive relationship with biofilm RR_N (Figure 4.5A), indicating an increased investment into production of enzymes for C acquisition (indicated by the high C:N ratio) under N limited conditions. Note that the x-axis is always negative suggesting consistent N limitation. Enzyme C:P demonstrated no relationship with biofilm RR_P, indicating that that enzymatic allocation for C and P acquisition is not in line with nutrient limitation based on bioassays (Figure 4.5B).

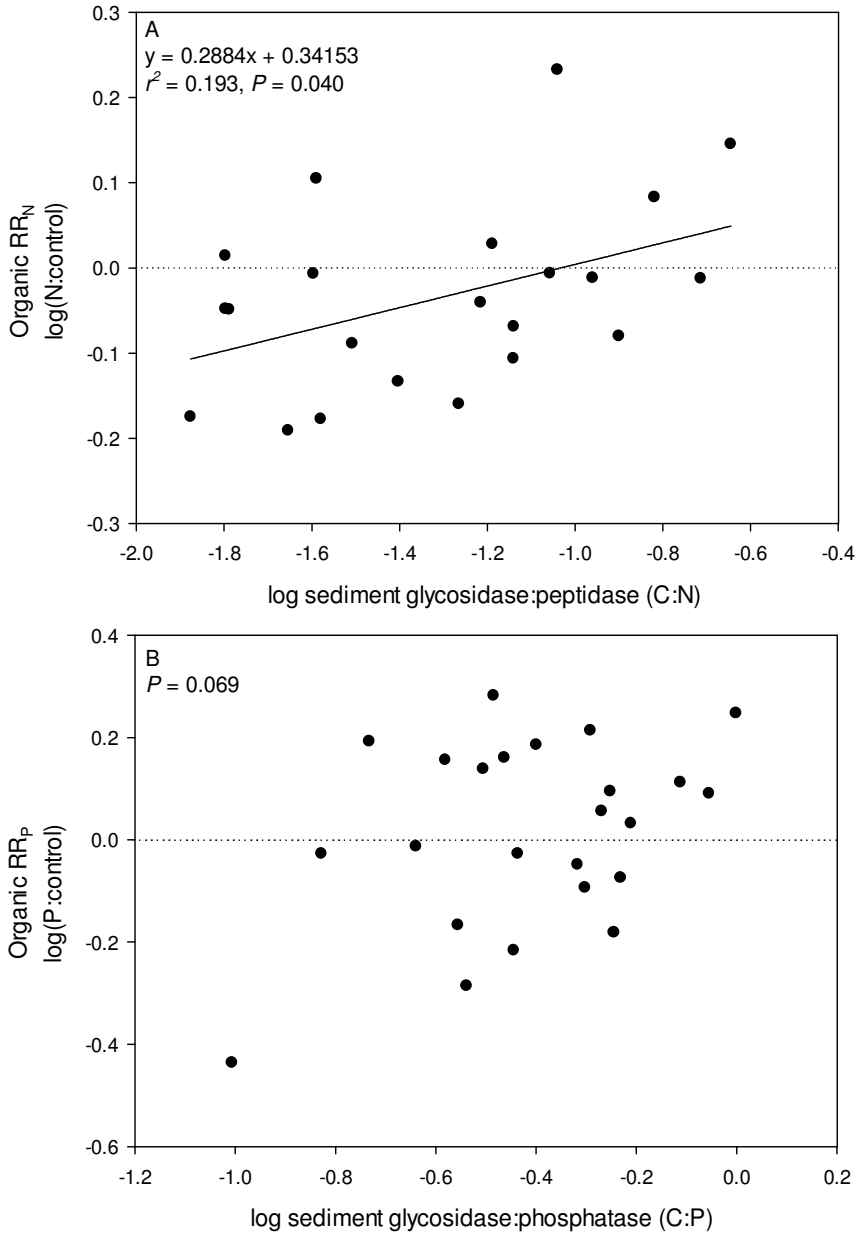


Figure 4. 5. Response ratios on organic biofilms for A) the response on nitrogen enriched biofilms against the enzyme ratio of C:N and B) the response on phosphorus enriched biofilms against the enzyme ratio of C:P, both in Auckland and Christchurch Summer. The dotted line at 0 on the y-axis indicates no response.

Biofilm RR_N and RR_{NP} were stronger with lower enzyme activity (Figure 4.5). These trends are not consistent with the expectations of a positive association between enzyme activity and water column nutrients (Figure 4.1). A similar pattern was found for RR_{NP}, which showed an increase in enzyme activity as RR's decreased. RR_P showed no trends when plotted against enzyme activity.

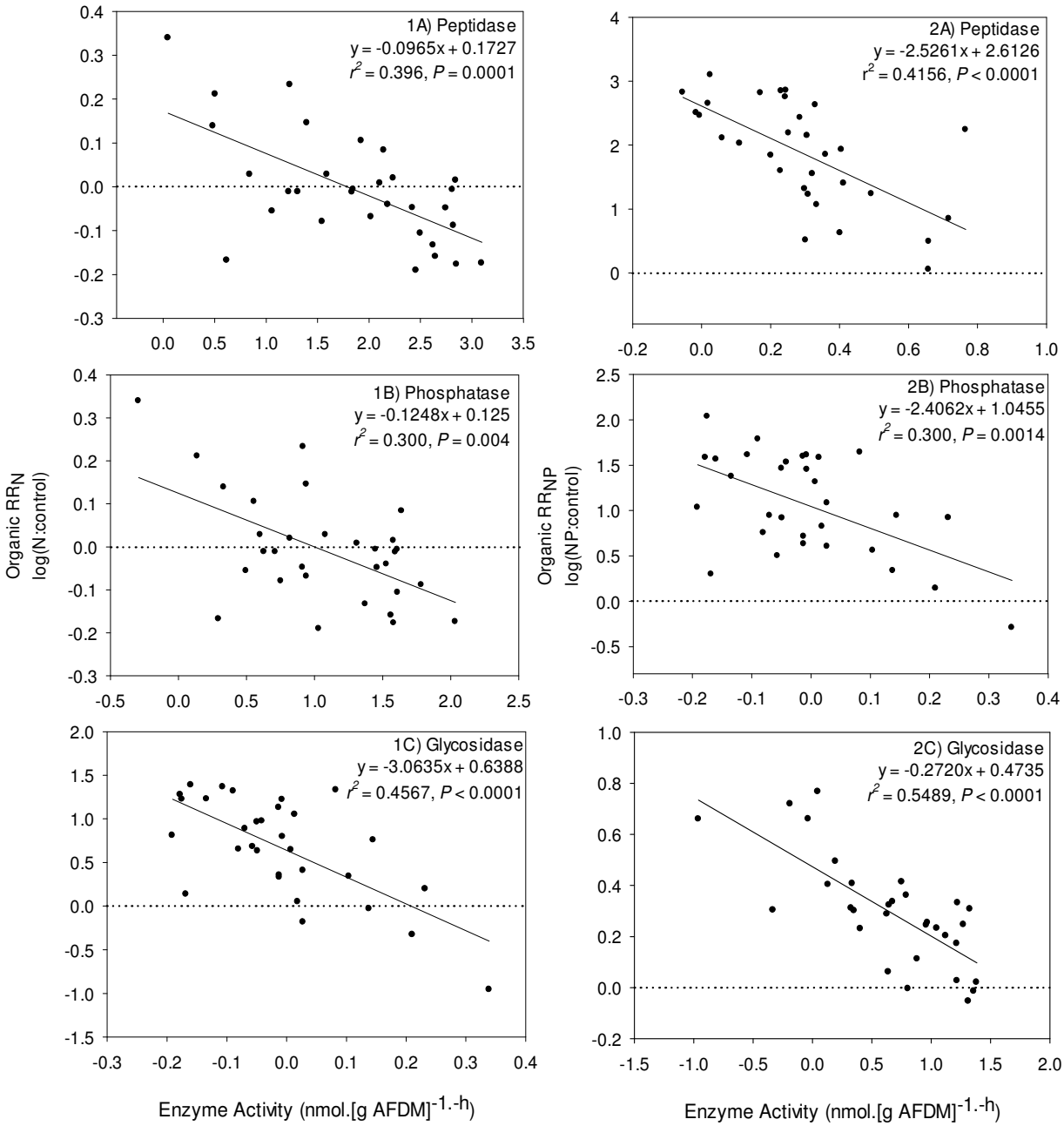


Figure 4. 6. Response ratio of 1) nitrogen and 2) phosphorus on organic biofilms against sediment enzyme activity fitted with log regression line. Dotted line at 0 indicates no response on biofilms; negative responses indicate lower responses on treatments than controls.

Nutrient limitation as suggested by inorganic RR's and enzyme ratios was, like organic biofilms, often not consistent with one another (Figure 4.7). There were fewer instances of inorganic biofilms producing significant interactions. RR_{NP} on inorganic biofilms against enzyme C:N, show different trends between Spring and Summer in Auckland (Figure 4.8A,B). In Auckland Spring RR_{NP} was strongest when C:P was higher, both consistent with some form of P limitation. This was similarly the case for Christchurch however this regression did not meet significance (Figure 4.7C). Nutrient limitation suggested by RR_N in Auckland Spring is not consistent with limitation patterns as suggested by enzyme ratios N:P or C:N (Figure 4.8A,B).

Enzyme N:P and C:N were not significantly related to other inorganic biofilm responses ($P > 0.05$).

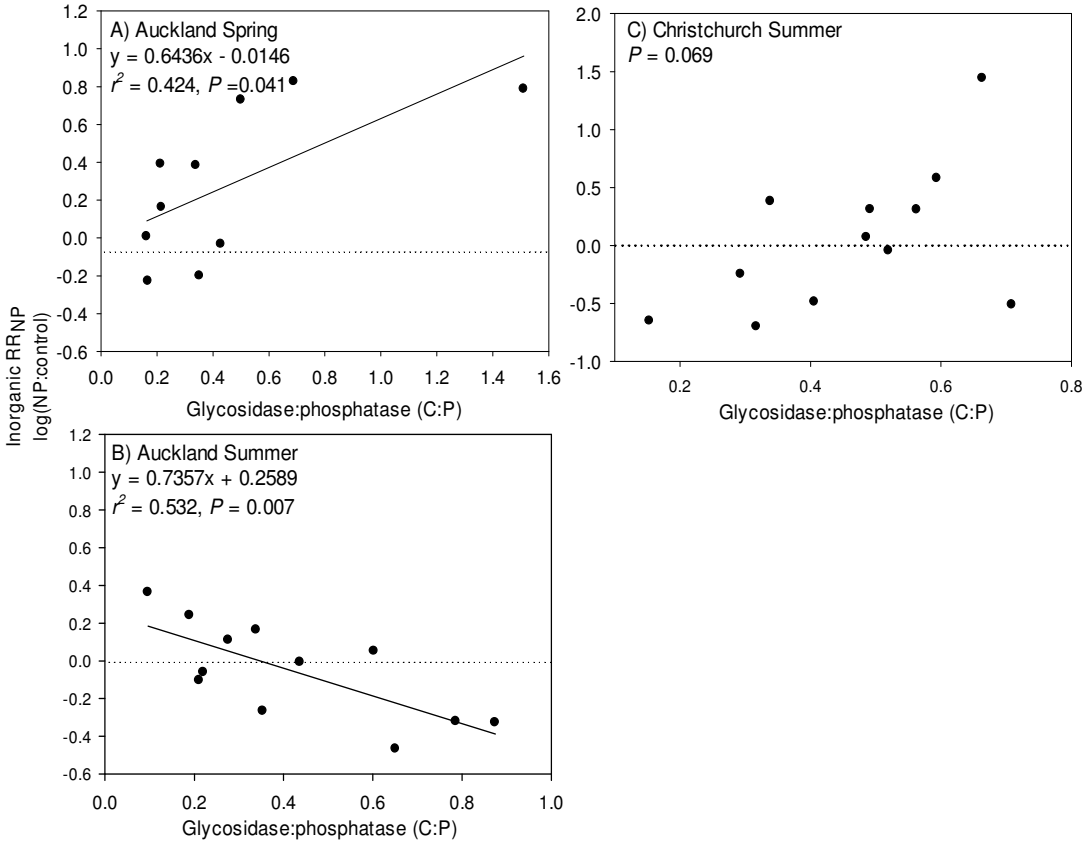


Figure 4. 7. Response ratio of nitrogen and phosphorus on inorganic biofilms against the ratio of glycosidase: phosphatase activity for A) Auckland Spring, B) Auckland Summer, and C) Christchurch Summer.

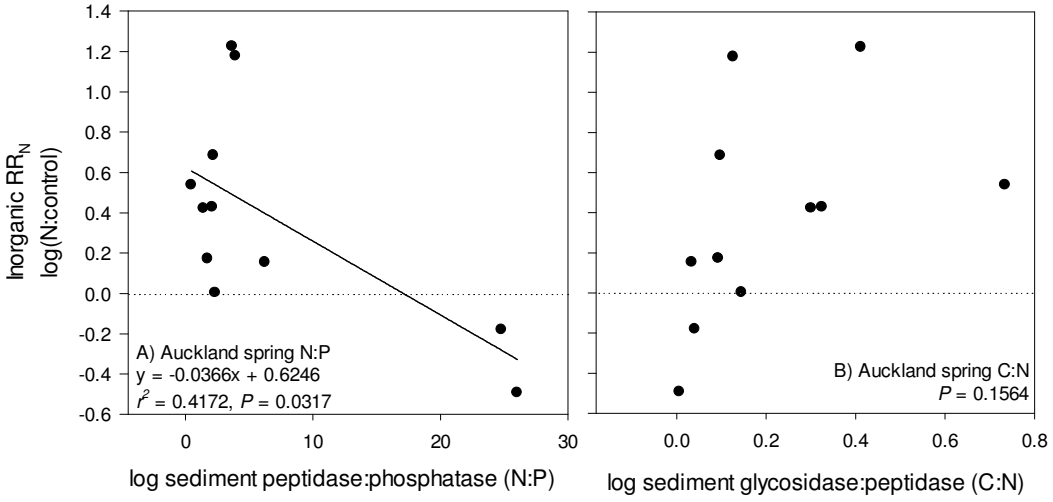


Figure 4. 8. Relationship between the response of nitrogen addition for inorganic biofilms and enzyme activity ratios in Auckland Spring. Graph A is fitted with a linear regression line. The dotted line at 0 indicates no response.

4.3.3. Enzyme activity on organic nutrient diffusing substrata

In St. Albans Stream (unlimited) enzyme stoichiometry was not reflective of nutrient limitation based on bioassays (Figure 4.2 & 4.9A). Enzyme N:P ratios were significantly larger on +P (ANOVA, P = 0.049) and +NP (ANOVA, P = 0.003) sponge than control sponge, indicating an increase allocation of enzymes towards N acquisition. Enzyme C:P ratios were larger on sponge enriched with +P (ANOVA, P = 0.010) and + NP (ANOVA, P < 0.0001) compared to control sponges. Enzyme C:N ratios increased on +P (ANOVA, P = 0.041) and +NP (ANOVA, P = 0.001) compared to control sponges, indicating increased enzymatic allocation towards C acquisition.

Bioassays and enzyme stoichiometry demonstrated agreement for P limitation in Whangamarie Stream (Figure 4.9C). Enzyme N:P ratios were larger on sponges enriched with +P (ANOVA, P = 0.007) and +NP (ANOVA, P = 0.028) when compared to the control treatment, suggesting a greater allocation of energy enzymes for N acquisition. Ratios of enzyme C:P were also larger on +P sponges (ANOVA, P = 0.002), as enzymes allocate more energy into acquisition of carbon relative to phosphorus.

Enzyme stoichiometry at West Hoe (N limited) demonstrated responses that were of mixed consistency with expectations (Figure 4.9B). On control sponges enzyme N:P ratios were high, suggesting greater allocation of enzyme effort for N acquisition, following expectations. Accordingly, enzyme N:P ratio declined on +N treatments (ANOVA, P = 0.016), but this also occurred when P alone was added (ANOVA, P = 0.030). Enzyme C:N ratios followed the predicted trend, increasing significantly on +NP compared to the control treatment (ANOVA, P = 0.021) but not when N was added alone (ANOVA, P = 0.452).

Enzyme stoichiometry at Otara Creek (co-limited) was not always consistent with expectations (Figure 4.9D). Control sponges had N:P ratios significantly larger than +P (ANOVA, P = 0.010), and +NP (ANOVA, P = 0.001) sponges, but not +N (ANOVA, P = 0.070), inconsistent with the theoretical model (Figure 4.2). Sponges enriched with +P had greater C:P ratios than control (ANOVA, P = 0.002), +N (ANOVA, P = 0.003), and +NP (ANOVA, P = 0.017), consistent with expectations. Enzyme C:N ratio was larger on +P (ANOVA, P = 0.047) and +NP (ANOVA, P = 0.050) sponges than control, suggesting a greater allocation to carbon.

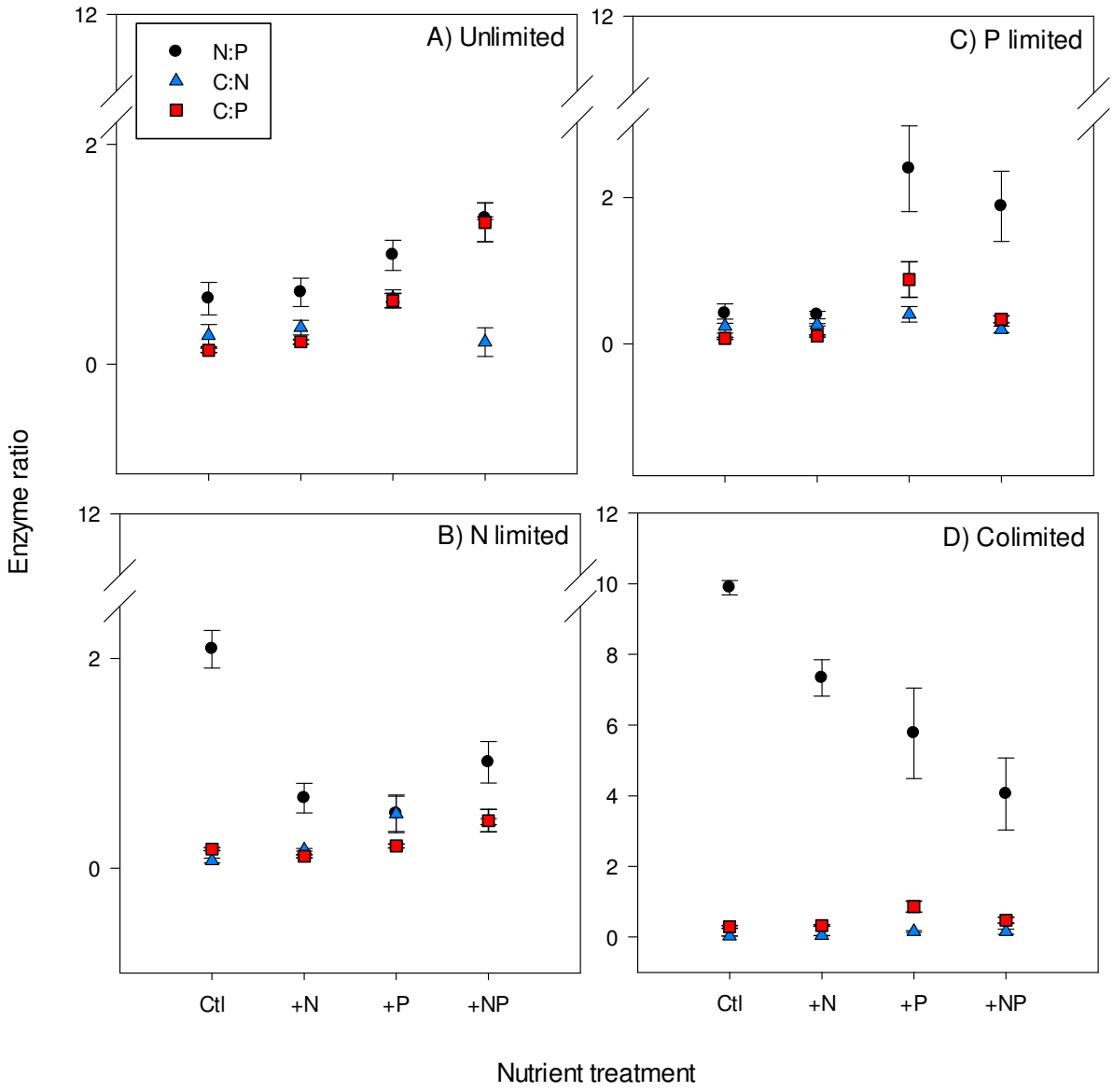


Figure 4. 9. Mean enzyme ratios (± SE) on organic biofilms, limitation patterns which were found at each site through respiration assays are noted on the top right of each plot. Note graph scales have been broken where appropriate to fit the data.

4.4. Discussion

The expectation of nutrient limitation studies is that limitation trends should be consistent between compartments of streams, with nutrient management aiming to control a single limiting nutrient (McDowall *et al.,* 2009; Death *et al.,* 2007). In my study sediment EEA and sponge biofilm RR's often demonstrated counterintuitive trends; indicating differences in the nutrient requirements of biofilms depending on their surrounding environment. My study also took the novel approach of measuring enzyme activity on organic sponge from nutrient diffusers, permitting me to directly relate enzyme and respiration response on the same substrate under experimental manipulation. This revealed that enzyme production and community respiration by microbes were not always in agreement with one another, especially regarding more complex limitation patterns.

4.4.1. Coherence in nutrient limitation trends across methods

Nutrient limitation based on sediment EEA and sponge biofilm RR's demonstrated no coherent responses. These measurements of limitation were often found to be inversely related, contrary to expectations (Figures 4.1 & 4.4). This suggests that microbial organisms on benthic sediments have distinctly different nutrient requirements compared to biofilms on organic and inorganic substrates in the water column. Biofilms on experimental glass frits demonstrated fewer responses to sediment EEA than biofilms on cellulose sponge. This, in combination with their lack of correlation with water column nutrients (chapter 2), suggests that glass frits may a poor predictor of nutrient limitation. The lack of agreement between nutrient limitation as described by response ratios or sediment EEA suggests that these two variables differ in terms of their nutrient requirements, due to alterations in nutrients, carbon, or physiochemical factors as discussed in chapter 3 (Mulholland, 1996). Other studies have similarly found no agreement between nutrient limitation as indicated by sediment EEA and water chemistry (Romani *et al.*, 2004; Harbott and Grace, 2005; Hill *et al.*, 2010a), but agreement between sediment EEA and sediment nutrient analysis and nutrient uptake (Hill *et al.*, 2006; Hill *et al.*, 2010a; Hill *et al.*, 2012). Thus, we can consistently expect a divergence of limitation trends dependant on which habitat (benthic vs water column) is being assessed; indicating differences in structural and functional characteristics of these biofilms (Romani *et al.*, 2004). The lack of consistent nutrient limitation trends between sediment enzymes and water column nutrients or biofilm responses, coupled with the agreement between responses on organic biofilms suggests that heterotrophic biofilms may be a more sensitive tool for consistently gauging microbial response to nutrient limitation.

Differences in responses may also be related to the parameters used to measure nutrient limitation. The link between sediment EEA and community respiration has not been explored in depth; however results from my study and others suggest that these are weakly linked (Chrost, 1991; Sinsabaugh and Foreman, 2001). Again, this relationship may have been weak due to the difference in experimental substrates (cellulose sponge versus sediments). Non-specificity of the enzymes used (e.g. peptidases can target both C and N) may also be a source of error, as discussed in chapter three. When sediment respiration and EEA are measured alongside one another these were found to demonstrate agreement, emphasising the divergence between in-stream habitats and nutrient limitation trends (Hill *et al.*, 2012). Differences in community structure and species composition may also influence observed limitation trends. Biofilms on sponge would have been relatively young and likely had a higher fungal dominance compared to more complex biofilms which can take months to form, likely altering species composition and potentially nutrient sources within the biofilm (Kirchman, 2012). Additionally, whilst microbes on cellulose sponge biofilms were provided with a labile carbon source, sediment carbon sources are likely to be much more variable (variable lability and available N and P) influencing the classes of enzyme microbes produced for carbon acquisition. This would not have been detected in this study as only glycosidase (cellulose degradation) was measured; although this has been found to be the dominant carbon source in freshwaters (Hill *et al.*, 2006; Sinsabaugh and Shah, 2012). There may have also been differences in the autotrophic and heterotrophic composition of each biofilm, due to the competition (see chapter two). Cellulose sponge may have had a smaller autotrophic component than sediment which may reduce the amounts of carbon and nutrients available to heterotrophs (e.g. algal exudates, cell lysis) (Romani and Sabater, 1999; Rier *et al.*, 2007).

4.4.2. Enzyme allocation on nutrient diffusers

I expected patterns of enzyme activity to be tightly coupled to respiration on the NDS sponges because both were measured on the same microbial communities. However, limitation patterns as described by enzyme activity were not always consistent with limitation trends as described by respiration assays (Figure 4.9). Whilst previous invariances have been thought to be related to resources and physiochemical variations, this possibility has been eliminated by conducting assays on the same biofilms, exposed to the same environmental conditions and resources. Trends were more consistent on sponge from the N limited and P limited sites than co-limited or unlimited sites. This suggests that these latter two limitation categories may be more difficult to detect through enzyme assays or that enzyme assays provide more detailed information on limitation trends which could not be picked up through community respiration

assays. No other studies have measured enzyme stoichiometry on organic or inorganic substrates from nutrient diffusers, so my results are difficult to compare. However, Scott *et al* (2009) measured phosphatase activity on glass frits from nutrient diffusers and found that N enrichment in an N limited system stopped N_2 fixation and increased phosphatase activity. Other studies which have looked at enzyme allocation have also found a similar trend of nutrient addition affecting enzyme expression (Sinsabaugh and Moorhead, 1994; Wright and Reddy, 2001; Hill *et al.*, 2012; Williams *et al.*, 2012).

Findings of my study suggest that increasing nutrient availability does not consistently down-regulate enzymatic responses. Differences may be due to methodologies, all microbial organisms respire but not all microbes may be producing enzymes and enzyme production may differ, as different microbes have different nutrient requirements (Hoellein *et al.*, 2010; Sinsabaugh *et al.*, 2010). This may also make EEA a more accurate representation of nutrient limitation status, as enzymes are only produced in response to a shortage of nutrients as they are energetically expensive (Lehto and Hill, 2013). However, it is also possible that enzymes may have yielded different patterns due to the ability of microbial organism to use enzymes for the acquisition of different nutrients (e.g. peptidases for N or C cycling). This may also explain why the pattern enzyme stoichiometry at the P limited site was more consistent, as phosphatase is specific to phosphorus acquisition. Furthermore, as microbes have a communal lifestyle they can benefit from nutrient acquisition or nutrient stores from other microbes (Lear *et al.*, 2012), affecting expression of enzymes due to nutrient enrichment and communal use of resources, potentially altering enzyme stoichiometry. The mechanisms behind the inconsistencies in EEA stoichiometry and respiration are unclear; however, results suggest that these are not as tightly coupled as theorised. More complex limitation patterns such as co-limitation may not be consistent between parameters; however EEA may be useful in detecting a broad switch from N to P limitation and may even detect more accurate limitation patterns. Further work should be done on this topic to develop further applications of this work; as enzymatic assays may provide an inexpensive and quick method of assessing in-stream nutrient limitation status.

4.5. Conclusion

This study has demonstrated the variability in predicted microbial nutrient limitation according to various methodologies. Enzymes on sediment were rarely in agreement with water column nutrient concentrations or biofilms, emphasising the difference in limitation between stream compartments. This also suggests that streambed sediments may not be a useful tool for the assessment of water column nutrient limitation, unless there is a specific focus on the

benthos, due to the divergence in limitation trends. Assessments of EEA stoichiometry on organic biofilms from nutrient diffusers were more in-line with respiration and water chemistry trends. This indicates that that nutrient limitation as predicted by EEA on organic biofilms in the water column may be useful for predicting changes in broad nutrient limitation trends.

Chapter 5

Synthesis

Eutrophication is a serious threat affecting New Zealand's freshwaters, this is especially pronounced in urban landscapes where nutrient concentrations are often elevated (Larned *et al.*, 2004; Walsh *et al.,* 2005; Ministry for the Environment, 2007; Neale, 2012). Understanding how nutrients are processed in streams is therefore crucial for efficient management. In chapter two I demonstrated that nutrient loading associated with urbanisation is likely to alter to magnitude and identity of limiting nutrients for stream microbial communities. Increasing N concentrations were associated with a shift towards P limitation in urban and agricultural sites. This is significant for management as it suggests substantial downstream export for N in urban sites with impervious areas over 30% and in agricultural areas over 25%. This may have consequences for estuarine areas which tend to be susceptible to N enrichment (Howarth and Marino, 2006). Moreover, co-limitation or secondary limitation were the prevalent responses amongst heterotrophic biofilms across all land-uses emphasising that where nutrient management does occur it should focus on both nutrients rather than one to improve ecosystem health (Allgeier *et al.*, 2011). Additionally, responses on biofilms were not consistent between seasons owing to variations in ambient nutrient concentrations and physiochemical parameters; assessments of nutrient limitation should therefore be carried out across different seasons where feasible (Johnson *et al.*, 2009a).

In chapters three and four I demonstrated that nutrient limitation is not tightly coupled among compartments in streams as theorised. There has been a recent effort to assess nutrient limitation in streams using biological metrics as these are thought to be a better representation of biotic limitation than water samples alone (Francoeur, 2001; Hill *et al.*, 2006; Hill *et al.*, 2012). My data suggests that when inferred limitation from sediment enzymes is compared to limitation from other stream compartments (water column chemistry or biofilms) these do not align. But nutrient limitations inferred from metrics in the water column were roughly in-line with one another. Assessments of nutrient limitation should account for differences in biological limitation within streams and take into consideration that nutrient limitation is not exclusively linked to water chemistry. Thus, when using EEA to describe nutrient limitation, it would be useful to collect biofilms from different compartments (e.g. benthos, woody debris, rocks); as each surface is likely to have different microbial communities (autotrophs vs heterotrophs) and various access to resources depending on their surrounding environment. This finding is

significant for our understanding of in-stream nutrient processing, as nutrient loading may not directly affect water column biota (beyond a degradation threshold) but it may have consequences for microbial communities in sediments; which control a significant proportion of energy cycling in streams (Hill *et al.*, 2006). For stream management this suggests that the dominant paradigm of streams being P limited is not strictly accurate; controls should therefore also be placed on N inputs (Schindler, 1977). Currently there is strong opposition to enforcing N limits in New Zealand's freshwaters due to the perceived idea leading from early studies that freshwaters are P limited (Schindler, 1977; Sharpe, 2012; Piddock, 2013). My data suggests that water column biofilms are dominantly N and P co-limited or secondarily limited by N, and stream sediments are dominantly N limited. Therefore, managing a stream as P limited is a simplification of stream limitation patterns and discounts differential limitation of the benthos. Furthermore, based on chapter two results it can be assumed that native or natural systems are N limited; thus the idea of controlling only for P may not help improve overall stream health. My data therefore suggest that nutrient limitation needs to be perceived as spatially and temporally variable and simplifications such as a sole focus on P management should be avoided.

Canterbury earthquakes caused tonnes of liquefaction to enter streams which is still visible today. My research has demonstrated that earthquake damage is still affecting stream microbial communities more than three years after the first earthquake. Biofilms demonstrated no nutrient limitation in sites impacted by heavy liquefaction and the phenomenon of nutrient suppression. In stream sediments, microbial communities demonstrated stronger N limitation; likely related to elevated SRP, and seven-fold lower DIN concentrations following sewerage leaks. Leading to the conclusion that stream nutrient processing was altered, likely through elevated denitrification rates (Wells *et al.,* 2013). Therefore, continued disturbance from sewage inputs and liquefaction are affecting microbial communities which has clear effects on nutrient limitation between stream compartments. This finding is in opposition to other studies which have suggested stream communities are robust to disasters (Wells *et al.,* 2013; ESR, 2012a, ESR, 2012b; Rutherford and Hudson, 2011; Allison and Martiny, 2008). Follow-up studies on this would be useful in understanding what implications this may have for stream recovery.

Data highlights the importance of using biological metrics to assess nutrient limitation. Nutrient limitation was specifically tested using nutrient diffusers accounting for autotrophic and heterotrophic components of biofilm communities, allowing nutrient limitation patterns to be accurately distinguished and compared to commonly used threshold of water chemistry and enzyme activity. This approach allowed for the viability of other methodologies to be assessed; with the conclusion that some commonly used methods may not provide coherent results (e.g.

sediment, glass frits), indicating that many studies may have inaccurately described nutrient limitation. Heterotrophic nutrient limitation roughly followed limitation patterns as predicted by inorganic nutrient ratios (Redfield ratio). However, more detailed patterns cannot be ascertained from nutrient ratios such as streams which were unlimited, colimited, or secondary limited. Nutrient limitation should therefore be experimentally tested rather than inferred from ratios before management actions are taken.

Robust sampling design has allowed microbial nutrient limitation patterns to be described across differences in land-uses. Only a few studies have looked into the impacts of urbanisation on nutrient limitation in microbial communities (Johnson *et al.*, 2009a; Hoellein *et al.*, 2011a). Those studies did not explore the relationship between biofilm responses and water column nutrient ratios or thresholds of urbanisation. This is a novel approach provides the first evidence that nutrient limitation according to heterotrophic biofilms can be roughly predicted from benchmark ratios of inorganic nutrients in the water column. The agreement between water chemistry and responses on organic biofilms suggests that these may be a more sensitive tool for consistently gauging microbial response to nutrient limitation, when compared to inorganic substrates. As such these should be prioritised in future nutrient limitations studies to develop the wider applicability of this methodology. Furthermore, EEA represents a promising tool for an in-expensive and quick measure of ecosystem health. EEA stoichiometry demonstrated differences with urbanisation intensity, suggesting that this is a potentially viable tool for the assessment of ecosystem health. However results indicate that sediment EEA is of limited use for predicting nutrient limitation trends within the water column. Unlike water chemistry biofilms are robust to intermittent changes in discharge or other disturbances indicating that they may provide a more accurate analysis of stream conditions (Hill *et al.,* 2010a). More studies should be done on this to develop the wider applicability of EEA as a bio-assessment tool.

This research has also identified several other research opportunities for future work. I have demonstrated that nutrient limitation according to biofilm and EEA responses is consistent across urban environments in Auckland and Christchurch; despite their environmental differences. Further studies focussing on other urban centres would be interesting in order to establish the wider applications of these findings. Mechanisms behind nutrient suppression have not been explained despite this being a commonly observed phenomenon; this has been identified as a priority for further research. A particular research focus is the relationship between enrichment concentrations and nutrient toxicity. Another mechanism which has not been explained is the high incidence of no-limitation according to glass frits which is common in NDS studies, this may suggest that either urban sites were infrequently nutrient limited, glass

fritted discs are not ideal colonisation substrates, or other factors were controlling primary productivity. Future studies should consider using other chlorophyll *a* measurement methods (e.g. flurometric method) to determine if this reduces error in measurements or trailing other inorganic surfaces.

Future studies should also consider looking at the relationship between C, N, and P in urban streams, as this has not been well characterised (Bechtold *et al.,* 2012). The cycles of each of these elements are tightly linked, and limitation may be affected by organic carbon availability (Allan and Castillo, 2007; Johnson, 2009b). Additionally, analysis of C, N, and P in streambed sediments may help to improve our understanding of nutrient limitation in the benthos and validate the hypothesis that limitation is variable between stream compartments due to biofilm access to absorbed phosphorus and additional carbon sources. Another method used to gauge nutrient limitation is whole-stream uptake rates (e.g. Bechtold *et al.*, 2012). Uptake includes all biotic compartments of streams and would be interesting to compare uptake measurements against nutrients limitation assessed in this study. On a smaller spatial scale, divisions of biofilm microbes into specific autotrophic or heterotrophic components or a taxonomic analysis (e.g. Lear and Lewis, 2009) of stream biofilms indicative of different nutrient limitation patterns could be carried out to better understand the effects of urbanisation on microbial communities. These analyses were beyond the scope of my study, but would provide an ideal opportunity for researchers interested in nutrient stoichiometry to expand upon the robust data set presented in this study.

References

Ainsworth, A. M. and R. Goulder. 2000 Downstream change in leucine aminopeptidase activity and leucine assimilation by epilithic microbiota along the River Swale, northern England, *Science of the Total Environment,* 251–252(0): 191-204.

Allan, D. J. 2004 Landscapes and Riverscapes: The Influence of Land Use on Stream Ecosystems, *Annual Review of Ecology and Systematics,* 35: 257-287.

Allan, D. J. and M. M. Castillo. 2007 *Stream Ecology: Structure and Function of running waters*, Springer Publishing, The Netherlands.

Allgeier, J. E., A. D. Rosemond and C. A. Layman. 2011 The frequency and magnitude of non-additive responses to multiple nutrient enrichment, *Journal of Applied Ecology,* 48: 96-101.

Allison, S. D. 2005 Cheaters, diffusion and nutrients constrain decomposition by microbial enzymes in spatially structured environments, *Ecology Letters,* 8: 626-635.

Allison, S. D. and J. B. H. Martiny. 2008 Resistance, resilience, and redundancy in microbial communities, *PNAS,* 105(1): 11512-11519.

Ancion, P., G. Lear and G. D. Lewis. 2010 Three common metal contaminants of urban runoff (Zn, Cu & Pb) accumulate in freshwater biofilm and modify embedded bacterial communities, *Environmental Pollution,* 158: 2738-2745.

ANZECC and ARMCANZ. 2000 *Australian and New Zealand guidelines for fresh and marine water quality. Volume 1. The guidelines*, Australia and New Zealand Environment and Conservation Council and Agriculture and Resource Management Council of Australia and New Zealand., Canberra, Australia.

American Public Health Association (APHA). 2000 *Standard Methods for the Examination of Water and Wastewater*, American Public Health Association, Washington.

Atkinson, B. L., M. R. Grace, B. T. Hart and K. E. N. Vanderkruk. 2008 Sediment instability affects the rate and location of primary production and respiration in a sand-bed stream, *Journal of the North American Benthological Society,* 27(3): 581-592.

Auckland Council, 2012 *State of the Environment Monitoring River Water Quality Annual Report 2010*, Auckland Council, Auckland, New Zealand.

Auckland Council, 2011 *Auckland Council draft Annual Plan 2011 – 2012*, Auckland Council, Auckland, New Zealand.

Barlett, M. A. and L. G. Leff. 2010 The effects of N:P ratio and nitrogen form on four major freshwater bacterial taxa in biofilms, *Canadian Journal of Microbiology,* 56: 32-43.

Beach, D. 2001 *Coastal Sprawl: The Effects of Urban Design on Aquatic Ecosystems in the United States*, Pew Oceans Commission, Arlington, Virginia.

Bechtold, H. A., A. M. Marcarelli, C. V. Baxter and R. S. Inouye. 2012 Effects of N, P, and organic carbon on stream biofilm nutrient limitation and uptake in a semi-arid watershed, *Journal of Limnology and Oceanography,* 57(5): 1544-1554.

Bernhardt, E. S. and G. E. Likens. 2004 Controls on periphyton biomass in heterotrophic streams, *Freshwater Biology,* 49: 14-27.

Bernot, M. J., D. J. Sobota, R. O. Hall, P. J. Mulholland, W. K. Dodds, J. R. Webster, J. L. Tank, L. R. Ashkenas, L. W. Cooper, C. N. Dahm, S. V. Gregory, N. B. Grimm, S. K. Hamilton, S. L. Johnson, W. H. McDowell, J. L. Meyer, B. Peterson, G. C. Poole, H. M. Valett, C. Arango, J. J. Beaulieu, A. J. Burgin, C. Crenshaw, A. M. Helton, L. Johnson, J. Merriam, B. R. Niederlehner, J. M. O'Brien, J. D. Potter, R. W. Sheibley, S. M. Thomas and K. Y. M. Wilson. 2010 Inter-regional comparison of land-use effects on stream metabolism, *Freshwater Biology,* 55(9): 1874-1890.

Bibby, R. L. and J. G. Webster-Brown. 2005 Characterisation of urban catchment suspended particulate matter (Auckland region, New Zealand); a comparison with non-urban SPM, *Science of the Total Environment,* 35: 177-197.

Biggs, B. J., C. Kilroy and R. L. Lowe. 1998 Periphyton development in three valley segments of a New Zealand grassland river: Test of a habitat matrix conceptual model within a catchment, *Archiv für Hydrobiologie,* 143: 147-177.

Black, A. 2012 *A review of the environmental impacts from the Ōtautahi/Canterbury Earthquakes*, Whenua Solutionz Ltd, Christchurch, New Zealand.

Blakely, T. J. and J. S. Harding. 2005 Longitudinal patterns in benthic communities in an urban stream under restoration, *New Zealand Journal of Marine and Freshwater Research,* 39(1): 17-28.

Borchat, M. A. 1996 Nutrients. In: *Algal ecology: freshwater benthic ecosystems*, R. J. Stevenson, Bothwell, M.L. and Lowe, R.L. eds. San Diego, Academic Press.

Bowen, J. L., B. B. Ward, H. G. Morrison, J. E. Hobbie, I. Valiela, L. Deegan and M. L. Sogin. 2011 Microbial community composition in sediments resists perturbation by nutrient enrichment, *The ISME Journal,* 5: 1540-1548.

Bratbak, G. 1987 Carbon flow in an experimental microbial ecosystem, *Marine Ecology - Progress Series,* 36: 267-276.

Brussard, C. P. D. and R. Riegman. 1998 Influence of bacteria on phytoplankton cell mortality with phosphorus or nitrogen as the algal growth limiting nutrient, *Aquatic Microbial Ecology* 14: 271-280.

Burns, R. G., J. L. DeFrost, J. Marxsen, R. L. Sinsabaugh, M. E. Stromberger, M. D. Wallestein, M. N. Weintraub and A. Zoppini. 2013 Soil enzymes in a changing environment: Current knowledge and future directions, *Soil Biology & Biochemistry,* 58: 216-234.

Canterbury District Health Board, 14/01/2013 *Media Release - 14/01/2013: Potential contamination of river*, http://www.cdhb.govt.nz/communications/media/2013/130114.htm, Retrieved 09/08/2013.

Cairns, L. 08/02/2013 *Sewage leaks into Avon River near Hagley*, http://www.stuff.co.nz/the-press/news/8280146/Sewage-leaks-into-Avon-River-near-Hagley, Retrieved 09/08/2013.

Chappell, K. R. and R. Goulder. 1994 Enzymes as river pollutants and the response of native epilithic extracellular-enzyme activity, *Environmental Pollution,* 86: 161-169.

Cheeseman, B. C., P. E. Hutton and J. M. Burch. 1992 Limiting nutrients for periphyton growth in sub-alpine, forest, agricultural and urban streams, *Freshwater Biology,* 28: 349-361.

Chrost, R. J. 1991 Environmental control of the synthesis and activity of aquatic microbial ectoenzymes. In: *Microbial Enzymes in Aquatic Environments*, R. Chrost, J. ed. New York, Springer-Verlag.

Classens, L., C. L. Tague, P. M. Groffman and J. M. Melack. 2010 Longitudinal assessment of the effect of concentration on stream N uptake rates in an urbanizing watershed, *Biogeochemistry,* 98: 63-74.

Cleveland, C. C. and D. Liptizin. 2007 C:N:P stoichiometry in soil: is there a ‘‘Redfield ratio’’ for the microbial biomass?, *Biogeochemistry,* 85: 235-252.

Clinton, S. M., R. T. Edwards and S. E. G. Findlay. 2010 Exoenzyme activities as indicators of dissolved organic matter composition in the hyporheic zone of a floodplain river, *Freshwater Biology,* 55: 1603-1615.

Cockrum, L. D. 1996 Responses of chlorophyll-a, organic matter, and macroinverebrates to nutrient additions in rivers flowing through agricultural and forested land, *Archive Hydrobiologica,* 136(3): 391-411.

Christchurch City Council. 2003 *Waterways, Wetlands and Drainage Guide: Part B: Design*, Christchurch City Council, Christchuch, New Zealand.

Christchurch City Council. 2005 *Contextual Historical Overview*, Christchurch City Council, Christchurch, New Zealand.

Christchurch City Council. 2007 *State of the Environment Monitoring: Indicator Reporting Sheet, Population Density*, Christchurch City Council, Chruistchurch, New Zealand.

Christchurch City Council. 2010 *State of the Environment Monitoring: Indicator Reporting Sheet, Land Use*, Christchurch City Council, Christchurch, New Zealand.

Christchurch City Council. 2011 *Waterways cleared of sewer overflows*, Christchurch City Council, Christchurch, New Zealand.

Christchurch City Council, 20/06/2013 *Civil Defence prepared and on stand-by during bad weather*, http://www.ccc.govt.nz/thecouncil/newsmedia/mediareleases/2013/201306203.aspx, Retrieved 09/08/2013.

Covino, T. P., B. L. McGlynn and R. A. MacNamara. 2010 Quantifying stream nutrient stream nutrient uptake from ambient to saturation with instantaneous tracer additions, *Limnology and Oceanography: Methods,* 8: 484-498.

Cox, S. C., H. K. Rutter, A. Sims, M. Manga, J. J. Weir, T. Ezzy, P. A. White, T. W. Horton and D. Scott. 2012 Hydrological effects of the MW 7.1 Darfield (Canterbury) earthquake, 4 September 2010, New Zealand, *New Zealand Journal of Geologyand Geophysics,* 55(3): 231-247.

Currie, D. J. and J. Kalff. 1984 Can Bacteria Outcompete Phytoplankton for Phosphorus? A Chemostat Test, *Microbial Ecology,* 10(3): 205-216.

Dairy NZ. 2013 *Sustainable Dairying: Water Accord*, Dairy NZ, Hamilton, New Zealand.

Danger, M., C. Oumarou, D. Benest and G. Lacroix. 2007 Bacteria can control stoichiometry and nutrient limitation of phytoplankton, *Functional Ecology,* 21: 202-210.

Davis, M. D., R. Jayaratne, J. Ansen, M. and M. Timperley. 2010 Characterisation of Urban Stormwater Quality in the Auckland Region (New Zealand), *World Environmental and Water Resources Congress 2010*: 3485-3501.

Dodds, W. K. 2003 Misuse of Inorganic N and Soluble Reactive P Concentrations to Indicate Nutrient Status of Surface Waters, *Journal of the North American Benthological Society,* 22(2): 171-181.

Environment Canterbury, 16/01/2013 *Water contamination in the Christchurch area after the 22 February and 13 June 2011 earthquakes*, http://ecan.govt.nz/services/online-services/monitoring/pages/water-contamination-christchurch-post-22-feb-2011-earthquake.aspx, Retrieved 20/03/2013.

ESR. 2012a *Sewage-Contaminated Liquefaction Silt Study*, ESR, Christchurch New Zealand. Client Report FW11082.

ESR. 2012b *Microbial Quality of the Avon River: The effect of major earthquakes and subsequent sewage discharges on the quality of the water and sediments in the Avon River, Final Report*, Technical Report FW12015, ESR, Christchurch, New Zealand.

Findlay, S. 2010 Stream microbial ecology, *Journal of the North American Benthological Society*, 29(1): 170-181.

Fischer, H., S. A. Steinberg and M. T. Pusch. 2002a Differential retention and utilization of dissolved organic carbon by bacteria in river sediments, *Limnology and Oceanography,* 47: 1702-1711.

Fischer, H., S. C. Wanner and M. Punsch. 2002b Bacterial Abundance and Production in River Sediments as Related to the Biochemical Composition of Particulate Organic Matter (POM), *Biogeochemistry,* 61(1): 37-55.

Fore, L. S. and C. Grafe. 2002 Using diatoms to assess the biological condition of large rivers in Idaho (U.S.A.), *Freshwater Biology,* 47: 2015-2037.

Francoeur, S. N. 2001 Meta-analysis of lotic nutrient amendment experiments: detecting and quantifying subtle responses, *Journal of the North American Benthological Society,* 20: 358-368.

Francoeur, S. N., B. J. Biggs, R. A. Smith and R. L. Lowe. 1999 Nutrient Limitation of Algal Biomass Accrual in Streams: Seasonal Patterns and a Comparison of Methods, *Journal of the North American Benthological Society,* 18(2): 242-260.

Frost, P.C., Benstead, J.P., Cross, W.F., Hillebrand, H., Larson, J.H., Xenopoulos, M.A. and Yoshida, T. 2006 Threshold elemental ratios of carbon and phosphorus in aquatic consumers, *Ecology Letters*, 9, 774–779.

Gage, M. 1980 *Ledgends in the Rocks: An Outline of New Zealand Geology*, Whitcoulls Publishers, Christchurch, New Zealand.

German, D. P., M. N. Weintraub, S. A. Grandy, C. L. Lauber, Z. L. Rinkes and S. D. Allison. 2011 Optimization of hydrolytic and oxidative enzyme methods for ecosystem studies, *Soil Biology & Biochemistry,* 43: 1387-1397.

Gibson, C. E. 1979 Nutrient Limitation, *Water Pollution Control Federation,* 43(12): 2436-2440.

Gorman, P. 25/10/2011 *Silt chokes Christchurch rivers*, http://www.stuff.co.nz/environment/5843796/Silt-chokes-Christchurch-rivers, Retrieved 10/01/2013.

Greenwood, J. L., A. D. Rosemond, J. B. Wallace, W. F. Cross and H. S. Weyers. 2007 Nutrients stimulate leaf breakdown rates and detritivore biomass: bottom-up effects via heterotrophic pathways, *Oecologia,* 151(4): 637-649.

Grimm, N. B. and S. G. Fisher. 1986 Nitrogen Limitation in a Sonoran Desert Stream, *Journal of the North American Benthological Society,* 5(1): 2-15.

Gucker, B. and M. T. Pusch. 2006 Regulation of nutrient uptake in eutrophic lowland streams, *Journal of Limnology and Oceanography,* 51(3): 1443-1453.

Hansson, L. A. 1989 The influence of a periphytic biolayer on phosphorus exchange between substrate and water, *Archiv für Hydrobiologie,* 115: 21-26.

Harbott, E. L. and M. R. Grace. 2005 Extracellular enzyme response to bioavailability of dissolved organic C in streams of varying catchment urbanization, *Journal of North America Benthological Society,* 24(3): 588-601.

Harding, J. S., J. E. Clapcott, J. M. Quinn, J. W. Hayes, M. K. Joy, R. G. Storey, H. S. Greig, J. Hay, T. James, M. A. Beech, R. Ozane, A. S. Meredith and I. K. G. Boothroyd. 2009 *Stream Habitat Assessment Protocols for wadeable rivers and streams of New Zealand*, School of Biological Sciences, University of Canterbury, Christchurch, New Zealand.

Harding, J. S. and M. J. Winterbourn. 1997 *New Zealand ecoregions a classification for use in stream conservation and management*, Department of Conservation Technical Series number 11, Department of Conservation, Wellington, New Zealand.

Hill, B. H., C. M. Elonen, A. M. Cotter, A. S. Trebitz and P. Danz. 2006 Sediment microbial enzyme activity as an indicator of nutrient limitation in Great Lakes coastal wetlands, *Freshwater Biology,* 51: 1670-1683.

Hill, B. H., C. M. Elonen, T. M. Jicha, D. W. Bolgrein and M. F. Moffett. 2010a Sediment microbial enzyme activity as an indicator of nutrient limitation in the great rivers of the Upper Mississippi River basin, *Biogeochemistry,* 97: 195-209.

Hill, B. H., C. M. Elonen, L. R. Seifert, A. A. May and E. Tarquinio. 2012 Microbial enzyme stoichiometry and nutrient limitation in US streams and rivers, *Ecological Indicators,* 18: 540-551.

Hill, B. H., F. H. McCormick, B. C. Harvey, S. L. Johnson, M. L. Warren and C. M. Elonen. 2010b Microbial enzyme activity, nutrient uptake and nutrient limitation in forested streams, *Freshwater Biology,* 55: 1005-1019.

Hill, W. R., P. J. Mulholland and E. R. Marzolf. 2001 Stream ecosystem responses to forest leaf emergence in spring, *Ecology*, 82: 2306–2319.

Hill W. R. 1996 Effects of light, in Stevenson, R.J., Bothwell, M. L. and Lowe, R.L. eds. *Algal Ecology*, Academic Press, California.

Hilton, J., M. O'Hare, M. J. Bowes and I. Jones. 2006 How green is my river? A new paradigm of eutrophication in rivers, *Science of the Total Environment,* 365: 66-83.

Hoellein, T. J., C. P. Arango and Y. Zak. 2011a Spatial variability in nutrient concentration and biofilm nutrient limitation in an urban watershed, *Biogeochemistry,* 106: 265-280.

Hoellein, T. J., J. L. Tank, S. A. Entrekin, E. Rosi-Marshall, M. L. Stephen and G. A. Lamberti. 2011b Effects of Benthic Habitat Restoration on Nutrient Uptake and Ecosystem Metabolism in Three Headwater Streams, *River Research and Applications,* 28(9): 1451-1461.

Hoellein, T. J., J. L. Tank, J. J. Kelly and E. Rosi-Marshall. 2010 Seasonal variation in nutrient limitation of microbial biofilms colonizing organic and inorganic substrata in streams, *Hydrobiologia,* 649: 331-345.

Hoellein, T. J., J. L. Tank, E. J. Rosi-Marshall and S. A. Entrekin. 2009 Temporal variation in substratum-specific rates of N uptake and metabolism and their contribution at the stream-reach scale, *Journal of the North American Benthological Society,* 28(2): 305-318.

Howarth, R. W. and R. Marino. 2006 Nitrogen as the limiting nutrient for eutrophication in coastal marine ecosystems: Evolving views over three decades, *Limnology and Oceanography,* 51(1/2): 364-376.

Hudson, J. C. and N. Rutherford. 2011 *Effects of wastewater overflows on oxygen and ammonia in the Avon and Heathcote Rivers*, Prepared for Environment Canterbury, Christchurch, New Zealand.

Humphris, A. 10/07/2013 Streets and lighting - *Building and funding streets,* http://www.TeAra.govt.nz/en/photograph/24476/tarsealing-1925*,* Retrieved 04/08/2013.

James, A. and S. McMurtrie. 2011 *Christchurch February earthquake - Effect on freshwater fish of the upper Avon River*, EOS Ecology, Christchurch, New Zealand.

Jinyu, L. 2009 *Relationship between in-stream ecology and urbanization,* unpublished Master of Science thesis, The University of Auckland, Auckland, New Zealand.

Johnson, L. T., J. L. Tank and W. K. Dodds. 2009a The influence of land use on stream biofilm nutrient limitation across eight North American ecoregions, *Canadian Journal of Fisheries and Aquatic Sciences,* 66: 1081-1094.

Johnson, L. T., Tank, J.L., Arango, C.P. 2009b The effect of land use on dissolved organic carbon and nitrogen uptake in streams, *Freshwater Biology,* 54: 2335-2350.

Keck, F. and F. Lepori. 2012 Can we predict nutrient limitation in streams and rivers?, *Freshwater Biology,* 27: 1410-1421.

Kemp, M. J. and W. K. Dodds. 2002 The influence of ammonium, nitrate, and dissolved oxygen concentrations on uptake, nitrification, and denitrification rates associated with prairie stream substrata, *Limnology and Oceanography,* 47: 1380-1393.

Kirchman, D. L. 2012 *Processes in Microbial Ecology*.Oxford University Press, New York.

Klotz, R. L. 1985 Factors controlling phosphorus limitation in stream sediments, *Limnology and Oceanography,* 30: 543-553.

Larned, S. T., V. I. Nikora and J. F. Biggs. 2004 Mass transfer-limited nitrogen and phosphorus uptake by stream periphyton: a conceptual model and experimental Evidence, *Limnology and Oceanography,* 49(6): 1992-2000.

Larned, S. T., M. Scarsbrook, T. H. Snelder and B. J. Biggs. 2004 Water quality in low-elevation streams and rivers of New Zealand: recent state and trends in contrasting land-cover classes, *New Zealand Journal of Marine and Freshwater Research,* 38: 347-366.

Law, J. 19/04/2013 *A Look Back at Summer*, http://blog.metservice.com/2013/04/a-look-back-at-Summer/, Retrieved 20/05/2013.

Lear, G., A. Dopheide, P. Ancion, K. Roberts, V. Washington, J. K. Smith and G. Lewis. 2012 *Biofilms in Freshwater: Their Importance for the Maintenance and Monitoring of Freshwater Health*, Caister Academic Press, Norfolk, UK.

Lear, G. and G. D. Lewis. 2009 Impact of catchment land use on bacterial communities within stream biofilms, *Ecological Indicators,* 9: 848-855.

Lehto, L. L. P. and B. H. Hill. 2013 The effect of catchment urbanization on nutrient uptake and biofilm enzyme activity in Lake Superior (USA) tributary streams, *Hydrobiologia,* 713: 35-51.

Leopold, L. M., M. G. Wolman and J. R. Miller. 1964 *Fluvial processes in geomorphology*, San Francisco, California.

Lohman, K., J. R. Jones and C. Baysinger-Daniel. 1991 Experimental Evidence for Nitrogen Limitation in a Northern Ozark Stream, *Journal of the North American Benthological Society,* 10(1): 14-23.

Marcarelli, A. M., H. A. Bechtold, A. T. Rugenski and R. S. Inouye. 2009 Nutrient limitation of biofilm biomass and metabolism in the Upper Snake River basin, southeast Idaho, USA, *Hydrobiologia,* 620: 63-76.

Marcarelli, A. M. and W. A. Wurstsbaugh. 2006 Temperature and nutrient supply interact to control nitrogen fixation in oligotrophic streams: An experimental examination *Limnology and Oceanography,* 51(5): 2279.

Marcarelli, A. M. and W. A. Wurstsbaugh. 2007 Effects of upstream lakes and nutrient limitation on periphytic biomass and nitrogen fixation in oligotrophic, subalpine streams, *Freshwater Biology,* 52: 2211-2255.

McDowell, R. W., S. T. Larned and D. J. Houlbrooke. 2009 Nitrogen and phosphorus in New Zealand streams and rivers: Control and impact of eutrophication and the influence of land management, *New Zealand Journal of Marine and Freshwater Research,* 43(4): 985-995.

Meyer, J. L., M. J. Paul and K. W. Taulbee. 2005 Stream Ecosystem Function in Urbanizing Landscapes, *Journal of North American Benthological Society,* 24(3): 602-612.

Mineau, M. M., F. R. Fatemi, I. J. Fernandez and K. S. Simon. 2013b Microbial enzyme activity at the watershed scale: response to chronic nitrogen deposition and acute phosphorus enrichment, *Biogeochemistry,* DOI 10.1007/s10533-013-9869-6.

Mineau, M. M., C. M. Rigsby, D. Ely, I. J. Fernandez, S. A. Norton, T. Ohno, M. H. Vallet and K. S. Simon. 2013a Chronic catchment nitrogen enrichment and stoichiometric constraints on the bioavailability of dissolved organic matter from leaf leachate, *Freshwater Biology,* 58: 248-260.

Ministry for the Environment. 2007 *Environment New Zealand, 2007*, Ministry for the Environment, Wellington, New Zealand.

Montuelle, B. and B. Volat. 1998 Impact of Wastewater Treatment Plant Discharge on Enzyme Activity in Freshwater Sediments, *Ecotoxicology and Environmental Safety,* 40: 154-159.

Morse, C. C., A. D. Huryn and C. Cronan. 2003 Impervious Surface Area as a Predictor of the Effects of Urbanization on Stream Insect Comminities in Maine, U.S.A., *Environmental Monitoring and Assessment,* 89: 95-127.

Mosisch, T. D., S. E. Bunn, P. M. Davies, and C. J. Marshall. 1999 Effects of shade and nutrient manipulation on periphyton growth in a subtropical stream, *Aquatic Botany*, 64: 167–177.

Mulholland, P. J. 1996 Role in Nutrient Cycling in Streams. In: *Algal Ecology: Freshwater Benthic Ecosystems*. R. J. Stevenson, Bothwell, M.L. and Lowe, R.L. eds. San Diego, Academic Press.

Neale, M. W. 2012 *State of the Environment Monitoring: River Water Quality Annual Report 2010, Auckland Council Technical Report 2012/006*, Auckland, New Zealand.

Newall, P. and C. J. Walsh. 2005 Response of epilithic diatom assemblages to urbanization influences, *Hydrobiologia,* 532: 53-67.

Niyogi, D. K., K. S. Simon and C. R. Townsend. 2003 Breakdown of tussock grass in streams along a gradient of agricultural development in New Zealand, *Freshwater Biology,* 48(9): 1698-1708.

O'Brien, P. J. and J. D. Wehr. 2010 Periphyton biomass and ecological stoichiometry in streams within an urban to rural land-use gradient, *Hydrobiologia,* 657: 89-105.

Olander, L. P. and P. M. Vituosek. 2000 Regulation of soil phosphatase and chitinase activity by N and P availability, *Biogeochemistry,* 49: 175-190.

Paul, M. J. and J. L. Meyer. 2001 Streams in the Urban Landscape, *Annual Review of Ecological Systems,* 32: 333-365.

Peterson, B. J., L. Deegan, J. Helfrich, J. E. Hobbie, M. Hullar, B. Moller, T. E. Ford, A. Hershey, A. Hiltner, G. Kipphut, M. A. Lock, D. M. Fiebig, V. McKinley, M. C. Miller, R. J. Vestal, R. Ventullo and G. Volk. 1993 Biological Responses of a Tundra River to Fertilization, *Ecology,* 74(3): 653-672.

Piddock, G. 14/06/2013 *Dairy sector must show 'human side'*, http://www.stuff.co.nz/business/farming/fieldays/8792646/Dairy-sector-must-show-human-side, Retrieved 10/08/2013.

Postel, S. and S. Carpenter. 1997 *Freshwater Ecosystem Services*, Island Press, Washington D.C.

Potapova, M. and D. F. Charles. 2003 Distribution of benthic diatoms in U.S. rivers in relation to conductivity and ionic composition, *Freshwater Biology,* 48: 1311-1328.

Quinn, J. M., B. A. Cooper, R. J. Davies-Colley, C. J. Rutherford and R. B. Williamson. 1997 Land use effects on habitat, water quality, periphyton, and benthic invertebrates in Waikato, New Zealand, hill country streams, *New Zealand Journal of Marine and Freshwater Research,* 31: 579-597.

Quinn, J. M. and C. W. Hickey. 1990 The magnitude of the effects of substrate particle size, recent flooding, and catchment development on benthic invertebrates in 88 New Zealand Rivers, *New Zealand Journal of Marine and Freshwater Research,* 24: 411-427.

Quinn, J. M. and M. J. Stroud. 2002 Water quality and sediment and nutrient export from New Zealand hill-land catchments of contrasting land use, *New Zealand Journal of Marine and Freshwater Research,* 36: 409-429.

Rasband, W. S. 2012 *Image J 1997-2012*, U. S. National Institutes of Health, Maryland, U.S.A.

Redfield, A. C. 1958 The biological control of chemical factors in the environment, *American Scientist,* 46: 205-221.

Rier, S. T., K. A. Kuhn and S. N. Francoeur. 2007 Algal regulation of extracellular enzyme activity in stream microbial communities associated with inert substrata and detritus, *Journal of the North American Benthological Society,* 26(3): 439-449.

Rier, S. T., K. S. Nawrocki and J. C. Whitley. 2011 Response of biofilm extracellular enzymes along a stream nutrient enrichment gradient in an agricultural region of north central Pennsylvania, USA, *Hydrobiologia,* 669: 119-131.

Romani, A. M., J. Artigas and I. Ylla. 2012 *Extracellular Enzymes in Aquatic Biofilms: Microbial Interactions versus Water Quality Effects in the Use of Organic Matter*, Caister Academic Press, Norfolk, UK.

Romani, A. M., A. Butturini, F. Sabater and S. sabater. 1998 Heterotrophic metabolism in a forest stream sediment: surface versus subsurface zones, *Aquatic Microbial Ecology,* 16: 143-151.

Romani, A. M., A. A. Giorgi and S. Sabater. 2004 The influence of substratum type and nutrient supply on biofilm oranic matter utilization in streams, *Limnology and Oceanography,* 49: 1713-1721.

Romani, A. M. and S. Sabater. 1999 Effect of primary producers on the heterotrophic metabolism of a stream biofilm, *Freshwater Biology,* 41: 729-736.

Rutherford, J. C. and N. Hudson. 2011 *Effects of wastewater overflows on oxygen and ammonia in the Avon and Heathcote Rivers*, NIWA, Christchurch, New Zealand.

Sardans, J., A. Rivas-Ubach and J. Penuelas. 2012 The elemental stoichiometry of aquatic and terrestrial ecosystems and its relationships with organismic lifestyle and ecosystem structure and function: a review and perspectives, *Biogeochemistry,* 111: 1-39.

Scarsbrook, M. 2006 *State and trends in the National River Water Quality Network (1989-2005), Prepared for Ministry for the Environment*, NIWA Client report HAM2006-131.

Scarsbrook, M., M. Mittinty and S. Wadhwa. 2007 *An assessment of Regional Council water quality data (1996-2002) in support of national SoE reporting (ENZ07), Prepared for Ministry for the Environment*, NIWA Client report HAM2007- 045.

Schade, J. D., K. Macneill, S. A. Thomas, F. C. McNeely, J. R. Welter, J. Hood, M. Goodrich, M. E. Power and J. C. Finlay. 2011 The stoichiometry of nitrogen and phosphorus spiralling in heterotrophic and autotrophic streams, *Freshwater Biology* 56: 424-436.

Schindler, D. W. 1977 Evolution of Phosphorus Limitation in Lakes, *Science,* 195(4275): 260-262.

Schindler, D. W. and E. J. Fee. 1974 Experimental Lakes Area: Whole-Lake Experiments in Eutrophication, *Journal of the Fisheries Research Board of Canada,* 31(5): 937-953.

Schindler, D. W., E. J. Fee and T. Ruszcynski. 1978 Phosphorus Input and Its Consequences for Phytoplankton Standing Crop and Production in the Experimental Lakes Area and in Similar Lakes, *Journal of the Fisheries Research Board of Canada,* 35: 190-196.

Schwartz, J. S., A. Simon and L. Klimetz. 2009 *Developing Linkages between Biological Impairment and Stream Siltation: Case Study in the Northern Great Plains Ecoregion*, World Environmental and Water Resources Congress 2009: 1-11.

Scott, T. S., D. A. Lang, R. S. King and R. D. Doyle. 2009 Nitrogen fixation and phosphatase activity in periphyton growing on nutrient diffusing substrata: evidence for differential nutrient limitation in stream periphyton, *Journal of the North American Benthological Society,* 28(1): 57-68.

Searle, E. J. 1981 *City of Vocanoes: A Geology of Auckland*, Longman Paul Limited, Auckland, New Zealand.

Sharpe, M. 26/09/2012 *Farmers to fight limit on nitrogen*, http://www.stuff.co.nz/business/farming/7728590/Farmers-to-fight-limit-on-nitrogen, Retrieved 20/08/2013.

Simon, K. S., M. A. Simon and E. F. Benfield. 2009 Variation in ecosystem function in Appalachian streams along an acidity gradient, *Ecological Applications,* 19(5): 1147-1160.

Sinsabaugh, R. L. 2009 *Fluorescence-Based Protocol Using MUB & MUC-Linked Substrates*. HYDROLASE ASSAYS WITH COUMARIN SUBSTRATES, Center for Dead Plant Studies, University of Toledo.

Sinsabaugh, R. L. and S. Findlay. 1995 Microbial Production, Enzyme Activity, and Carbon Turnover in Surface Sediments of the Hudson River Estuary, *Microbial Ecology,* 30(2): 127-141.

Sinsabaugh, R. L., S. Findlay, P. Franchini and D. Fischer. 1997 Enzymatic analysis of riverine bacterioplankton production, *Limnology and Oceanography,* 41(1): 29-38.

Sinsabaugh, R. L. and C. M. Foreman. 2001 Activity profiles of bacterioplankton in a eutrophic river, *Freshwater Biology,* 46: 1239-1249.

Sinsabaugh, R. L., B. H. Hill and J. J. F. Shah. 2009 Ecoenzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment, *Nature Letters,* 462(10): 795-798.

Sinsabaugh, R. L., C. L. Lauber, M. N. Weintraub, B. Ahmed, S. D. Allison, C. Crenshaw, A. R. Contosta, D. Cusack, S. Frey, M. E. Gallo, T. B. Gartner, S. E. Hobbie, K. Holland, B. L. Keeler, J. S. Powers, M. Stursova, C. Takacs-Vesbach, M. P. Waldrop, M. D. Wallenstein, D. R. Zak and L. H. Zeglin. 2008 Stoichiometry of soil enzyme activity at global scale, *Ecology Letters,* 11: 1252-1264.

Sinsabaugh, R. L. and D. L. Moorhead. 1994 Resource allocation to extracellular enzyme production: a model for nitrogen and phosphorus control of litter decomposition, *Soil Biology & Biochemistry,* 26: 1305-1311.

Sinsabaugh, R. L. and J. J. F. Shah. 2012 Ecoenzymatic Stoichiometry and Ecological Theory, *Annual Review of Ecological Systems,* 43: 313-343.

Sinsabaugh, R. L., D. J. Van Horn, J. J. Follstad and S. Findlay. 2010 Ecoenzymatic Stoichiometry in Relation to Productivity for Freshwater Biofilm and Plankton Communities, *Microbial Ecology,* 60: 885-893.

Statistics New Zealand, 2009 *Mapping Trends in the Auckland Region*, Statistics New Zealand, Wellington, New Zealand.

Sterner, R.W. and Elser, J.J. 2002 *Ecological Stoichiometry: The Biology of Elements from Molecules to the Biosphere*, Princeton University Press, New Jersey.

Stelzer, R.S., Heffernan, J., and Likens, G.E. 2003 The influence of dissolved nutrients and particulate organic matter quality on microbial respiration and biomass in a forest stream, *Freshwater Biology*, 48(11): 1925–1937.

Stoddard, J. L., D. P. Larsen, C. P. Hawkins, R. K. Johnson and R. H. Norris. 2006 Setting Expectations for the Ecological Condition of Streams: The Concept of Reference Condition, *Ecological Applications,* 16(4): 1267-1276.

Stream Solute Workshop, 1990 Concepts and methods for assessing solute dynamics in stream ecosystems, *Journal of the North American Benthological Society,* 9: 95-119.

Tank, J. and W. K. Dodds. 2003 Nutrient limitation of epilithic and epixylic biofilms in ten North American streams, *Freshwater Biology,* 48: 1031-1049.

Tank, J. L., M. J. Bernot and E. J. Rosi-Marshall. 2006 Nitrogen limitation and uptake. In: *Methods in stream ecology*, Hauer, F.R. and Lamberti.G.A. eds. New York, Elsevier: 213-238.

Tank, J. L. and W. K. Dodds. 2003 Nutrient limitation of epilithic and epixylic biofilms in ten North American streams, *Freshwater Biology,* 48: 1031-1049.

Tank, J. L., E. J. Rosi-Marshall, M. A. Baker and R. O. Hall. 2008 Are Rivers Just Big Streams? A Pulse Method to Quantify Nitrogen Demand in a Large River, *Ecology,* 89(10): 2935-2945.

Tank, J. L. and J. R. Webster. 1998 Interaction of Substrate and Nutrient Availability on Wood Biofilm Processes in Streams, *Ecology,* 76(6): 2168-2179.

Tank, J. L. and M. J. Winterbourn. 1996 Microbial activity and invertebrate colonisation of wood in a New Zealand forest stream, *New Zealand Journal of Marine and Freshwater Research,* 30: 271-280.

Taulbee, K. W., S. D. Cooper and J. M. Melack. 2005 Effects of nutrient enrichment on algal biomass across a natural light gradient, *Archiv für Hydrobiologie,* 164(4): 449-464.

Taylor, M. and W. Blair. 2011 *Effects of Seismic Activity on Inaka spawning grounds on City Rivers*, Aquatic Ecology Ltd., Prepared for: Christchurch City Council and Environment Canterbury, Christchurch, New Zealand.

The Royal Society of New Zealand, 2012 *The Canterbury Earthquakes: Scientific answers to critical questions*, The Royal Society of New Zealand, Chritchurch, New Zealand.

Tiquia, S. M. 2011 Extracellular Hydrolytic Enzyme Activities of the Heterotrophic Microbial Communities of the Rouge River: An Approach to Evaluate Ecosystem Response to Urbanization, *Microbial Ecology,* 62: 679-689.

Van Horn, D. J., R. L. Sinsabaugh, K. R. Takacs-Vesback and C. N. Dahm. 2011 Response of heterotrophic stream biofilm communities to a gradient of resources, *Aquatic Microbial Ecology,* 64: 149-161.

Vinten, A. J. A., R. R. E. Artz, N. Thomas, J. M. Potts, L. Avery, S. J. Langan, H. Watson, Y. Cook, C. Taylor, C. Abel, E. Reid and B. K. Singh. 2011 Comparison of microbial community assays for the assessment of stream biofilm ecology, *Journal of Microbiological Methods,* 85: 190-198.

Von Schiller, D., E. Marti, J. L. Riera and F. Sabater. 2007 Effects of nutrients and light on periphyton biomass and nitrogen uptake in Mediterranean streams with contrasting land uses, *Freshwater Biology,* 52(5): 891-906.

Waikato Regional Council. 1991 *Pukekohe / Waiuku Natural Resources Issues: Discussion Paper*, Waikato Regional Council, Waikato, New Zealand.

Walsh, C. J. 2000 Urban impacts on the ecology of receiving waters: a framework for assessment, conservation and restoration, *Hydrobiologia,* 431: 107-114.

Walsh, C. J., A. H. Roy, J. W. Feminella, P. M. Groffman and R. P. Morganm. 2005 The urban stream syndrome: current knowledge and the search for a cure, *Journal of North America Benthological Society,* 24(3): 806-723.

Weekes, J. 14/10/2012 *'Vile' storm hits country*, http://www.nzherald.co.nz/nz/news/article.cfm?c_id=1&objectid=10840401, Retrieved 10/02/2013.

Wells, N. S., T. J. Clough, L. M. Condron, T. W. Baisden, J. S. Harding, G. D. Lewis and G. Lear. 2013 Biogeochemistry and community ecology in a Spring-fed urban river following a major earthquake, *Environmental Pollution,* 182: 190-200.

Wenger, S. J., A. H. Roy, R. C. Jackson, E. S. Bernhardt, T. L. Carter, S. Filoso, C. A. Gibson, W. C. Hession, S. S. Kaushal, M. Martí, J. L. Meyer, M. A. Palmer, M. J. Paul, A. H. Purcell, A. Ramírez, A. D. Rosemond, K. A. Schofield, E. B. Sudduth and C. J. Walsh. 2009 Twenty-six key research questions in urban stream ecology: an assessment of the state of the science, *Journal of the North American Benthological Society,* 28(4): 1080-1098.

Wilczek, S., H. Fisher and M. T. Pusch. 2005 Regulation and seasonal dynamics of extracellular enzyme activities in the sediments of a large lowland river, *Microbial Ecology,* 50: 253-267.

Williams, C. J., A. B. Scott, H. F. Wilson and M. A. Xenopolose. 2012 Effects of land use on water column bacterial activity and enzyme stoichiometry in stream ecosystems, *Aquatic Sciences,* 74: 483-494.

Williams, C. J., Y. Yamashita, H. F. Wilson, R. Jaffe and M. A. Xenopolose. 2010 Unraveling the role of land use and microbial activity in shaping dissolved organic matter characteristics in stream ecosystems, *Limnology and Oceanography,* 55(3): 1159-1171.

Winterbourn, M. J. 1990 Interactions among nutrients, algae and invertebrates in a New Zealand mountain stream, *Freshwater Biology,* 23(3): 463-474.

Withers, P. J. A. and H. P. Jarvie. 2008 Delivery and cycling of phosphorus in rivers: A review, *Science of the Total Environment,* 400: 379-395.

Wood, P. and P. D. Armitage. 1997 Biological Effects of Fine Sediment in the Lotic Environment, *Environmental Management,* 21(2): 203-217.

Wright, A. L. and K. R. Reddy. 2001 Phosphorus Loading Effects on Extracellular Enzyme Activity in Everglades Wetland Soils, *Soil Science Society of America Journal,* 65: 588-595.

APPENDIX A

Appendix A. 1. Summary of Auckland habitat assessment data, collected over Summer immediately following NDS incubation. Sites are coded by land-use category, reference (REF), native (N), urban (U), suburban (SU), pastoral (P).

Site Name	Average Stream width (m)	Average Stream Depth (m)	Sediment Classification (Φ)	Average Discharge (L/s)	Riparian Score 1(low)-5(high)
Cascades Stream (REF, N)	5.2(0.3)	0.2(0.0)	-5.2(0.3)	89.3(19)	4.6
Wairoa Tributary (REF, N)	2.3(0.2)	0.1(0.0)	-5.0(0.4)	29(16)	4.6
West Hoe (REF, N)	1.0(0.1)	0.1(0.0)	2.0(0.0)	0.3(0.3)	4.4
Otaki Creek (U)	2.3(0.2)	0.2(0.1)	3.0(0.4)	0.6(0.1)	2.2
Oakley Creek (U)	1.9(0.2)	0.2(0.0)	-2.8(0.5)	16.9(3)	3.0
Pakuranga Creek (U)	2.1(0.2)	0.3(0.1)	-1.0(0.6)	6.9(3.8)	2.9
Lucas Creek (SU)	2.1(0.3)	0.2(0.0)	-3.4(0.6)	5.7(1.3)	2.5
Otara Creek (SU)	2.6(0.1)	0.3(0.0)	-1.9(0.7)	11.4(2)	2.3
Puhinui Stream (SU)	1.9(0.1)	0.1(0.0)	-3.6(0.5)	2.4(0.4)	3.9
Kaukapakapa River (P)	4.3(0.9)	0.3(0.03)	2.6(0.4)	2.4(0.6)	1.5
Ngakaroa Stream (P)	1.4(0.4)	0.2(0.0)	-1.3(0.8)	237.0(234)	1.8
Whangamarie Stream (P)	3.2(0.18)	0.3(0.1)	7.0(0.0)	30.0(10)	1.8

Appendix A. 2. Summary of Christchurch habitat assessment data, collected over Summer during NDS incubation. (Steamwharf Stream was hard bottomed before quake). Sites are coded by land-use category, reference (REF), rural-suburban (R-SU), urban-wetland (UW), and urban (U).

Site Name	Average Stream width (m)	Sediment Classification (Φ)	Average Stream Depth (m)	Average Discharge (L/s)	Riparian Score: 1(low)-5(high)
Styx River Upper (REF, R-SU)	2.3(0.4)	3.6(0.4)	0.46(0.02)	116.9(4)	3.0
Smacks creek (REF, R- SU)	2.2(0.1)	-1.8(0.3)	0.10(0.03)	85.1(10)	3.9
Crosers Stream (UW)	2.7(0.07)	4.0(0.0)	0.56(0.03)	334.1(10)	3.0
Papanui Stream (U)	2.2(0.2)	-1.0(0.4)	0.15(0.03)	109.2(18)	2.9
Shirley Stream (U)	2.5(0.1)	3.0(0.0)	0.15(0.03)	64.4(7)	2.5
Okeover Stream (U)	2.6(0.2)	-1.8(0.3)	0.09(0.01)	106.7(7)	2.7
St Albans Stream (U)	1.8(0.1)	2.5(0.1)	0.11(0.03)	32.75(3)	3.0
Steamwharf Stream (U)	2.5(0.2)	3.7(0.2)	0.13(0.03)	56.8(8)	2.3
Upper Avon River (U)	3.8(0.1)	-0.5(0.3)	0.20(0.04)	131.4(22)	2.6
Upper Heathcote River (U)	2.8(0.1)	-1.0(0.3)	0.16(0.03)	91.8(16)	2.3
Waimairi Stream Tributary (U)	2.3(0.2)	-0.6(0.2)	0.07(0.02)	103.0(3)	2.2
Wairarapa Stream (U)	2.4(1.0)	1.5(0.3)	0.15(0.01)	84.2(7)	2.2

Appendix A. 3. Response ratios across Auckland sites in Spring and Summer for both respiration (organic) and chlorophyll *a* (inorganic). Sites marked with n/a indicate missing data, due to loss of diffusers in streams.

Site	Season	Respiration				Chlorophyll *a*			
		RR_N	RR_P	RR_{NP}	RR_N:RR_P	RR_N	RR_P	RR_{NP}	RR_N:RR_P
Cascades Stream	Spring	0.34	-0.02	0.66	-21.53	0.68	0.65	0.16	1.05
	Summer	0.10	-0.02	0.41	-4.68	1.36	-0.06	0.05	-23.86
Kaukapakapa River	Spring	-0.68	-0.65	-0.50	1.04	-0.18	-0.56	-0.12	0.32
	Summer	-0.24	-0.43	-0.05	0.56	-0.02	-0.30	0.36	0.06

Lucas Creek	Spring	-0.01	0.02	0.31	-0.50	1.18	-1.07	0.73	-1.10
	Summer	-0.01	-0.09	0.36	0.07	0.50	-0.01	-0.06	-45.16
Ngakaroa Stream	Spring	-0.17	0.52	0.40	-0.32	0.43	-0.24	0.83	-1.82
	Summer	-0.01	0.12	0.30	-0.10	-0.55	0.10	-0.01	-5.66
Oakley Creek	Spring	n/a				n/a			
	Summer	0.03	0.14	0.23	0.19	-0.21	0.18	-0.10	-1.20
Otaki Creek	Spring	0.01	0.04	0.06	0.18	0.15	0.06	0.39	2.43
	Summer	-0.29	-0.15	-0.58	1.86	0.20	-0.37	-0.27	-0.53
Otara Creek	Spring	n/a				0.54	-0.25	-0.20	-2.19
	Summer	-0.01	0.00	0.20	7.42	-0.61	-0.77	0.16	0.80
Pakuranga Stream	Spring	0.02	0.42	0.77	0.05	-0.49	-0.22	-0.23	2.24
	Summer	-0.04	0.10	0.25	-0.41	-0.28	-0.68	0.11	0.41
Puhinui Stream	Spring	0.03	0.35	0.72	0.08	0.17	-0.49	0.01	-0.35
	Summer	-0.07	-0.04	0.11	1.80	-0.64	0.03	-0.33	-21.55
Wairoa Tributary	Spring	0.21	-0.06	0.30	-3.79	0.00	-0.08	0.38	-0.03
	Summer	0.15	0.03	0.41	5.62	0.79	n/a	-0.47	n/a
West Hoe Stream	Spring	0.14	-0.06	0.66	-2.15	0.42	0.12	-0.03	3.39
	Summer	0.23	-0.01	0.49	-30.12	0.03	0.87	0.24	0.04
Whangamarie Stream	Spring	-0.06	0.47	0.33	-0.12	1.22	1.31	0.79	0.94
	Summer	-0.08	0.32	0.32	-0.25	0.38	0.00	-0.32	-188.61

Appendix A. 4. Response ratios in Christchurch Summer sites for both respiration (organic) and chlorophyll *a* (inorganic).

Site	Respiration				Chlorophyll *a*			
	RR_N	RR_P	RR_{NP}	RR_N:RR_P	RR_N	RR_P	RR_{NP}	RR_N:RR_P
Crosers Stream	-0.17	-0.08	0.03	2.23	-0.30	-1.32	-0.65	0.23
Okeover Stream	0.08	0.28	0.31	0.29	0.25	-0.83	0.07	-0.30
Papanui Stream	-0.13	0.11	0.33	-1.25	-0.27	-0.61	-0.51	0.44
Shirely Stream	-0.19	-0.11	-0.01	1.73	0.43	-0.28	0.58	-1.54
Smacks Creek	-0.05	0.09	0.24	-0.56	0.54	0.06	-0.70	8.73
St. Albans Stream	-0.11	-0.05	-0.02	1.98	-0.04	-0.67	0.31	0.05
Steamwharf Stream	-0.16	-0.22	0.02	0.71	1.29	0.78	1.44	1.67
Styx River Upper	-0.05	0.24	0.29	-0.20	0.57	1.25	-0.05	0.45
Upper Avon River	0.01	0.15	0.23	0.09	-0.40	-0.48	-0.25	0.83
Upper Heathcote River	-0.09	-0.30	-0.05	0.30	0.20	-0.42	0.38	-0.49
Waimairi Stream	-0.01	0.17	0.17	-0.04	-0.23	-0.80	-0.49	0.28
Wairapapa Stream	-0.18	0.14	0.25	-1.30	-0.10	-0.70	0.31	0.14

APPENDIX B

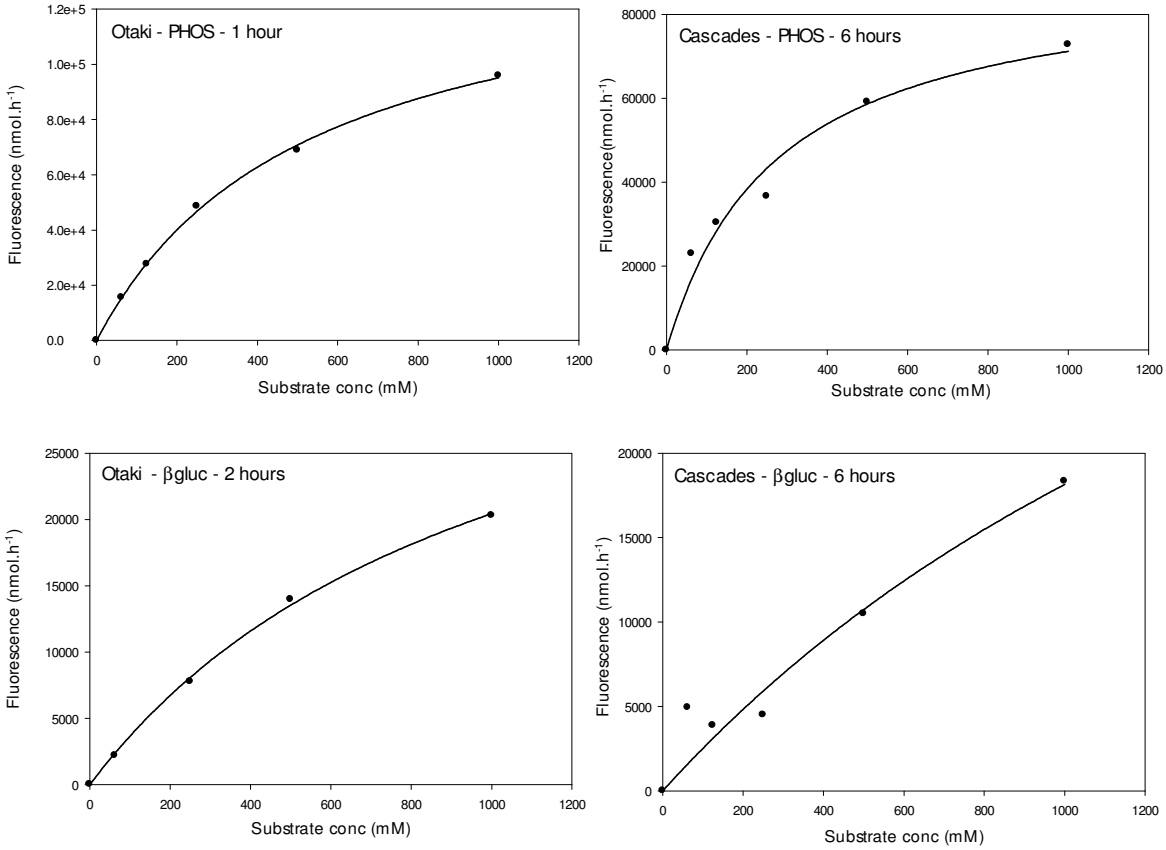
Appendix B. 1. Auckland site and catchment characteristics, land-use was derived from the LUCAS database and additional site descriptors from the REC database, GPS co-ordinates were recorded during site visits.

Site Name	Land use description	Land use						Catchment Area (ha)	Stream Order	Distance to coast (km)	Elevation (m)	GPS (NZMG)	
		% Urban	% Native	% Pastoral	% Exotic	% Horticulture	% Other					Northing	Easting
Cascades Stream	Native	0.0	100.0	0.0	0.0	0.0	0.0	1390	3	13.5	125.6	6478297	2645984
Hoteo River	Native/ Pastoral	0.3	23.3	53.4	22.9	0.2	0.0	26728	5	15.8	21.3	6534238	2645689
Kaukapakapa River	Pastoral	5.0	12.4	75.0	6.1	1.1	0.4	6163	1	12.0	17.2	6506757	2646355
Kumeu Stream	Pastoral	1.9	11.2	76.2	5.6	4.9	0.1	4568	4	40.9	30.0	6490524	2649724
Lower Tamaki River	Urban	98.1	1.9	0.0	0.0	0.0	0.0	125	1	2.0	27.4	6481185	2676984
Lucas Creek	Suburban	60.8	14.5	23.0	1.7	0.0	0.0	609	3	2.7	19.9	6496265	2661870
Mahurangi River	Native/ Pastoral	1.7	28.3	44.1	25.4	0.5	0.0	4805	4	9.5	49.1	6532171	2659385
Makarau Stream	Pastoral	0.0	19.3	74.5	5.7	0.0	0.5	2137	5	4.4	17.3	6514693	2646607
Marawhara Stream	Native	0.0	99.5	0.5	0.0	0.0	0.0	382	1	0.5	13.0	6472278	2641032
Meola Creek	Urban	99.0	0.5	0.0	0.0	0.0	0.5	1474	3	0.7	9.6	6480315	2663624
Motions Stream	Urban	96.0	4.0	0.0	0.0	0.0	0.0	350	2	1.4	15.7	6480878	2663696
Ngakaroa Stream	Pastoral	0.0	1.8	63.3	3.6	27.8	3.5	449	2	7.0	35.0	6443280	2685525
Oakley Creek	Urban	97.9	2.0	0.0	0.1	0.0	0.0	1224	3	1.6	21.4	6479023	2662295
Okura Stream	Native/ Pastoral	0.1	65.5	24.6	9.7	0.0	0.1	552	3	1.3	18.4	6500417	2661869
Omaru Creek	Suburban	77.32	0.66	0.0	0.7	0.0	22.03	472	1	2.6	36.2	6478465	2676387
Opanuku Stream	Native/ Pastoral	1.0	81.8	15.3	0.9	1.0	0.0	1568	3	11.0	60.8	6477297	2652538
Otaki Creek	Urban	100.0	0.0	0.0	0.0	0.0	0.0	160	2	0.5	2.6	6468910	2674710
Otara (East Tamaki Rd)	Urban	85.1	0.2	12.9	0.0	0.0	1.8	869	2	2.6	15.8	6469418	2677778
Otara (Kennel Hill)	Suburban	30.0	5.7	62.1	1.8	0.4	0.1	190	3	2.9	10.4	6470056	2678735
Pakuranga Creek (Botany)	Urban	99.5	0.2	0.2	0.0	0.0	0.0	738	3	0.7	15.5	6474688	2680726
Pakuranga Creek (GM)	Urban	97.3	0.0	0.3	2.3	0.0	0.0	220	2	0.4	11.9	6473114	2680302
Papakura Stream	Pastoral	6.0	8.5	73.0	7.5	4.6	0.4	4752	4	2.1	13.1	6461943	2681589
Puhinui Stream	Suburban	44.2	10.9	43.3	1.5	0.0	0.1	624	3	9.5	37.1	6464804	2680411
Wairoa River	Native/ Pastoral	0.2	22.5	62.8	14.3	0.1	0.1	11383	5	8.1	14.5	6463378	2693084
Wairoa Trib	Native	0.0	100.0	0.0	0.0	0.0	0.0	216	2	27.4	92.6	6454464	2697094
Waitangi Stream	Pastoral	0.1	3.4	80.6	0.7	14.0	1.2	1857	3	0.3	15.0	6440229	2664691
Waiwera River	Native/ Pastoral	0.0	49.4	43.5	6.5	0.5	0.1	3016	1	2.8	19.1	6515334	2659277
Wekatahi Stream	Native	0.0	100.0	0.0	0.0	0.0	0.0	227	2	0.8	39.0	6472059	2641507
West Hoe	Native	0.0	100.0	0.0	0.0	0.0	0.0	53	2	0.5	51.5	6512307	2658832
Whangamarie Stream	Pastoral	0.8	0.0	57.9	0.0	41.25	0.1	790	2	9.7	38.9	6446266	2673997

Appendix B. 2. Christchurch site and catchment characteristics, land-use and additional site descriptors were derived from the REC database, GPS co-ordinates were recorded during site visits.

Site Name	Earthquake impact as liquefaction	Land use description	Land use					Catchment Area (ha)	Stream Order	Distance to coast (km)	Elevation (m)	GPS (NZMG)	
			% Urban	% Native	% Pastoral	% Exotic	% Other					Northing	Easting
Addington Brook	Nil	Urban-industrial	89.0	0.0	0.0	10.0	1.0	213	1	18.8	10.6	5741402	2479361
Anzac Stream	Heavy	Urban-wetland	57.0	2.0	37.0	2.0	2.0	458	3	4.9	2.4	5745682	2485842
Ballintines Stream	Nil	Semi-Urban	73.0	0.0	26.0	0.0	1.0	68	1	18.1	10.9	5737743	2477705
Cavedish Stream	Nil	Urban	92.0	0.0	7.0	0.0	1.0	40	1	20.0	13.5	5748420	2478385
Centaurus Drain	Nil	urban	57.0	7.4	26.0	8.0	1.6	7327	5	11.0	7.9	5737374	2480991
Crosers Stream	Heavy	Urban-wetland	81.0	0.0	17.0	0.0	2.0	43	1	5.1	2.8	5745567	2485502
Cross Stream	Light	Urban	100.0	0.0	0.0	0.0	0.0	261	2	22.3	8.9	5743777	2478484
Curletts Rd Stream	Nil	Urban-industrial	42.0	2.0	54.0	0.7	1.3	2023	3	19.2	15.7	5739354	2476910
Dudley Creek	Heavy	Urban	85.0	0.0	14.0	0.0	1.0	622	2	14.0	7.2	5744887	2481788
Gardeners Drain	Nil	Rural-suburban	9.0	0.0	90.0	0.0	1.0	126	2	21.3	17.2	5748428	2477639
Nottingham Stream	Light	Semi-Urban	14.0	0.0	85.0	0.0	1.0	356	2	21.5	9.8	5735297	2475246
Okeover Stream	Nil	Urban	96.0	0.4	1.0	1.0	1.6	7861	3	24.8	17.9	5742824	2476231
Otukikino River	Nil	Rural	4.0	1.1	82.0	11.0	1.9	8119	5	12.7	15.5	5750859	2478264
Papanui Stream	Light	Urban	67.0	0.0	32.0	0.0	1.0	88	1	17.2	10.6	5745549	2478948
Riccarton Stream	Nil	Urban	95.0	0.0	0.0	4.0	1.0	370	2	16.8	7.1	5741658	2478989
Shirley Stream Tributary	Heavy	Urban	81.0	0.0	18.0	0.0	1.0	1371	2	11.2	4.0	5744081	2482817
Shirley Stream	Heavy	Urban	91.0	0.0	7.0	0.4	1.6	134	1	14.0	6.3	5744928	2482331
Smacks creek	Nil	Rural-suburban	3.0	15.0	78.0	0.0	4.0	264	2	23.0	21.2	5749544	2476842
St Albans Stream	Heavy	Urban	100.0	0.0	0.0	0.0	0.0	427	1	16.8	8.2	5743675	2480092
Steamwharf Stream	Heavy	urban	100.0	0.0	0.0	0.0	0.0	66	1	3.1	0.2	5739503	2484969
Styx River Upper	Nil	Rural-suburban	6.0	0.0	93.0	0.0	1.0	298	2	22.2	20.0	5748840	2476782
Upper Avon River	Nil	Urban	81.0	0.4	13.0	5.0	0.6	495	2	24.2	15.0	5742382	2476239
Upper Heathcote River	Nil	Urban	32.0	0.0	67.0	0.0	1.0	521	2	20.8	19.4	5738513	2475922
Waimairi Stream	Nil	Urban	35.0	0.0	63.0	0.7	1.3	1092	3	24.9	18.3	5743193	2476260
Waimairi Stream Tributary	Nil	Urban	100.0	0.0	0.0	0.0	0.0	32	1	23.4	14.8	5743170	2476253
Wairarapa Stream*	Light	Urban	88.0	0.0	10.0	1.0	1.0	856	3	24.1	12.1	5743547	2477158

Appendix B. 3. Plots of fluorescence measured at different substrate concentrations and time points (1, 6 hours), fitted with Michaelis–Menten kinetics to determine the maximum substrate concentration (V_{max}).



Appendix B. 4. Summary of Auckland Summer physiochemical, <5 indicates that the sample was under the instrument detection limit. Sites are coded with land uses: reference (REF), native (N), suburban (SU), urban (U), native/pastoral (NP), and pastoral (P). Values marked with a star (*) are over ANZECC & ARMCANZ (2000) trigger values; NO_X=444 µg/L, SRP=10 µg/L, NH_4^+=21 µg/L, pH= 7.2-7.8).

Site Name	DO (mg/L)	Temperature (°C)	pH	Conductivity (µS/cm)	NH_4^+ (µg N/L)	NO_X (µg N/L)	SRP (µg P/L)	DIN: SRP
Cascades stream (REF, N)	9.6	20.2	7.0	60	<5	<5	11*	1
Marawhara stream (REF, N)	9.2	19.3	7.5	90	<5	5	15*	1
Wairoa Tributary (REF, N)	9.9	14.7	7.3	94	<5	95	41*	5
Wekatahi stream (REF, N)	9.1	19.4	7.3	170	<5	11	20*	1
West Hoe (REF, N)	8.4	15.1	7.0	162	<5	<5	6	2
Lower Tamaki River (U)	5.0	21.0	7.7	289	74*	226	28*	24
Meola Creek (U)	2.3	18.1	6.9	147	1119*	5146*	317*	44
Motions Stream (U)	9.0	21.7	6.9	139	45*	3004*	91*	74
Oakley Creek (U)	9.1	21.1	7.3	222	<5	658*	14*	108
Otaki creek (U)	13.9	23.5	7.9	743	89*	477*	13*	97
Otara @ ETR (U)	7.9	20.5	7.1	461	45*	203	12*	46
Pakuranga @ Botany (U)	4.7	20.9	7.4	186	118*	334	10	104
Pakuranga @ Greenmount (U)	3.4	18.3	7.1	201	89*	52	6	51
Lucas creek (SU)	5.6	19.3	7.6	185	17	64	11*	16
Otara @ KH (SU)	4.4	22.0	7.2	176	<5	6	7	3
Puhinui @ Totara Park (SU)	7.9	19.0	7.5	152	10	392	31*	29
Omaru Stream(SU)	8.1	22.7	7.4	213	41	217	20*	29
Hoteo stream (NP)	8.9	23.0	8.8	133	<5	6	13*	2
Mahurangi river (NP)	7.9	21.6	7.7	132	6	13	8	5
Okura stream (NP)	3.0	18.2	7.3	204	67*	99	10	37
Opanuku stream (NP)	9.2	19.9	7.4	93	<5	43	5	19
Wairoa River (NP)	7.6	20.3	7.3	87	<5	15	14*	3
Waiwera river (NP)	4.7	19.4	7.6	127	34*	21	7	18
Kaukapakapa (P)	6.4	20.3	7.4	152	9	16	18*	3
Kumeu river (P)	4.1	20.4	7.2	112	28*	281	9	72
Makarau river (P)	4.3	17.8	7.5	140	<5	6	<5	5
Ngakaroa stream (P)	6.3	18.6	7.1	137	<5	7199*	<5	4059
Papakura (P)	8.6	22.0	7.2	111	9	70	29*	6
Waitangi Falls (P)	5.2	19.9	6.9	138	<5	407	<5	187
Whangamarie stream (P)	3.1	19.2	7.1	207	<5	11750*	6	4129

Appendix B. 5. Summary of Auckland Spring physiochemical variables, <5 indicates that the sample was under the instrument detection limit. Sites are coded with land uses: reference (REF), native (N), suburban (SU), urban (U), native/pastoral (NP), and pastoral (P). Values marked with a star (*) are over ANZECC & ARMCANZ (2000) trigger values; NO_X=444 µg/L, SRP=10 µg/L NH_4^+=21 µg/L pH= 7.2-7.8).

Site Name	DO (mg/L)	Temperature (°C)	pH	Conductivity (µS/cm)	NO_X (µg N/L)	NH_4^+ (µg N/L)	SRP (µg P/L)	DIN: SRP
Cascades stream (REF, N)	11.1	11.7	8.0	125	<5	<5	8	1
Marawhara stream (REF, N)	10.8	12.6	7.6	203	<5	<5	<5	15
Wairoa Tributary (REF, N)	10.6	12.2	7.8	110	52	<5	28*	4
Wekatahi stream (REF, N)	10.7	12.7	7.8	200	<5	<5	9	1
West Hoe (REF, N)	9.6	13.0	7.8	171	<5	<5	<5	5
Lower Tamaki River (U)	7.7	15.5	7.6	497	114	54*	5	78
Meola Creek (U)	6.1	16.5	6.6	213	5723*	280*	81*	163
Motions Stream (U)	9.8	16.6	7.3	208	4490*	34*	96*	105
Oakley Creek (U)	10.2	14.4	7.6	198	984*	<5	16*	138
Otaki creek (U)	7.4	13.2	7.8	578	2332*	82*	5	1016
Otara @ ETR (U)	10.6	17.8	7.8	181	1090*	95*	9	278
Pakuranga @ Botany (U)	7.3	16.2	7.8	255	975*	64*	<5	571
Pakuranga @ Greenmount (U)	10.6	17.8	7.8	268	858*	40*	<5	458
Lucas creek (SU)	10.3	14.9	7.8	203	166	<5	5	72
Omaru Stream(SU)	9.8	15.9	7.8	245	436	<5	11*	86
Otara @ KH (SU)	10.9	17.0	7.8	218	92	<5	9	23
Puhinui @ Totara Park (SU)	10.0	12.2	7.8	175	568*	8	9	145
Hoteo stream (NP)	9.0	14.5	7.9	201	<5	<5	7	1
Mahurangi river (NP)	7.3	14.6	7.8	180	36	8	5	20
Opanuku stream (NP)	11.8	12.8	7.8	127	36	<5	7	12
Okura stream (NP)	9.9	11.8	8.2	217	370	31*	15*	58
Wairoa River (NP)	10.0	13.4	7.8	105	363	<5	12*	67
Waiwera river (NP)	9.1	14.3	7.8	196	24	8	6	12
Kaukapakapa (P)	9.2	14.4	7.7	206	121	10	12*	24
Kumeu river (P)	10.6	14.8	7.3	146	296	14	6	115
Makarau river (P)	8.2	12.1	7.4	185	117	9	<5	74
Ngakaroa stream (P)	9.7	14.5	7.5	149	6275*	<5	<5	3173
Papakura (P)	10.4	17.6	7.8	167	300	<5	17*	38
Waitangi Falls (P)	10.4	16.6	7.8	187	3301*	<5	<5	1771
Whangamarie stream (P)	16.1	12.8	7.8	277	13950*	<5	6	4906

Appendix B. 6. Summary of physiochemical variables over Spring in Christchurch, with standard errors in parentheses, <5 indicates that the sample was under the instrument detection limit. Sites are coded with land uses: reference (REF), rural-suburban (R-SU), semi-urban (SE-U), urban-wetland (UW), urban-industrial (UI), and urban (U). In addition to liquefaction status as: light (LL) or heavy liquefaction (HL), unmarked sites were not affected by liquefaction. Values marked with a star (*) are over ANZECC & ARMCANZ (2000) trigger values; NO_X=444 µg/L, SRP=10 µg/L, NH_4^+=21 µg/L, pH= 7.2-7.8).

Site Name	DO (mg/L)	Temp (°C)	pH	Cond. (µS25/cm)	NO_X (µgN/L)	NH_4^+ (µgN/L)	SRP (µgP/L)	DIN: SRP
Smacks Creek (REF, R-SU)	6.6	12.2	8.1	109	879*	<5	6	353
Styx River upper (REF, R-SU)	5.7	12.6	7.8	109	1737*	<5	<5	995
Otukikino River (REF, R)	10.1	11.6	8.0	84	309	<5	5	142
Gardners Drain (REF, R-SU)	8.2	12.3	8.1	124	639*	<5	<5	366
Addington Brook (UI)	7.7	13.7	7.5	331	1156*	92*	<5	624
Anzac Drive (UW, HL)	8.4	16.4	7.7	3653	318	29*	21*	36
Ballintines Stream (SE-U)	5.8	13.1	7.5	263	4811*	5	<5	3330
Cavendish Stream (U)	11.3	13.3	7.9	-	57	<5	<5	35
Centaurus Drain (U)	13.9	14.1	9.7	569	<5	8	37*	1
Crosers Stream (UW, HL)	7.5	15.5	6.8	1765	252	16	18*	32
Cross Stream (U, LL)	5.5	12.9	7.7	137	1880*	9	<5	1015
Curletts Rd stream (UI)	2.3	13.1	7.5	354	1312*	102*	7	474
Dudley Creek (U, HL)	9.1	12.7	7.9	152	295	61*	21*	38
Nottingham Stream (SE-U, LL)	6.6	15.1	7.7	223	2686*	17	6	957
Okeover Stream (U)	8.9	13.9	8.2	130	1634*	<5	5	670
Papanui Stream (U, LL)	7.7	14.0	8.0	129	587*	25*	<5	345
Riccarton Stream (U)	9.8	13.5	7.7	216	3594*	<5	<5	2173
Shirley Stream tributary (U, HL)	8.7	13.2	8.1	143	231	148*	27*	31
Shirley Stream (U, HL)	5.3	13.2	7.6	145	152	165*	18*	40
St.Albans Stream (U, HL)	7.6	12.8	8.1	126	356	74*	25*	38
Steamwharf Stream (U, HL)	8.8	17.9	8.0	183	856*	28*	19*	104
Upper Avon River (U)	8.4	13.6	7.3	170	3525*	6	6	1330
Upper Heathcote River (U)	3.5	12.9	6.8	322	7630*	<5	5	3503
Waimairi Stream (U)	9.0	13.4	7.1	141	2423*	10	<5	1748
Waimairi tributary (U)	8.5	12.8	7.7	146	2339*	<5	<5	1565
Wairarapa Stream (U, LL)	9.2	12.4	8.0	134	1531*	10	6	609

Appendix B. 7. Summary of physiochemical variables over Summer in Christchurch, with standard errors in parentheses, <5 indicates that the sample was under the instrument detection limit. Sites are coded with land uses: reference (REF), rural-suburban (R-SU), semi-urban (SE-U), urban-wetland (UW), urban-industrial (UI), and urban (U). In addition to liquefaction status as: light (LL) or heavy liquefaction (HL), unmarked sites were not affected by liquefaction. Values marked with a star (*) are over ANZECC & ARMCANZ (2000) trigger values; NO_X=444 µg/L SRP=10 µg/L, NH_4^+=21 µg/L, pH= 7.2-7.8).

Site Name	DO (mg/L)	Temp (°C)	pH	Cond (µS25/cm)	NO_X (µgN/L)	NH_4^+ (µgN/L)	SRP (µgP/L)	DIN: SRP
Smacks Creek (REF, R-SU)	5.6	15.7	7.2	114	439	<5	8	118
Styx River upper (REF, R-SU)	5.6	13.9	7.3	119	709*	<5	5	303
Otukikino River (REF, R)	4.6	16.7	7.0	82	90	<5	9	23
Gardners Drain (REF, R-SU)	3.6	15.1	6.8	147	548*	64*	20*	67
Addington Brook (UI)	6.4	15.0	7.6	379	2285*	168*	14*	383
Anzac Drive (UW, HL)	9.4	18.0	7.8	2496	84	357*	334*	3
Ballintines Stream (SE-U)	6.0	14.5	6.9	254	6937*	<5	6	2678
Cavendish Stream (U)	6.0	16.7	6.7	84	84	8	23*	9
Centaurus Drain (U)	9.0	15.3	7.9	1174	2892*	229*	205*	34
Crosers Stream (UW, HL)	6.2	17.5	7.8	502	237	<5	17*	31
Cross Stream (U, LL)	4.3	16.0	6.7	86	1513*	22*	39*	88
Curletts Rd stream (UI)	9.9	16.2	7.4	258	1802*	316*	207*	23
Dudley Creek (U, HL)	7.8	14.6	7.4	143	448*	46*	20*	55
Nottingham Stream (SE-U, LL)	5.0	14.1	7.0	140	1111*	39*	39*	65
Okeover Stream (U)	8.9	15.2	7.2	170	4387*	<5	9	1100
Papanui Stream (U, LL)	6.2	14.9	7.1	68	424	25*	8	118
Riccarton Stream (U)	8.8	15.1	7.3	216	2731*	24*	8	722
Shirley Stream tributary (U, HL)	8.0	18.1	7.7	180	430	143*	27*	48
Shirley Stream (U, HL)	2.6	15.1	6.9	114	176	174*	18*	43
St.Albans Stream (U, HL)	7.8	16.6	7.4	283	96	363*	<5	266
Steamwharf Stream (U, HL)	7.8	16.3	7.7	169	752*	8	14*	121
Upper Avon River (U)	6.1	14.9	7.1	174	5214*	6	5	2409
Upper Heathcote River (U)	12.2	18.5	8.0	284	2513*	23*	30*	184
Waimairi Stream (U)	8.3	16.1	7.3	173	4540*	<5	7	1428
Waimairi tributary (U)	7.5	15.9	7.3	120	1802*	21	34*	119
Wairarapa Stream (U, LL)	7.5	14.5	7.5	147	886*	15	10	196