Utility of the MBT/CBT paleotemperature proxy in lake sediments: Spatial variation in bacteria and bacterial lipid distribution in two New Zealand lake catchments.

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Abstract

Branched glycerol dialkyl glycerol tetraethers (GDGTs), produced by unidentified bacteria, have been found ubiquitously in terrestrial and marine environments. The methylation and cyclisation of branched GDGTs is known to change in soils due to mean annual air temperature and pH, respectively. The identification of branched GDGTs produced within the water column and sediments of lakes indicates strong potential for the development of a temperature proxy for lake environments. In order to help develop the applicability of using branched bacterial GDGT lipids as a temperature proxy in lacustrine environments, this research set out to determine the distribution and provenance of bacterial communities and their corresponding GDGT lipids present in lake sediments in two small New Zealand lakes, the Karori Upper Dam and Lake Pounui. The Karori Upper Dam is a small lake, directly fed by two tributaries. Lake Pounui, in comparison, is much larger and fed by tributaries which are buffered by swamps.

Water and sediment samples from Lake Pounui and its catchment indicate a predominantly autochthonous production of branched GDGTs. However, the lake calibrated MBT/CBT paleotemperature proxy is not applicable to sites similar to that of the Karori Upper Dam which have a strong terrestrial branched GDGT signature. This research concluded that it can be expected that a terrestrial GDGT signature of some extent will be present in all lacustrine sites; however, only some sites will display a strong authochthonous lacustrine GDGT signal suitable for the application of the MBT/CBT paleotemperature proxy. Through the use of ARISA DNA analysis this research identified bacterial species which statistically explain a significant portion of variance in branched GDGT abundances. Based on the seasonal fluctuations of measured environmental controls it can be assumed that species abundance will also fluctuate. Future work will need to be undertaken in order to further understand this relationship as the sample size for this research was too small to determine the seasonal pattern of these bacteria.

Acknowledgements

First and foremost, I would like to thank my supervisors, Professor Rewi Newnham and Dr Marcus Vandergoes, for your guidance and feedback throughout this thesis. Dr Susie Wood, thank you for helping me undertake the DNA extraction and analysis, and for ensuring I understood these processes fully. Dr Klaus-Gerhard Zink and Professor Lorenz Schwark, my greatest gratitude to you both for undertaking my GDGT analysis and for answering all the questions that I bothered you with. Andrew Rees, thank you so much for your help regarding statistics and for your guidance throughout the interpretation and analysis of my data. Your help was invaluable. To Zealandia wildlife sanctuary and James Cameron, thank you for letting me undertake this research at my respective sites.

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1. Introduction

1.1 MBT/CBT paleotemperature proxy

Paleoclimate reconstructions are fundamental to understand past climate change and therefore aid in the development of accurate climate models (Cohen, 2003; Le Truet et al, 2007: Huddart & Scott, (2010). Accurate climate reconstructions require an independent temperature proxy, one which predominantly responds to changes in temperature (Powers et al, 2004). Lake sediments are ideal to reconstruct past environments (Cohen, 2003). Sedimentation rates within a lacustrine environment are generally higher than those in a marine environment due to a high level of primary production combined with sediment contribution from soil erosion and tributaries (Cohen, 2003). This allows organisms, or molecules and cells of organisms which once lived within the lake, or the surrounding lake catchment, to be preserved (Blaga et al, 2009). The chemical make-up of an organism strongly reflects the habitat in which it lived. Therefore, organisms found within lake sediment have the potential to facilitate the reconstruction of past habitats of the lake catchment (Meyers, 2003).

Branched glycerol dialkyl glycerol tetraethers (GDGTs), membrane lipids of currently unknown bacterial species, have been found ubiquitously in soils, peats, hot springs, rivers and sea and lake sediments (Weijers et al, 2006; Liu et al, 2010; Schouten et al, 2007; Wu et al, 2013; Fietz et al, 2011). Weijers et al. (2007) identified that the organic structure of these cells in soils changes in response to fluctuations in mean annual air temperature and pH. This allowed the MBT/CBT (methylation of branched tetraethers/cyclisation of branched tetraethers) paleoproxy, based on the abundance and distribution of branched GDGTs in soils, to be developed as a paleoreconstruction tool. Due to the ubiquitous nature of these branched GDGTs, this proxy has been applied to lacustrine sediments. Recently, an *in situ* lake production of branched GDGTs has been identified, leading to the development of lacustrine calibrated versions of this proxy (Zink et al, 2010; Tierney et al, 2011).

In order to assess the potential for using branched bacterial ether lipids to reconstruct past temperatures, the origin and species of bacterial lipids present in lake sediments must be determined. Recent research has shown that lipids produced in soils, tributaries,

and within a lake system all have distinctly different structures and respond to different environmental controls (Tierney & Russell, 2009). A study undertaken by Blaga et al. (2009) found that lake surface sediment samples retrieved from all 47 sites researched included branched bacterial GDGTs produced within soil environments in proportions varying from traces up to 40% of total GDGTs. This suggests that a soil lipid signature can be expected to extend into a lake environment to varying extents depending upon the erosion of soil and the contribution of sediment from streams and rivers (Tierney & Russell, 2009). This spatial variability of lipid distribution and provenance in lacustrine sediments may, under some conditions, reduce the utility of temperature proxies derived from branched GDGTs.

Another outstanding issue with the MBT/CBT paleoproxy is the lack of knowledge about the phyla and origin of the lipid producing bacteria. Although extensive research has been undertaken to determine the viability of using branched bacterial GDGTs to reconstruct past climates in lacustrine sediments, there has been little research into identifying the lipid producing bacteria. Weijers et al. (2006) determined that the branched GDGT lipids were produced by unknown bacteria due to the structure and chemistry of the organisms. Due to the environments in which GDGT lipids have been found, researchers suggest that the bacteria may be anaerobic bacteria; however, this has yet to be determined.

Statistical analysis has suggested a correlation between bacterial lipid composition and temperature (Weijers et al, 2006); however, identification of the GDGT lipid producing bacteria will help establish the interaction between bacterial lipid composition and environmental controls, including seasonal controls, in a natural environment. This will enable future cultivation of bacteria which may determine the effect that specific environmental controls have on the composition of bacterial GDGTs. Identifying the bacteria phyla which produce branched GDGT lipids in lacustrine environments is necessary to determine the viability of this paleoproxy.

1.2 Aims

This research aims to:

1. determine the spatial variation of bacterial communities and corresponding branched GDGTs in the sediments of two small New Zealand lakes;
2. determine the seasonal cycle of environmental controls such as temperature, pH and conductivity on the composition of bacterial communities and corresponding lipids in the water column of two New Zealand lakes; and
3. determine bacterial species which are significant to branched GDGT abundance in lacustrine sediments.

Investigating these aims will help determine the viability and utility of using bacterial lipids as a paleotemperature proxy in small New Zealand lacustrine systems.

1.3 Research Contributors

This research is part of a current wider project by the Institute of Geological and Nuclear Sciences Ltd (GNS) titled, *Bacterial geo-thermometer: A new, precise indicator of climate change.* The GNS project aims to accurately reconstruct climate estimates for the Last Glacial Maximum in New Zealand, through the use of multiple proxies, including bacterial lipids, thereby determining the viability of using bacterial lipids in lake sediments to reconstruct past climate through the comparison of pre-existing climate records (Vandergoes, 2010).

Under this wider project the construction of the paleothermometer based on lipid composition will be undertaken by Dr Klaus-Gerhard Zink, Organic Geochemist contracted to GNS and Professor Lorenz Schwark, Organic Geochemist at Christian-Albrechts-University Kiel. This paleothermometer will be tested against pollen and chironomid records by Dr Marcus Vandergoes, Paleoclimate Scientist for GNS, Dr Ann Dieffenbacher-Krall, Paleoclimate Scientist at the University of Maine and Professor Rewi Newnham, Victoria University of Wellington.

Both this research and the wider GNS project are funded through the Marsden Fund Scheme administrated by the Royal Society of New Zealand.

1.4 Research Sites

The research for this thesis was undertaken at two field sites. Site 1 is the Karori Upper Dam at the Zealandia wildlife sanctuary, Karori, Wellington. The second field site is Lake Pounui, situated in the south-west Wairarapa, New Zealand.

1.4.1 Karori Upper Dam, Wellington

The Karori Upper Dam is a mesotrophic lake located at an altitude of 170m above mean sea level at a latitude of -41.298638 and a longitude of 174.744559. The dam is approximately 100m in length, has a catchment size of 2.6km^2 and a maximum depth of 8.7m (Vidal & Maris-McArthur, 1973).

The Karori Upper Dam is an artificial lake constructed in 1908 to provide water to the Wellington Region. The reservoir was closed in 1991 due to the risk posed by the active Wellington Fault which lies under the dam (Astwood & Fell, 2012). Although this site has been anthropogenically altered in the past, and is therefore not a pristine environment, the development of the area as a wildlife sanctuary in 1995 has resulted in minimal human influence since then.

The Karori Upper Dam has two main tributaries from the surrounding catchment, although it can be expected that runoff would also enter the dam from the surrounding slopes. This site is bordered by vegetation on the north, south and west banks and is bordered by a concrete dam to the east. Figure 1.1 shows the site and surrounding catchment.

Figure 1.1: Karori Upper Dam catchment

1.4.2 Lake Pounui, Wairarapa

Lake Pounui, Site 2, is situated in the foothills of the Rimutaka Ranges, located in the south-western Wairarapa, at a latitude and longitude of -41.344757 and 175.113572 respectively. Lake Pounui is a mesotrophic lake, approximately 1.2 km in length and has an approximate area of 46 ha (Jellyman, 2010; Persse, date unknown; Perrie & Milne, 2012). The catchment of Site 2 covers some pastoral land and the vegetated slopes of the Rimutaka Ranges (Figure 1.2). The maximum measured depth of Lake Pounui was 9.5m.

As can be seen on Figure 1.2 below, Lake Pounui is bordered by pastoral land on the eastern bank of the lake. Lake Pounui is part of the QEII National Trust (which aims to preserve areas of environmental and cultural importance (QEII National Trust, 2011)), suggesting that there would be little anthropogenic influence on this site therefore making it a feasible location for this research. In particular, as with the Karori Upper Dam, it was anticipated that there would be minimal anthropogenic influence on bacterial community distribution both within the lakes and in their surrounding catchments.

Figure 1.2 also shows the bathymetry of Lake Pounui as identified by Persse (date unknown).

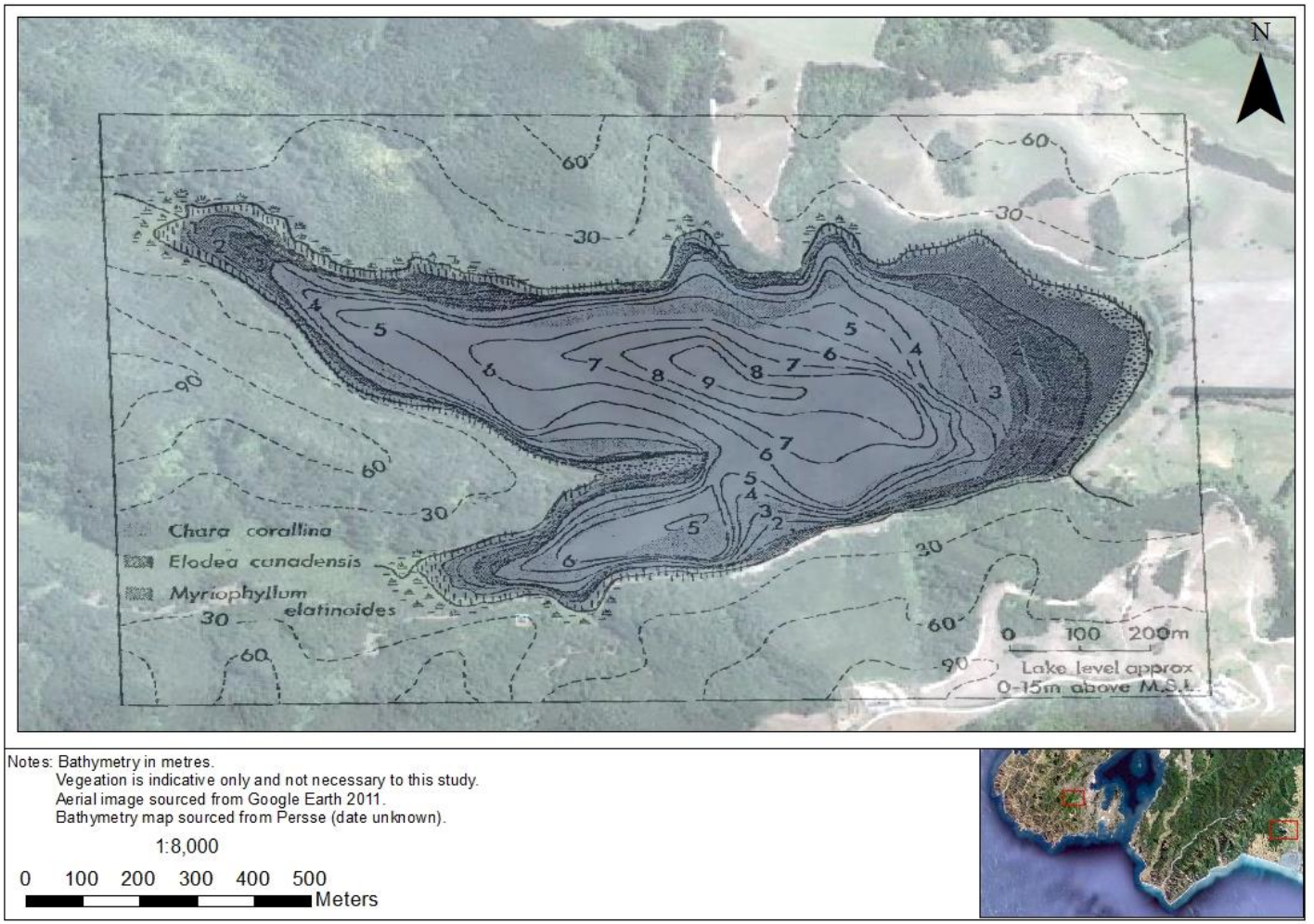

Figure 1.2: Lake Pounui catchment.

1.5 Methods

The research for both sites was carried out using a three-step methodology. Chapter 3 will expand on the methods used.

1. To determine the spatial variation in bacterial communities, the lake floor and surrounding catchments were extensively sampled. Surface sediment samples were taken from both within the lakes and their surrounding catchments to undergo DNA analysis. DNA was extracted from these samples before undergoing DNA finger printing to determine the bacterial community at each site and the variance between environments.

2. To determine the spatial variation in branched GDGTs, duplicate surface sediment samples were taken from the DNA sample sites to undergo geochemical and GDGT analysis. Sampling from an array of environments at each site enables the provenance of the branched GDGTs to be determined, and variation between terrestrial and lacustrine lipids to be ascertained. This work will help to establish the extent to which any possible terrestrial lipid signature needs to be considered when determining paleotemperature reconstructions from lacustrine sites such as these.

3. To determine the seasonal cycle of environmental controls on the composition of bacterial communities and the corresponding lipids, monthly field work was undertaken at both sites over a seasonal cycle (2012- 2013). This included collecting water samples near the surface and bottom of the lake for GDGT analysis, a separate surface water sample for DNA extraction, and measuring environmental controls with a Hanna multiparameter water meter throughout the water column at the lake depo-centre over a 12 to 22 month period. Surface water samples were filtered before undergoing DNA extraction, finger printing and DNA sequencing to determine both the nature of the bacterial communities and the bacterial species within the water column. The statistical relationship between the bacterial species and environmental controls was determined using ordinations and multiple linear regression, in order to validate the strength of the relationship between each environmental control and lipid-producing species. GDGT water samples were filtered and then freeze dried in order to extract the

bacteria from the water sample and to prime it for the organic chemistry analysis of the GDGT lipids. GDGT analysis of the water samples will not be made available for this thesis due to time constraints. However, assumptions based on the relationship between GDGT composition and bacterial community structure of lacustrine surface sediment samples will be made to infer the structure of bacterial lipids within the water column, and identify any seasonal trends.

These methods will help determine the utility of using bacterial GDGT lipids as a paleoclimate proxy in lacustrine systems.

1.6 Thesis Outline

This thesis is set out in six chapters. This chapter has identified the main problems to be considered in this thesis, and has outlined the aims of this research and the methods to be undertaken to address them. Chapter Two reviews previously published work relevant to these problems and identifies research gaps that this work is anticipated to inform. Chapter Three describes the methodologies used to address the aims of this research. Chapter Four sets out the results produced by this research. Chapter Five discusses these results, comparing them with current literature and determines the utility of the MBT/CBT paleotemperature proxy in small New Zealand lakes. Chapter Six then reiterates the main themes from my research and concludes this thesis.

2. A review of the development and applications of GDGT paleotemperature proxies

This chapter will outline current research being undertaken using branched GDGTs as a paleotemperature proxy and will elaborate on these limitations. The aim of this chapter is to identify current limitations within this field and present the necessity of my research.

Section 2.1 will first discuss the difference between archaea-derived and bacterial-derived lipids before briefly discussing the TEX_{86} paleotemperature proxy. This is briefly addressed as the TEX_{86} proxy is a paleoreconstruction tool that has been found to be applicable to some lacustrine sites. Section 2.2 then goes on to discuss the MBT/CBT paleotemperature proxy, a branched GDGT proxy that has been widely recognised to be applicable to a far greater range of lacustrine sites than the TEX_{86} proxy. This section will explore current knowledge gaps of this proxy and state how this research aims to help understand these research gaps.

2.1 Introduction

There is currently extensive literature about the applicability of lipid biomarkers as a proxy in reconstructing past climates (Blaga et al, 2009; Powers et al, 2004; Wei et al, 2011; Wutcher et al, 2004). Both archaeal and bacterial lipids are known to change their organic geochemistry make up in relation to environmental controls. This relationship between changes in environmental controls and the organic chemistry of lipids is thought to be relatively simple.

Archaea, the second domain of the prokaryotes, have isoprenoid lipids that consist of carbon chains bonded to glycerol moieties made up cyclopentane rings. Bacteria, the first domain of prokaryotes, have branched membrane lipids that are made up of methyl substituted C28 n-alkyl side chains that contain 4, 5 or 6 methyl substitutes (Castaneda & Schouten, 2011; Schouten & Sinninghe Damste, 2012).

The TEX_{86} index (TetraEther index of 86 carbon atoms) is a marine paleotemperature proxy that was developed by Schouten et al. (2002). It is based upon the observation that crenarchaeol isoprenoid lipids change their composition in relation to sea surface temperature. Past temperatures are reconstructed based on the following ratio.

$$TEX_{86} = \frac{([IV]+[V]+[VI])}{([III]+[IV]+[V]+[VI])}$$

The Arabic numbers correspond to the isoprenoid GDGTs in Figure 2.1.

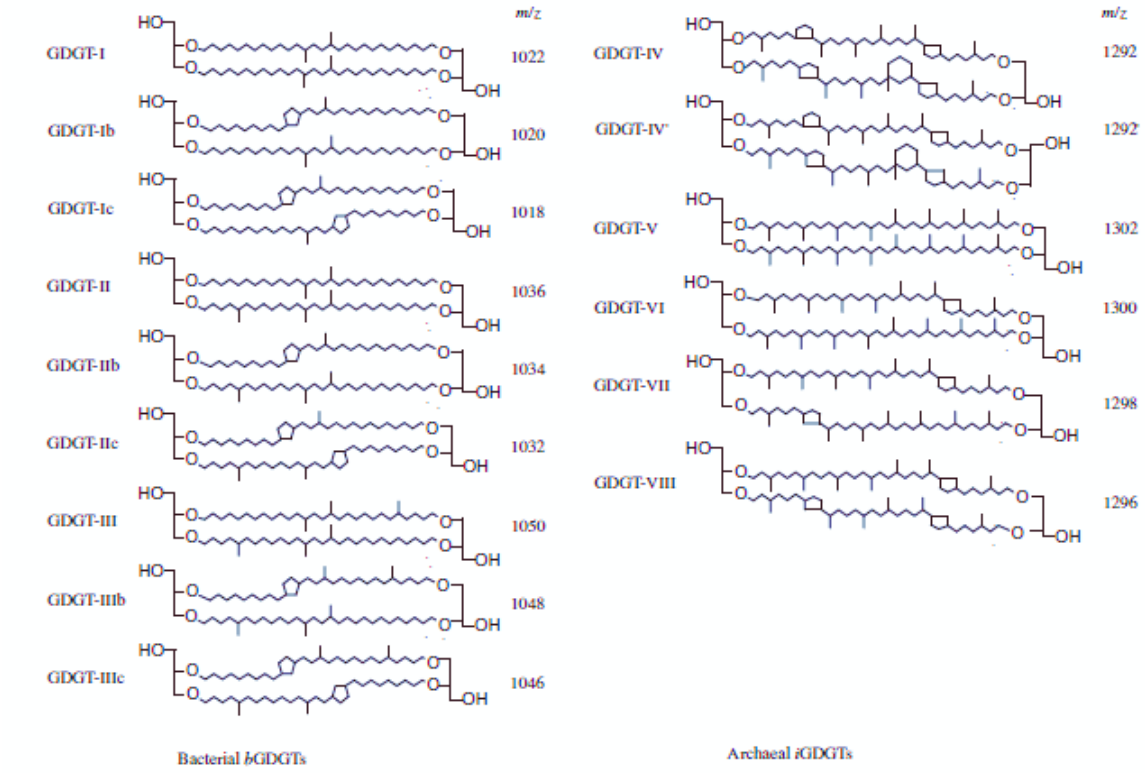

Figure 2.1: Structure of branched and isoprenoid GDGTs (Pearson et al, 2011).

Isoprenoid lipids are present in various environments and several researchers have applied the TEX_{86} temperature proxy to lacustrine sediments. While the TEX_{86} index has been found to accurately reconstruct temperatures in large lakes in which crenarchaeol concentrations are high (Powers et al, 2005; Tierney & Russell, 2009; Tierney et al, 2010; Woltering et al, 2011; Berke et al, 2012; Blaga et al, 2013), in smaller lakes the relationship between crenarchaeol abundance and temperature appears to be weak (Blaga et al, 2009; Powers et al, 2010). This may be because in smaller lakes, bacterial branched GDGT concentrations exceed archaea concentrations thus creating a bias when using the TEX_{86} proxy in small lake environments. The TEX_{86} temperature proxy is, therefore, thought to be inapplicable to the majority of lacustrine environments where organic matter, and therefore bacterial GDGT lipids, is dominant (Blaga et al, 2009; Powers et al, 2010).

Branched GDGTs were first identified in peats, and marine and lacustrine sediments by Sinnginghe Damste et al. (2000). However, these lipids, which have a distinctly different structure to isoprenoid GDGTs (Figure 2.1), have been found to be abundant in both small and large lakes, which suggests they may be a more promising paleoproxy for use in lacustrine environments.

2.2 MBT/CBT Paleoproxy

The MBT/CBT paleoproxy was first developed by Weijers et al. (2006) to reconstruct temperature and pH in soils. Weijers et al. (2006) found that the degree of methylation and cyclisation of bacterial branched GDGTs in soils responded to changes in these environmental controls, respectively. The MBT/CBT indices are calculated with the following equations, the roman numerals corresponding to the branched GDGTs in Figure 2.1.

$$MBT = \frac{([I] + [Ib] + [Ic])}{([I] + [Ib] + [Ic]) + ([II] + [IIb] + [IIc]) + ([III] + [IIIb] + [IIIc])}$$

$$CBT = -\log\left(\frac{[Ib]+[IIb]}{[I]+[II]}\right)$$

Mean annual air temperature (MAAT) and soil pH can then be calculated using the following equations.

$$MAAT = \frac{MBT}{0.86+0.096*pH} \qquad (R^2 = 0.82)$$

Or

$$MAAT = \frac{MBT}{0.122+0.187*CBT+0.020} \qquad (R^2 = 0.77)$$

$$pH = \frac{CBT}{3.33-0.38} \qquad (R^2 = 0.70)$$

The MBT/CBT proxy has been applied to other environments where branched GDGTs have been found to be abundant, including peats, bogs and lacustrine sediments (Weijers et al, 2006; Liu et al, 2010; Schouten et al, 2007; Wu et al, 2013; Fietz et al, 2012). Examples of some successful applications of this proxy in lacustrine sediments can be found in research carried out by Fawcett et al. (2011) and Niemann et al. (2012).

Fawcett et al. (2011) used the MBT/CBT paleoproxy to reconstruct mid-Pleistocene temperatures using lake sediments from a part of the Valles Caldera in New Mexico. Temperature reconstructions appeared accurate with interglacials being distinctly warmer than glacials, and with the highest reconstructed temperatures being comparable to present day temperatures at this site.

Niemann et al. (2012) applied the MBT/CBT paleoproxy to sediments of Lake Cadagno, Switzerland to reconstruct temperatures for the past 11000 years. Reconstructions again appeared accurate, reconstructing the Little Ice Age and the Medieval Warm Period; temperatures also aligned with other reconstructions from the region, indicating that this proxy was accurate for this site.

Kumar Das et al. (2012) applied the MBT/CBT paleoproxy to a highly polluted lake in South Africa. The aim of their study was to determine the applicability of this paleotemperature proxy to sites that are not pristine. Reconstructions appeared accurate in that they were consistent with instrumental records from this site, suggesting that the MBT/CBT paleoproxy is applicable despite traditionally confounding influences.

2.2.1 Current knowledge gaps of the MBT/CBT GDGT paleoproxy

Although many studies have shown the potential of the bacterial GDGT derived paleotemperature proxy in lacustrine environments, the literature also shows that there are current knowledge gaps that need to be addressed in order to assess the full potential of this proxy for temperature reconstructions (Sinninghe-Damste et al, 2009; Tierney & Russell, 2009). These knowledge gaps include: identification of the origin of branched GDGTs in lacustrine sediments, determining whether bacterial lipid distributions are spatially homogenous across lake systems, and identifying if temperature is the prominent environmental control on the composition of lake *in situ* bacterial lipids (Weijers et al, 2007; Tierney & Russell, 2009).

This section will first discuss the spatial variation and origin of branched GDGTs located in lacustrine sediments before discussing the importance of determining the autochthonous and allochthonous signals in lake sediments. It then explores the relationship between branched GDGT distributions and environmental controls before discussing current knowledge of the lipid producing bacteria.

2.2.1.1 Spatial variation of bacterial lipid s in lacustrine sediments

Branched GDGT lipids were first thought to be produced in terrestrial sediments and deposited in lacustrine environments through the processes of runoff and soil erosion (Hopmans et al, 2004; Niemaan et al, 2012). Therefore, the MBT/CBT proxy was thought to be applicable to lacustrine sediments. Recent research however, suggests that branched GDGTs are also produced *in situ*, both within the water column and in lacustrine sediments (Tierney & Russell, 2009; Niemann et al, 2012; Tierney et al, 2010; Wang et al, 2012). Studies focussing on the spatial variation of bacterial lipids in lacustrine sediments suggest that the soil calibrated MBT/CBT temperature proxy may not be viable at all sites. This is thought to be due to the extent of a terrestrial lipid signature in lacustrine sediments combined with *in situ* production rates of branched GDGTs.

For example, a study undertaken by Blaga et al. (2009) found that lake surface sediment samples retrieved from all 47 of their study sites included branched bacterial GDGTs produced within terrestrial soil environments in proportions varying from trace quantities up to 40% of total GDGTs. Similarly, Niemaan at el. (2012) found that lacustrine sediment samples at Lake Cadagno, Switzerland portrayed very similar GDGT signatures to the majority of terrestrial sediment samples, suggesting that lacustrine sediments were of terrestrial origin and transported into the lake by fluvial processes. Temperatures reconstructed with the soil calibrated MBT/CBT proxy in Lake Cadagno appeared consistent with previous paleotemperature reconstructions suggesting that, at this site, terrestrial matter had a dominant signal across the lake and that *in situ* production of branched GDGTs was limited and had no impact on the MBT/CBT proxy.

Several researchers, however, have found that the application of the soil MBT/CBT proxy at their sites underestimates temperature and pH (Zink et al, 2010; Pearson et al, 2011). Zink et al. (2010) sampled 11 sites in the South Island, New Zealand, and compared MBT/CBT air temperature reconstructions with temperatures previously reconstructed from chironomids. The results from their study showed a slight underestimate in mean annual air temperature reconstructions. Zink et al. (2010) suggested that this discrepancy may be due to both an autochthonous and allochthonous source of branched GDGTs in lake sediments. These researchers suggest that in order to

accurately reconstruct temperatures using GDGTs in lacustrine sediments in New Zealand, the MBT/CBT equation must be modified to account for this *in situ* source of GDGTs. A study of 90 sites along an Arctic to Antarctic transect, also found that the MBT/CBT ratio was not applicable and predicted much lower temperatures than previously reconstructed due to an *in situ* lake source of branched GDGTs (Pearson et al, 2011).

Loomis et al. (2011) found that surface sediments from all 29 lakes sampled in Western Uganda had different GDGT characteristics than terrestrial soil and river signatures. The results of this study indicated that *in situ* lake production of branched GDGTs was dominant compared to that of terrestrial input, regardless of elevation or site.

Wang et al. (2012) assessed the distribution of branched GDGTs in surface sediments and the surrounding soils of Lake Qinghai, China. The relative abundance of GDGTs differed between lake sediments and soils from the surrounding catchment. For example, concentrations of GDGT I were considerably higher in the lacustrine sediments. This supports the suggestion presented in previous studies (Zink et al, 2010; Pearson et al, 2011) that some branched GDGTs are produced within lakes.

Sun et al. (2011) suggest that precipitation is an important environmental control on the origin of bacterial lipid composition within lake systems. This is because precipitation will increase soil erosion within the surrounding catchment. These researchers go on to describe how this has the potential to compromise the validity of a lake calibrated MBT/CBT paleoproxy as the branched GDGT signal will no longer be representative of an autochthonous production of lipids. A study undertaken by Sinninghe Damste et al. (2009) also suggests that heavy rainfall affects the relationship between lipid composition and environmental controls. This research undertaken on Lake Challa observed an increase in branched GDGT abundances in lake systems that correlated with rainfall events suggesting that heavy rainfall resulted in an influx of soil-derived lipids.

The above research suggests that at some lake sites at least, a soil lipid signature may be present in lake sediments to varying extents depending upon soil mobility and sediment contribution from streams and rivers. This possibility may limit the applicability of the

MBT/CBT proxy to lacustrine sediments. To ensure the viability of the MBT/CBT temperature proxy in lacustrine sediments, both the terrestrial signature and the *in situ* lake production of branched GDGTs must first be determined. This understanding will allow for site-specific modifications to the MBT/CBT equation to ensure improved accuracy in paleotemperature reconstructions.

Tierney & Russell, (2009) suggest that branched GDGT lipids produced in soils, tributaries, and within a lake system all have distinctly different structures and respond to different environmental controls. Therefore, in order to assess the applicability of branched bacterial GDGT lipids as a temperature proxy in lacustrine environments, the spatial variation and origin of GDGT lipids present in lake sediments must be determined.

2.2.1.2 Environmental controls on the composition of in situ lake bacterial lipids

As discussed previously, recent research suggests that the production of branched GDGTs may vary under different environmental conditions (Tierney & Russell, 2009). The role of environmental factors must therefore also be assessed in order to determine the viability of the MBT/CBT paleotemperature proxy in lake sediments (Weijers et al, 2007; Tierney et al, 2010; Zink et al, 2010).

2.2.1.2.1 Temperature

Some studies have indicated that temperature is the most influential environmental control on branched GDGT abundances in lacustrine systems (Tierney & Russell, 2009). A study on the relationship between the variations in branched GDGTs and environmental controls in East African lakes, undertaken by Tierney et al. (2010), found that temperature was the main influence on branched GDGT variations. They also found a strong correlation between surface water pH and the composition of bacterial lipids located within lake sediments, concluding that pH is the second most significant factor influencing bacterial lipid distribution within lakes. In a study of 90 sites located on an Arctic to Antarctic transect, Pearson et al. (2011) also found that temperature explained the most variance in lipid composition, with conductivity being the second most influential environmental control.

Other studies, however, have suggested that temperature is not always the dominant control on production of branched GDGTs in lakes. For example, in a study of the

distribution of both archaeal and bacterial GDGT lipids in the hot springs of Yellowstone National Park, USA, (Schouten et al, 2007), no direct correlation between lipid composition and fluctuations in temperature was found. Schouten et al. (2007) suggest accordingly that environmental factors other than temperature must influence lipid distribution.

2.2.1.2.2 pH

A study undertaken by Weijers et al. (2007) focusing on the relationship between environmental controls and bacterial lipid composition found that there appeared to be an exponential relationship between the composition of bacterial GDGT lipids and soil pH. These researchers suggest that an exponential correlation between the abundance of branched GDGTs with cyclopentyl moieties (GDGTs possessing 'b' and 'c' configurations) and soil pH reflects a potential relationship between these two variables.

2.2.1.2.3 Other environmental controls affecting GDGT distribution

Tierney et al. (2010) suggested that other environmental parameters, such as salinity, depth and dissolved oxygen, have no significant impact on branched GDGT distribution within lake environments independent of temperature or pH at the majority of their sites. Nevertheless, they suggest that depth may be a significant factor in GDGT variation throughout the water column in some lake environments. For example, water depth had a significant role on the distribution of GDGTs Ic, IIc and IIIc in shallow lakes located at mid-elevation in Kenya. This suggests that these GDGTs may be of different origin to those GDGTs that showed no correlation with depth.

In summary, the majority of studies indicate that temperature is the dominant control on branched GDGT production, followed by pH (Table 2.1, Pearson et al, 2011). Nevertheless, it is clear from previous work that site-specific differences in the effect of other environmental variables can also occur and must therefore be considered in any assessment of the applicability of the MBT/CBT temperature proxy at a given site.

Table 2.1 Relationship between GDGT composition and environmental controls in lake sediments from an Arctic-Antarctic transect. Sourced from Pearson et al. (2011). The total values represent the total percentage of variation this environmental control explains in relation to GDGT abundances. The unique value explains percentage of variation solely explained by the specific environmental controls with all variation from other environmental controls removed.

Variable	All GDGTs		Isoprenoid GDGTs		Branched GDGTs	
	Total	Unique	Total	Unique	Total	Unique
Temperature	18.7	7.9	0.7*	0.6*	35.9	27.2
pH	19.9	1.5	0.1*	0.1*	10.9	2.8
Conductivity	35.4	10.9	3.7	5.1	13.6	0.9
Water depth	6.5	3.1	3.4	4.9	6.7	4.4
All variables	49.9		8.5		53.8	
Joint effects		26.5		-1.5		18.5

** indicates the variance that environmental control displays is statistically significant.*

2.2.1.3 Seasonal cycle of environmental controls on composition of bacterial lipids

Another possible confounding factor that may limit the potential of MBT/CBT as a temperature proxy is the role of seasonal climatic variations in the composition of bacterial lipids. For example, Zink et al. (2010) suggested that, while the composition of bacterial lipids in lake sediments would have no seasonal variation and would reflect the average annual temperature signal, this average annual temperature signal would most likely be biased towards summer months. They suggested that this bias would be due to the increase in organic matter biosynthesized, the process of conversion of substrates to amino acids, and lipids, throughout summer months thus resulting in an increase in abundance of the lipid producing bacteria species.

Sun et al. (2011) also suggested that the correlation between the MBT/CBT proxy and temperature is stronger throughout summer months than with the mean annual air temperature. Sun et al. (2011) suggested this is due to the warmer temperatures resulting in an increase in the abundance of bacteria. It is important to determine the season in which lipids are primarily produced as this could create bias towards a specific season when reconstructing past temperatures.

Zink et al. (2010) further stated that the location and elevation of lakes would influence how the composition of bacterial lipids reflects changes in temperature. Shanahan et al. (2013) found that when reconstructing temperatures in the arctic reconstructed values were consistent with summer annual air temperature. Zink et al. (2010) and Shanahan et al. (2013) suggest that bacterial lipids located in lakes present in alpine environments may experience a shift in temperature changes due to the high rates of bioproduction which occur throughout the summer months.

Tierney et al. (2010) also proposed that the elevation of a lake influences the seasonal cycle of environmental controls on the composition of bacterial lipids. These researchers suggested that both the surface and deep water temperatures of lakes situated in tropical regions have a strong correlation with the mean annual air temperature. However, they suggested that the surface water temperature of lakes situated in temperate and sub-tropical regions has a strong seasonal correlation with summer and winter air temperatures, whereas in sub-tropical and temperate regions, deep water temperatures of lakes are strongly correlated with the coldest winter air temperature. Tierney et al. (2010) accordingly suggested that establishing the location of GDGT production within the water column is fundamental to determining the seasonal cycle of environmental controls on the composition of bacterial lipids.

As discussed above, some studies (Zink et al, 2010; Tierney et al, 2009; Sun et al, 2011) have suggested that seasonal variability in certain lakes may interfere with the utility of the MBT/CBT paleotemperature proxy. Sun et al. (2011) suggested that more research is needed to determine the relationship between the seasonal cycle of environmental controls on the composition of bacterial lipids as there have been limited studies determining this relationship. As stated above, determining the relationship between the seasonal cycle of environmental controls on the composition of bacterial lipids is fundamental to determine if temperatures reconstructed with the MBT/CBT proxy will be biased towards a specific season.

2.2.2 Lacustrine bacterial community structure

Although, many studies have identified a correlation between branched GDGT structure and temperature, identification of the lipid producing bacteria is fundamental to:

1. identify the environment and season in which the bacteria are produced; and
2. understanding the relationship between the bacterial species and any environmental controls which may influence the location and abundance of the particular species.

Bacterial community structure within a lake system depends entirely on the conditions and environment of the specific lake. Lacustrine bacteria are generally grouped on the basis of the energy and carbon source upon which the synthesis of their body matter relies (Golterman, 1975: Lampert & Sommer, 2007).

Acidobacteria

Although the branched lipid producing bacteria have yet to be identified, it has been suggested by several researchers that they may belong to the acidobacteria phylum (Weijers et al, 2009; Peterse et al, 2012; Sinninghe-Damste et al, 2011) and originate in anaerobic environments due to the environments in which the bacteria have been found (Weijers et al, 2007); however, this has yet to be verified by other work.

Acidobacteria, a relatively newly documented phylum of bacteria, are thought to be one of the most diverse bacterial phyla and have been found to be ubiquitous in a range of environments including soils, peats, savannas, caves, lakes and marine environments (Barns et al, 1999; Barns et al, 2007; Araujo et al, 2012; Zimmerman et al, 2012). Originally with eight subtypes of bacteria, acidobacteria species have increasingly been identified with 26 subtypes recently being identified. Species in the acidobacteria phylum range from aerobic chemoorganotrophs, *Candidatus Chloracidobacterium thermophilum* to anaerobic-originating bacteria, *Geothrix* (Zimmerman et al, 2012). This suggests that acidiobacteria inhabit a range of environments and may therefore respond to different environmental controls; however, there is still little known about this bacterial phylum (Hugenholtz et al, 1998).

2.3 Conclusion

Although research has shown the potential for a bacterial GDGT-derived paleotemperature proxy, there remain several knowledge gaps which need to be further researched before the MBT/CBT paleoproxy can be widely utilised, with accuracy, at all lacustrine sites. A widely applicable MBT/CBT proxy will ensure paleoclimates can be reconstructed accurately and further our knowledge of past climates.

This research aims to help further understand the origin and spatial variation of branched GDGTs in lacustrine sediments. By identifying the spatial variation of branched GDGTs in lacustrine sediments, it can be determined whether the MBT/CBT proxy can be successfully applied to lacustrine sediments as a robust paleotemperature proxy.

A secondary aim of this research is to attempt to identify the bacteria or bacterial communities producing the branched GDGTs utilised in paleotemperature reconstructions. This will enable the relationship between environmental controls and the bacteria in a natural environment to be explored to further determine specific environmental controls on the bacteria and branched GDGT production. It will also help determine the environment in which the bacterial species are produced and whether any seasonal variation in relation to species abundance or habitat occurs. This will then allow for the cultivation of bacteria which will enable the determination of the environmental controls which influences the abundance and distribution of the bacterial species and the GDGT composition to be further explored and understood.

This thesis will address these aims by answering the following research questions:

1. What is the spatial variation of bacterial communities and branched GDGTs in two New Zealand lakes and surrounding catchments?
2. What are the seasonal variations of bacteria and bacterial lipids produced within the water column, and how are these influenced by environmental controls?
3. What bacterial species are likely producing the branched GDGTs in lacustrine sediments?
4. How does the spatial variation of branched GDGTs affect the validity of the MBT/CBT proxy as a paleotemperature reconstruction tool in lake sediments?

3 Methodology

This chapter outlines the methodologies used to help determine these research questions and the aims set out in Chapter One of this thesis. This chapter is structured into five sections. Section 3.1 describes why the research sites were chosen. Section 3.2 outlines the methods used to determine the spatial variation of branched GDGTs in lacustrine sediments at the Karori Upper Dam and Lake Pounui. Section 3.3 states the methods used to determine the spatial variation in bacterial communities in lake sediments at these sites. Section 3.4 outlines the methods used to understand the seasonal variability of environmental controls at these sites and the relationship between these environmental controls and the structure of bacterial communities in the water column. Section 3.5 outlines the methods used to infer the seasonal cycle of branched GDGTs within the water column at the Karori Upper Dam and Lake Pounui.

3.1 Site selection

Two unique sites were chosen for this research in order to determine the utility of the MBT/CBT paleotemperature proxy across a range of sites. These sites were chosen as they represent sites which have relatively minor human impact, typify the size and nature of sites from which we might apply a GDGT based paleoclimate reconstruction, and because of the geomorphic features explained in Table 3.1.

As stated in Chapter One, this research would ideally be undertaken in pristine environments that have had no previous anthropogenic modifications. This would ensure that the sampled bacterial communities and corresponding branched GDGTs truly reflected the sample locations and thus would provide an accurate record of branched GDGTs within lacustrine systems. Although the Karori Upper Dam has had historic anthropogenic alterations, the development of this site as a wildlife sanctuary has resulted in minimal human influence in recent years. Similarly, Lake Pounui is a part of the Queen Elizabeth II trust which aims to restore sites to their natural state. This indicates that the bacterial communities and corresponding branched GDGTs located within water and modern sediment samples from the lake beds should, most likely be indicative of the catchment structure, soil transport within the catchment and natural lake processes.

The unique geomorphic characteristics (Table 3.1) of the Karori Upper Dam and Lake Pounui will enable this research to determine if the MBT/CBT paleotemperature proxy is viable over a range of sites. Based on the different area and lengths of the two sites combined with the presumed hydrological response it has been assumed that these two sites will exhibit different levels of terrestrial input from the surrounding catchment. Karori Upper Dam is a small lake, surrounded by a steep catchment and has a presumed quick flushing time. It will therefore be expected that the Karori Upper Dam will exhibit a strong terrestrial signature. Comparatively, Lake Pounui is considerably larger and has a presumed slow flushing time due to the buffering of inflowing tributaries. This suggests that there may be limited terrestrial input into the lake and therefore it will be expected that Lake Pounui will have a high autochthonous organic matter source. By comparing two lakes with different organic matter sources this research will help determine if the MBT/CBT paleotemperature proxy can be applied to a range of sites.

Table 3.1 Geomorphic features of the Karori Upper Dam and Lake Pounui.

Site	**Latitude/Longitude**	**Maximum depth**	**Catchment Size**	**Nutrient Status**	**Length**	**Presumed hydrological response**	**Catchment description**
Karori Upper Dam	-41.298638 175.744559	8.5	260ha	Mesotrophic	100m	Quick flushing time due to the following variables: - Small lake with close proximity to terrestrial sources. - Two inflowing tributaries directly feeding the lake,	Regenerating indigenous and exotic vegetation. Two tributaries feed the Karori Upper Dam.
Lake Pounui	-41.344757 175.113572	9.5	627ha	Mesotrophic	1200m	Slow flushing time due to the following variables: - Large lake, - Inflowing tributaries buffered by swamps slowing down incoming water, - One small tributary discharging Lake Pounui.	Predominantly indigenous forest (Perrie & Milne, 2012). Two small tributaries, located on the North West bank, and one tributary located on the South West bank fed Lake Pounui. Swamp areas act as buffers between the lake and the tributaries (Jellyman, 1990).

3.2 Terrestrial GDGT lipid signature extent in lacustrine sediments

The first objective of this research (as stated in Chapter 1) was to determine the spatial variation of bacterial communities and corresponding branched GDGTs in the sediments of two small New Zealand lakes. This section will focus on the methods used to determine the extent to which terrestrial GDGT lipid signatures extend into lacustrine sediments at the Karori Upper Dam and Lake Pounui.

Twenty surface sediment samples and 17 surface sediment samples, at 0-2cm depth beneath surface, were taken from the Karori Upper Dam and Lake Pounui, respectively, and their surrounding catchments.

The selection of locations to be sampled were based on the morphology and hydrology of the catchments and the bathymetry of the lakes following the hypothesis that soil erosion occurring in the catchment surrounding the sites, combined with sediment contribution from tributaries will contribute to an influx of bacteria within the lakes. The samples were taken at the same locations as those taken for DNA analysis (Objective Two) to ensure continuity between samples analysed for each procedure. Table 3.2 below lists the location and environment type for each sample taken (refer to Figures 3.1and 3.2 for sample locations).

Table 3.2: Site descriptions of sediment samples that underwent geochemical, GDGT and DNA analysis for the Karori Upper Dam and Lake Pounui.

Site	Sample Type	Sample ID	Latitude	Longitude	Water Depth	Collection Date	Analyses
Karori	Lake Bed	K1	-41.298361	174.74475	Shallow (1m)	5/13/2013 10:36:05 AM	GDGT/DNA
Karori	Lake Bed	K2	-41.298417	174.744861	Intermediate (4.8m)	5/13/2013 10:53:17 AM	GDGT/DNA
Karori	Lake Bed	K3	-41.298611	174.744889	Deep (7.7m)	5/13/2013 11:07:20 AM	GDGT/DNA
Karori	Lake Bed	K4	-41.298972	174.744806	Deep (6.4m)	5/13/2013 11:25:32 AM	GDGT/DNA
Karori	Lake Bed	K5	-41.299222	174.744917	Intermediate (4.1m)	5/13/2013 11:39:23 AM	GDGT/DNA
Karori	Lake Bed	K6	-41.29925	174.744889	Shallow (0.8m)	5/13/2013 11:52:55 AM	GDGT/DNA
Karori	Lake Bed	K7	-41.299028	174.744472	Intermediate (5.4m)	5/13/2013 12:05:24 PM	GDGT/DNA
Karori	Lake Bed	K8	-41.298833	174.744194	Intermediate (3.3m)	5/13/2013 12:15:33 PM	GDGT/DNA
Karori	Lake Bed	K9	-41.298611	174.744306	Intermediate (4.5m)	5/13/2013 12:26:19 PM	GDGT/DNA
Karori	Lake Bed	K10	-41.298528	174.744028	Shallow (1.2m)	5/13/2013 12:55:34 PM	GDGT/DNA
Karori	Lake Bed	K11	-41.298778	174.743833	Shallow (0.9m)	5/13/2013 1:10:30 PM	GDGT/DNA
Karori	Lake Bed	K12	-41.299389	174.743861	Shallow (0.9m)	5/13/2013 1:25:45 PM	GDGT/DNA
Karori	Swamp	K13	-41.299944	174.743167	NA	5/13/2013 1:43:58 PM	GDGT/DNA
Karori	Swamp	K14	-41.299	174.743667	NA	5/13/2013 1:57:30 PM	GDGT/DNA
Karori	Soil	K15	-41.299306	174.745444	NA	5/13/2013 2:25:13 PM	GDGT/DNA
Karori	Soil	K16	-41.300083	174.744056	NA	5/14/2013 10:51:45 AM	GDGT/DNA
Karori	Stream	K17	-41.301861	174.742639	NA	5/14/2013 11:07:03 AM	GDGT/DNA
Karori	Stream	K18	-41.303389	174.741222	NA	5/14/2013 11:31:58 AM	GDGT/DNA
Karori	Soil	K19	-41.299889	174.742528	NA	5/14/2013 11:47:50 AM	GDGT/DNA
Karori	Soil	K20	-41.298361	174.74375	NA	5/14/2013 12:03:11 PM	GDGT/DNA

Pounui	Lake Bed	P1	-41.342222	175.112611	Shallow (1.1m)	5/09/2013 12:48	GDGT/DNA
Pounui	Lake Bed	P2	-41.342639	175.112694	Intermediate (4.4m)	5/09/2013 13:31	GDGT/DNA
Pounui	Lake Bed	P3	-41.34675	175.111389	Intermediate (5.2m)	5/09/2013 14:39	GDGT/DNA
Pounui	Lake Bed	P4	-41.344389	175.1165	Deep (8m)	5/10/2013 10:54	GDGT/DNA
Pounui	Lake Bed	P5	-41.344306	175.116111	Deep (9.5m)	5/10/2013 11:17	GDGT/DNA
Pounui	Lake Bed	P6	-41.343472	175.115611	Intermediate (5.5m)	5/10/2013 11:44	GDGT/DNA
Pounui	Lake Bed	P7	-41.343583	175.117944	Shallow (2.9m)	5/10/2013 12:59	GDGT/DNA
Pounui	Lake Bed	P8	-41.344444	175.118167	Intermediate (4.4m)	5/10/2013 12:45	GDGT/DNA
Pounui	Lake Bed	P9	-41.345472	175.117556	Intermediate (6.6m)	5/10/2013 13:10	GDGT/DNA
Pounui	Lake Bed	P10	-41.343917	175.108222	Intermediate (6.4m)	5/10/2013 13:50	GDGT/DNA
Pounui	Lake Bed	P11	-41.34325	175.106417	Shallow (1.6m)	5/10/2013 14:07	GDGT/DNA
Pounui	Soil	P17	-41.342194	175.111889	NA	5/09/2013 14:05	GDGT/DNA
Pounui	Soil	P18	-41.346722	175.10875	NA	5/09/2013 15:20	GDGT/DNA
Pounui	Soil	P19	-41.348111	175.11	NA	5/09/2013 15:38	GDGT/DNA
Pounui	Soil	P20	-41.341472	175.10525	NA	5/10/2013 14:42	GDGT/DNA
Pounui	Stream	P21	-41.341972	175.107	NA	5/10/2013 14:51	GDGT/DNA
Pounui	Swamp	P22	-41.342556	175.106917	NA	5/10/2013 15:13	GDGT/DNA

Each environment type (excluding swamp and stream environments at Lake Pounui[1]) was sampled at least twice to ensure the spatial variability of bacterial communities, each environment type could be confidently represented. Sampling within the lakes was undertaken intensively and divided into three categories, shallow water, intermediate water and deep water with respect to overall lake depth, in order to determine if the input of terrestrial material varied along bathymetric intervals.

Figures 3.1 and 3.2 show the sample locations for the Karori Upper Dam and Lake Pounui, respectively.

[1] Only one stream and one swamp was sampled at Lake Pounui due to time constraints.

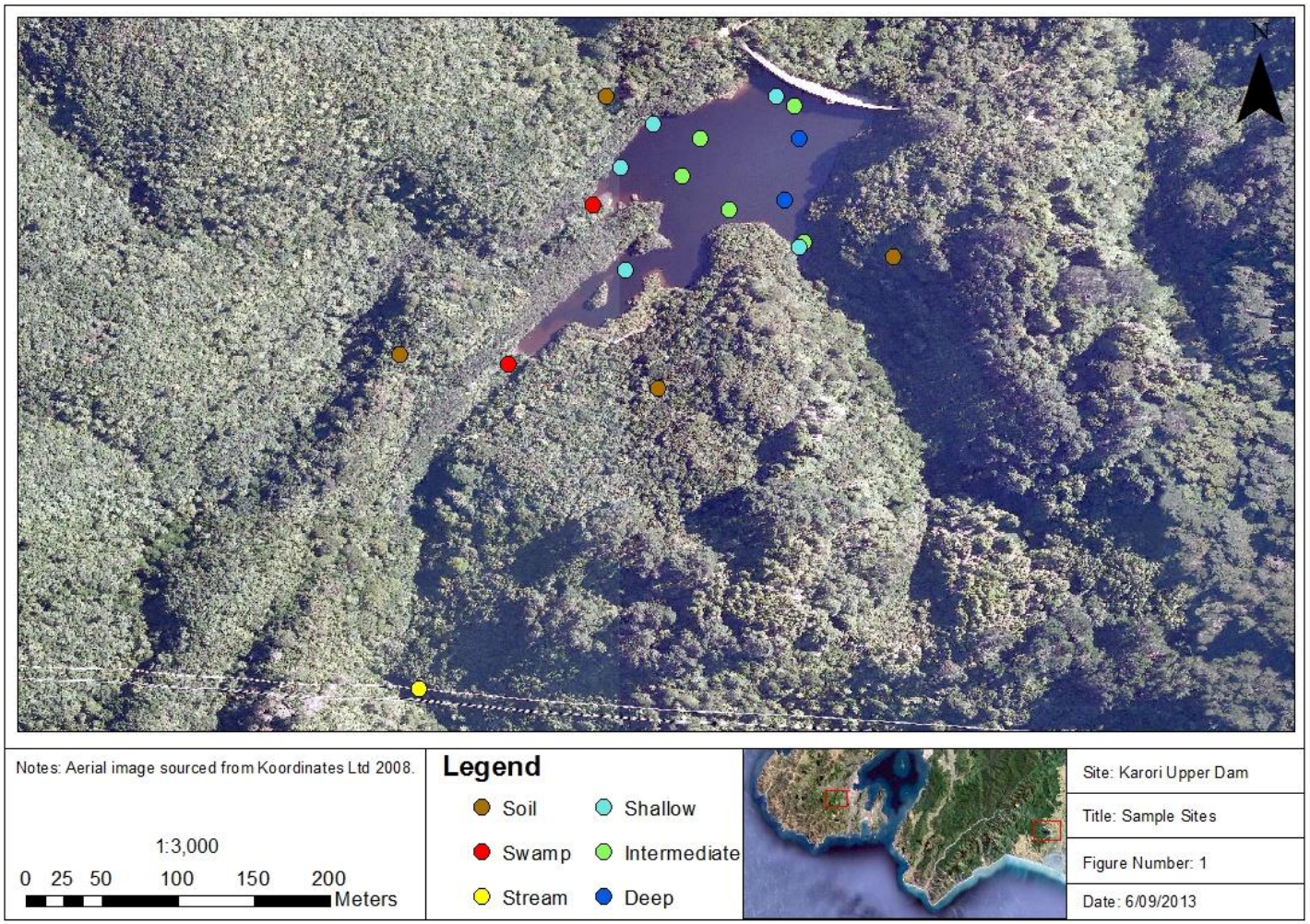

Figure 3.1: Sediment sample sites at the Karori Upper Dam.

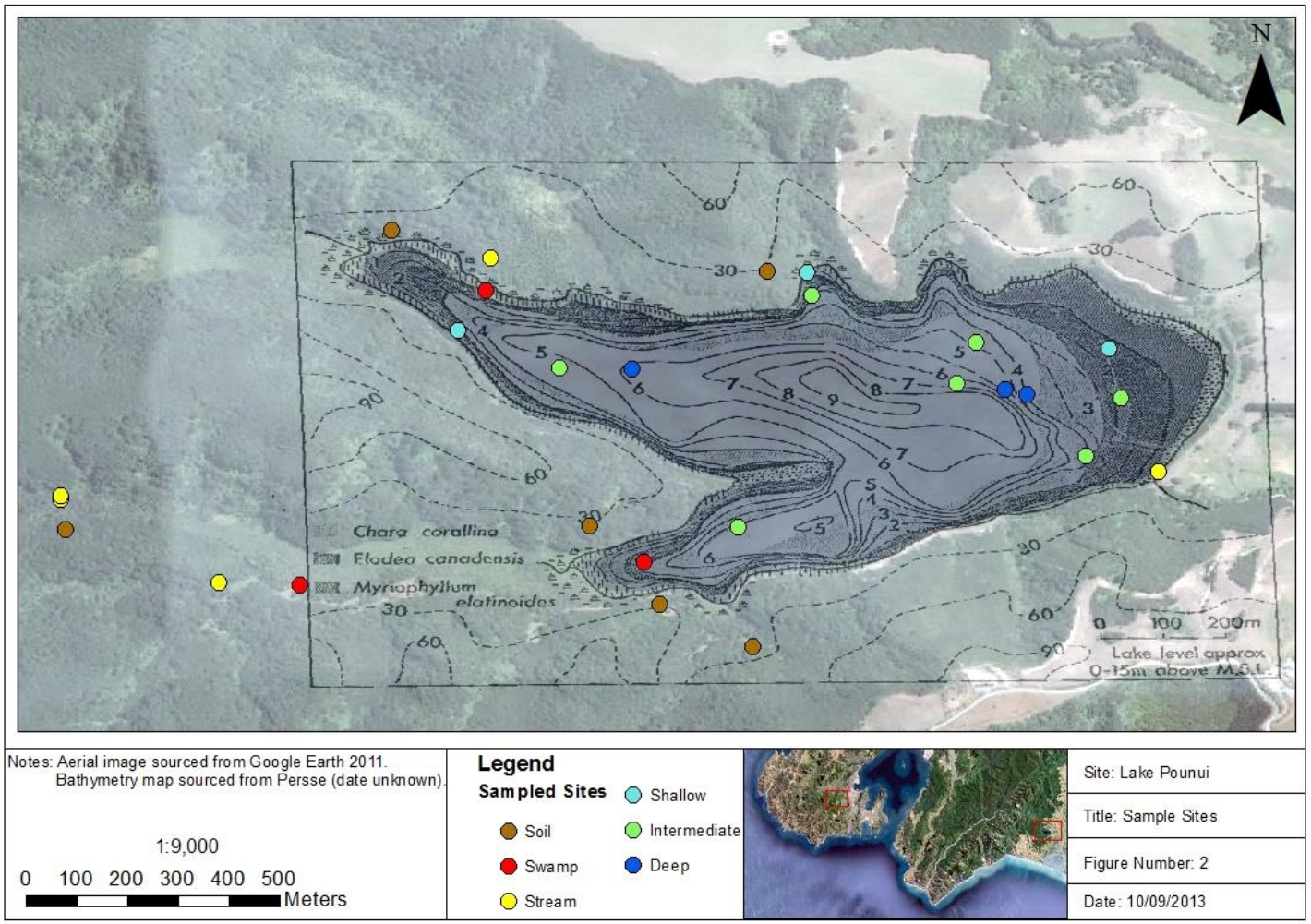

Figure 3.2: Sediment sample sites at Lake Pounui.

3.2.1 Sample collection

Lake floor samples were collected from a range of water depths. Sediment samples at 0-2 cm and 2-4 cm (archive samples) depth beneath the lake bottom were collected at each site using a Hongve-style gravity corer.

Terrestrial sediment samples were collected from a range of locations in the catchment (Figures 3.1 and 3.2) in order to get an accurate spatial representation of the catchments. Plant matter was scraped away before approximately 200g of surface sediment was extracted and placed in a sterile container using a spatula.

The samples were oven dried at 65°C before being homogenised by grinding with a mortar and pestle and sent to the Institute of Geosciences, University of Kiel, Germany, to undergo geochemical screening and GDGT extraction and analysis.

Sediment samples underwent geochemical analysis (total carbon, total nitrogen, total sulphur and total carbon dioxide) to determine the nature and origin of organic matter present in lake sediments (Hakanson & Jansson, 1983; Meyers & Teranes, 2001). This is important for this research as it will indicate whether the lacustrine sediment is dominated by allochthonous or authochthonous organic material. This will further aid the understanding of spatial variation and origin of bacterial communities and branched GDGTs at the Karori Upper Dam and Lake Pounui.

3.2.2 Geochemical and GDGT Extraction and Analysis

Geochemical analysis was undertaken by Dr Lorenz Schwark and Dr Klaus Zink by using an elemental analyser (Vario EL) according to standard laboratory procedures at the Institute of Geosciences, University of Kiel, Germany.

Samples undergoing bulk geochemical analysis for total carbon (TC), total sulphur (TS) and total nitrogen (TN) were first sieved through mesh before the fine material (<0.5 mm) was dried overnight in an oven at 45°C. Samples were then homogenized using a pestle and mortar.

Prior to elemental analysis the presence of carbonates was tested on a small aliquot (50 mg) by the addition of 10% hydrochloric acid (HCl). During this process no effervescent of CO_2 was identified, therefore TC is solely representative of TOC. 10-15 mg of sample material was weighted into tin capsules and analysed by dry combustion in an oxygen stream at a temperature of 1050°C. Full conversion of CO to O_2 was achieved by catalytic oxidation of combustion gases. Instrument calibration for TC, TS and TN was done using sulfanilic acid ($C_6H_7NO_3S$; C = 41.61 wt %, N = 8.09 wt %, S = 18.51 wt %,). Analytical precision for TC and TS is 0.02 wt%, for TN is 0.002wt %. TOC normalised extract yields (mgExt/gTOC) were calculated using the following equation:

$$\text{mgExt/gTOC} = \frac{\text{extract}}{\text{TOC \% x 10}}$$

All samples were analysed in duplicate with good reproducibility (Appendix 1). Geochemical results stated in this thesis are the average of the two duplicate results (standard deviation <5%) (Appendix 4).

Samples undergoing GDGT analysis were prepared using a method developed by the Royal Netherlands Institute for Sea Research in 2000, which has been successfully applied to other GDGT studies (Hopmans et al, 2004; Weijers et al, 2007; Weijers et al, 2011). This method uses high performance liquid chromatography/mass spectrometry (LC/MS) to determine GDGT peaks and compound ratios of GDGTs. Mass spectrometry records the positive molecular ions of the GDGTS (selected ion monitoring) which determines the individual compound peak areas. The individual compound peak areas are then used to determine compound ratios. The results of this study therefore may be directly compared with these other studies.

The GDGTs were extracted from the dried, homogenised sediment and separated into apolar and polar fractions by using solvents hexane/dichloromethane 9:1 using an Accelerated Solvent Extractor (ASE). The polar fractions containing the GDGTs were then cleaned and filtered before undergoing LC/MS which analyses the GDGT fraction using an LC column, cyanopropyl (2.1mm diameter, 150mm length, 3µm particle size of solid phase).

3.2.3 Statistical Analysis

MBT and CBT values were calculated for all sediment samples using the following equations. With the GDGT numbers corresponding to the lipid structures in Figure 2.1, Section 2.1.

$$MBT = \frac{[VI] + [VIb] + [VIc]}{([VI] + [VIb] + [VIc]) + ([VII] + [VIIb] + [VIIc]) + ([VIII] + [VIIIb] + [VIIIc]}$$

$$CBT = - \log \frac{[[Ib]+[IIb]]}{[I]+[II]}$$

Temperature estimates using TEX_{86} values were reconstructed. However, due to low abundances of isoprenoid GDGTs these values were not reliable and therefore were not used in this thesis.

3.3 Spatial Variation in Bacterial Communities

As stated in Chapter 1, the first objective of this thesis was to determine the spatial variation of bacterial communities and corresponding branched GDGTs in the sediments of two small New Zealand lakes. This section will focus on the methods used to determine the spatial variation in bacterial communities from both terrestrial and lacustrine environments at the Karori Upper Dam and Lake Pounui. To ensure continuity throughout this research the samples were taken at the same locations as the samples taken for Objective One.

3.3.1 Sample collection

A gravity corer was used to collect lake floor sediment samples. The top layer of lacustrine sediment was extracted from the gravity corer and stored in sterile containers. For samples taken from the soils, swamps and streams in the surrounding catchment, organic matter was scraped away using a spatula sterilised in 80% ethanol before approximately 200g of the surface sediment was collected from the environment and stored in sterile containers.

To avoid contamination, nitrile medical gloves were worn at all times throughout sampling and equipment was sterilised with 85% ethanol between sampling. Upon return from the field, samples were frozen at -20°C until DNA extraction could be carried out.

3.3.2 DNA Extraction and Fingerprinting

DNA extraction was undertaken at the Cawthron Institute, Nelson, New Zealand. 0.25g of sediment was taken from each sample to undergo DNA extraction. The supernatant from each sample was discarded before DNA was extracted using the Power Biofilm DNA Isolation Kit (MoBio Laboratories) in accordance with the MO BIO Laboratories protocol manual (Appendix 2). The extracted DNA was then amplified by polymerase chain reaction (PCR) using bacterial primers ITSF 5′-TCGTAACAAGGTAGCCGTA-3′ and ITSReub 5’-GCCAAGGCATCCACC-3’ (Cardinale et al, 2004).

The PCR samples consisted of 20µM samples made up of 12.5µM Plat Super Master Mix, 0.4µM of each primer and 15-20ng of extracted DNA. The PCR samples were held at a temperature of 94°C for 3 minutes before running for 30 cycles at intervals of,

45 seconds at 94°C, 60 seconds at 52°C, 120 seconds at 72°C, and 7 minutes at 72°C. Samples were then held at a temperature of 10°C until samples were removed from the PCR.

To determine if the PCR worked and to discount contamination between samples within the PCR the samples were run through an argose gel. If the argose gel implied that samples had been contaminated or did not contain adequate quantities of DNA, the samples were rerun through the PCR. Amplified samples were then diluted with ultra-pure (18MΩ) milli Q water, 1:20, and sent to the Waikato DNA Sequencing Facility, University of Waikato, New Zealand, to undergo automated ribosomal intergenic spacer analysis (ARISA), as described below.

In accordance with the manufacture's protocol amplified lengths, containing 0.25µL of GS1200LIZ ZyStandard (Applied Biosystems) were run on GeneScan mode on an ABI 3130xI Genetic Analyzer for 45 minutes. This determines the size of fluorescence units. Using Applied Biosystems' PeakSannerTM software (version 1.0) electropherograms were processed. Peaks with more than 30 fluorescence units and made up at least 0.1% of the signal were included in the analysis.

3.3.3 Next Generation Sequencing

Next generation sequencing was undertaken in order to determine the species in the bacterial communities at the Karori Upper Dam and Lake Pounui. Using previously extracted DNA (Section 3.3.2) samples were prepared for this process at the Cawthron Institute, Nelson, New Zealand. However, due to laboratory delays, next generation sequencing results were not made available for this thesis. The methods for extraction are outlined in Appendix 3.

3.3.4 Statistical Analysis

Relative abundances and presence/absence data were determined from the ARISA fragment lengths using multidimensional scaling applied with PRIME 6 software (Clarke & Gorley, 2006). Multidimensional scaling (MDS) using the Bray Curtis Similarity method was also used to determine the variation of bacteria communities between sites. Detrended correspondence analysis (DCA), which determines gradient length, was used to determine if unimodal analysis or linear analysis should be used. Gradient lengths greater than four correspond to the use of canonical correlation

analysis (CCA), while gradient lengths of less than two correspond to the use of redundancy analysis (RDA). CCA and RDA tests were run with species data, and geochemical and environmental data to determine the environmental variables causing any variation produced by the MDS.

Statistical analysis using forward selection was undertaken to determine bacterial species that explain the most variation of branched GDGT abundances in lacustrine sediments.

3.4 Seasonal variability of environmental controls on the composition of bacterial communities within the water column

The second objective of this research, as stated in Chapter 1, was to determine the seasonal cycle of environmental controls on the composition of bacterial communities and corresponding lipids in the water column of two New Zealand lakes. This section will focus on the methods used to determine the relationship between bacterial communities and environmental controls at the Karori Upper Dam and Lake Pounui.

Both the Karori Upper Dam and Lake Pounui were sampled and monitored monthly to determine the seasonal cycle of environmental controls on GDGT distribution within the water column. The Karori Upper Dam was monitored monthly from February 2012 to December 2013 (23 months) as part of this project, with further monitoring continuing until July 2014 in order to gain a better understanding of the relationship between environmental controls and seasonal cycles.

3.4.1 Monitoring of environmental controls within the water column

In order to determine the seasonal cycle of environmental controls on the structure of bacterial communities and the composition of corresponding bacterial lipids the following variables were measured at 1m intervals down the water column using a Hanna multiparameter water meter:

- Temperature: Temperature is measured as it is thought to be the variable in which lipid composition, and relative abundances, primarily responds to.
- pH: The acidity or alkalinity of a lake environment controls which species can live within lake systems, thus influencing branched GDGT concentrations. As discussed in Section 2.2 Weijers et al. (2006) determined that soil pH was the second most influential environmental control on branched GDGTs in soils. Water pH was therefore measured as it could impact on lipid composition and relative abundances of branched GDGTs in lacustrine systems.
- Dissolved oxygen: Dissolved oxygen levels were measured to determine lake ecosystem stability which may impact bacterial lipid composition.
- Conductivity: Conductivity measures the capacity of water to conduct electricity. It is influenced by both organic and inorganic compounds and is

therefore a measure of water quality, which may influence the bacterial community structure and therefore the relative abundance of branched GDGTs.

- Total dissolved solids (TDS): TDS measures the total organic and inorganic substances in a water body and therefore serves as a measure of water quality.
- Salinity: Salinity measures the dissolved salt content of water bodies. Salinity has been determined to have no influence on the applicability of the TEX_{86} paleotemperature proxy in lacustrine systems, however, this still needs to be determined for the MBT/CBT proxy (Trommer et al, 2009).
- Oxygen reduction potential (ORP): ORP measures the ability of the lake to break down waste and pollution. A low ORP value could result in a change in bacterial species abundances and therefore lipid composition due to an accumulation of waste or pollutants.

A sechii disk reading, which measures water clarity, and a surface temperature recording were also measured at each site.

3.4.2 Sample Collection

Sample collection for this commenced in June 2012 and November 2012, at the Karori Upper Dam and Lake Pounui, respectively. ARISA analyses were used to determine the seasonal cycle of bacterial communities produced within the water column at the Karori Upper Dam and Lake Pounui. 300mL of surface water was extracted from the water column monthly (Table 3.3). Where water column monitoring suggested that the bacterial community may differ at depth, for example the existence of anoxic conditions or high temperature differences between surface water and bottom water, a bottom water surface sample was also taken.

Table 3.3: DNA water sampling monitoring programme for the Karori Upper Dam and Lake Pounui.

Location	Date Sampled	DNA Label	Depth in water column
Pounui	5/11/2012	S	Surface water
Pounui	16/01/2013	F	Surface shallow water
Pounui	18/02/2013	T	Surface water
Pounui	18/02/2013	H	Surface water
Pounui	8/04/2013	G	Surface water
Pounui	8/04/2013	I	Bottom water
Pounui	2/05/2013	J	Surface water
Pounui	2/05/2013	E	Bottom water
Karori	13/06/2012	Q	Surface water
Karori	19/07/2012	M	Surface water
Karori	17/08/2012	P	Surface water
Karori	24/09/2012	L	Surface water
Karori	15/10/2012	R	Surface water
Karori	15/11/2012	N	Surface water
Karori	11/12/2012	O	Surface water
Karori	18/02/2012	A	Surface water
Karori	18/02/2012	B	Bottom water
Karori	9/04/2013	C	Surface water
Karori	9/04/2013	D	Bottom water
Karori	9/05/2013	K	Surface water

Approximately 20mLs of the sample was then filtered through 0.025µm filter paper in preparation for DNA extraction.

To avoid contamination gloves were worn at all times throughout filtering and equipment was sterilised with 85% ethanol. Samples were then frozen at -20°C until DNA extraction could occur.

3.4.3 DNA Extraction and Fingerprinting

DNA extraction was undertaken at the Cawthron Institute, Nelson, New Zealand. The filter papers were removed from their containers and inserted into PowerBead Tubes. The papers underwent bead beating for two minutes to ensure that the DNA material was mixed into the solution present within the tubes. DNA was then extracted following the procedures listed in Section 3.2.2 before being sent to the University of Waikato to undergo ARISA to determine the variability in bacterial communities.

3.4.4 Next Generation Sequencing

As stated in Section 3.3.3 next generation sequencing was undertaken on these samples to determine the bacterial species present within the water column at the Karori Upper Dam and Lake Pounui. Using previously extracted DNA (Section 3.5.2) samples were prepared for this process at the Cawthron Institute. However, due to laboratory delays, next generation sequencing results were not made available for this thesis. The methods for extraction are outlined in Appendix 2.

3.4.5 Data Analysis

The origin of bacterial species identified within the water columns was determined using the following assumptions:

- species found in soil must be of soil origin,
- species found in streams but not soils must be of stream origin,
- species found in swamps but not soils or streams must be of swamp origin, and
- species found in the water column but not present in any terrestrial environment must be have originated from the water column.

These assumptions are based on the downstream relationship of the samples at both sites (refer to Figures 3.1 and 3.2).

To determine the most abundant *in situ* produced bacterial species within the water column relative abundances, generated by the analysis of ARISA data, for all species of all water column samples were summed at each site. The fifteen most abundant species were then identified to be the most significant species for water column samples. It is important to note that ARISA data are able to distinguish between bacterial species (and assigns individual species a unique code) but does not, by itself enable taxonomic identifications to be made. ARISA data species classifications are represented as numeric codes throughout this thesis.

The Shannon diversity index was used to calculate species richness and diversity in water column species at Lake Pounui only. The Shannon diversity index uses both the abundance and evenness of species present. An increase in the Shannon index equates to an increase in species abundance and even species distribution (Beals et al, 2000).

Species richness was then plotted against variables that were deemed significant in literature (temperature, pH, DO and conductivity) to determine the relationship between these environmental controls and bacterial community structure in the water column at Lake Pounui. This statistical test was not applied to the Karori Upper Dam due to spatial distribution results (Section 4.1.2) indicating no distinct *in situ* signal of bacteria produced within the water column.

3.5 Seasonal variability of environmental controls on the composition of bacterial lipids within the water column

The second objective of this research, as stated in Chapter 1, was to determine the seasonal cycle of environmental controls on the composition of bacterial communities and corresponding lipids in the water column of two New Zealand lakes. This section will focus on the methods used to determine the relationship between bacterial lipids and environmental controls at the Karori Upper Dam and Lake Pounui.

3.5.1 Sample Collection

Each water column site was monitored monthly at a predefined location representing the maximum depth of each site. This depth was determined using a depth sounder.

At each site 10L of water was taken 1m from the surface and 1m from the bottom of the lake using a Van Dorn water sampler. The water samples were then filtered through 1.2µm and 0.6µm filter paper to extract the organic matter containing lipid material from the water samples. The samples were then frozen for a minimum of three days before being freeze dried for at least 24 hours. This ensured that the liquid was completely removed from the material, allowing for the preservation of the lipid material. The samples were sent to the Institute of Geosciences, University of Kiel, Germany, to undergo geochemical screening and GDGT extraction and analysis.

3.5.2 GDGT Analysis

Geochemical analysis was carried out following the methodology given in Section 3.2.2. GDGT analysis of the water filtrates is currently under development by Dr Klaus Zink and Dr Lorenz Schwark (University of Kiel) and results from this were not available for this thesis.

3.5.3 Data Analysis

Comparing the GDGT data from surface sediment samples and DNA ARISA data from both surface sediments and the water column will allow us to make assumptions about seasonal changes in GDGT structure, diversity and origin. This will help determine the seasonal variation of branched GDGTs produced within the water and the relationship with environmental controls.

3.6 Summary

This section described the results of the methods used to determine research aims set out in Chapter 1. The methods used to determine the spatial variation of bacterial communities and branched GDGTs in lake catchments were first explored before discussing the methods used to determine the temporal variations of bacterial communities and lipids in lacustrine environments. Chapter 4 will go on to state the results of the aims set out in Chapter 1.

4 Results

This chapter outlines the results of the research aims set out in Chapter 1. Section 4.1 sets out the spatial variation of bacterial communities and branched GDGTs in lake catchments, and is followed by the results of temporal variations of bacterial communities in lacustrine environments in Section 4.2.

Both sections will first present the results of measured environmental controls before discussing the structure of bacterial communities. Both sections will then conclude with linkages to branched GDGTs and the MBT/CBT indices.

4.1 Spatial variation of bacterial communities and lipids in two New Zealand lake catchments

4.1.1 Geochemistry

The results from the geochemical analysis undertaken on both the Karori Upper Dam and Lake Pounui surface sediment samples are listed in Appendix 4. Here, carbon/nitrogen ratios will first be presented followed by stating total organic carbon (TOC), normalised extract yields and sulphur values for both the Karori Upper Dam and Lake Pounui.

Carbon/nitrogen (C/N) ratios calculated for the Karori Upper Dam lacustrine sediments ranged from 12.0 to 17.1, compared to values ranging from 12.0 to 22.0 in terrestrial sediments (Figure 4.1A). In comparison, C/N ratios for Lake Pounui ranged from 7.6 to 9.1 in lacustrine sediments and 8.2 to 24.4 in terrestrial sediments (Figure 4.1B).

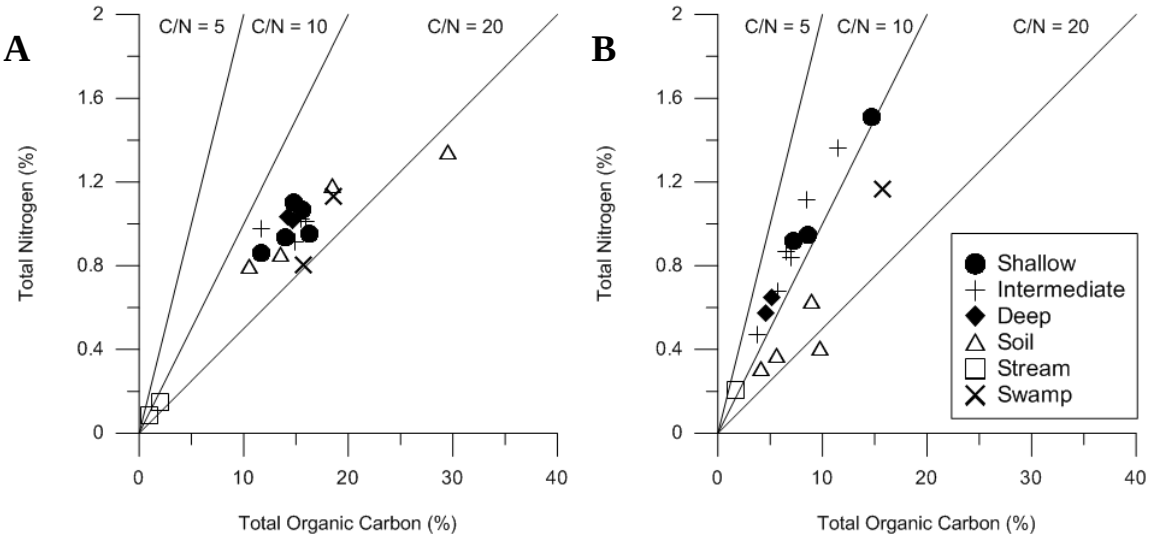

Figure 4.1: Carbon/Nitrogen ratio plots for the Karori Upper Dam (A) and Lake Pounui sediment samples (B).C/N ratios at the Karori Upper Dam showed little variation between environments whereas C/N ratios differed significantly between environments at Lake Pounui.

When plotted against total organic carbon (TOC), normalised extract yields calculated for lacustrine sediments at the Karori Upper Dam and Lake Pounui showed comparative differences (Figure 4.2). Lake sediment samples at the Karori Upper Dam clustered together with average mgExt/gTOC values of 57, 57 and 58 in shallow, intermediate and deep water sediments, respectively. Terrestrial sediments had distinctly lower mgExt/gTOC concentrations with average values of 40, 26 and 34 in soils, streams and swamps, respectively (Figure 4.2A).

Lacustrine sediments at Lake Pounui did not show strong clustering between depths with average mgExt/gTOC values of 81, 71 and 53 in shallow, intermediate and deep water sediments, respectively. Terrestrial sediments had varied mgExt/gTOC concentrations with average values of 53, 13 and 58 in soils, streams and swamps respectively (Figure 4.2B).

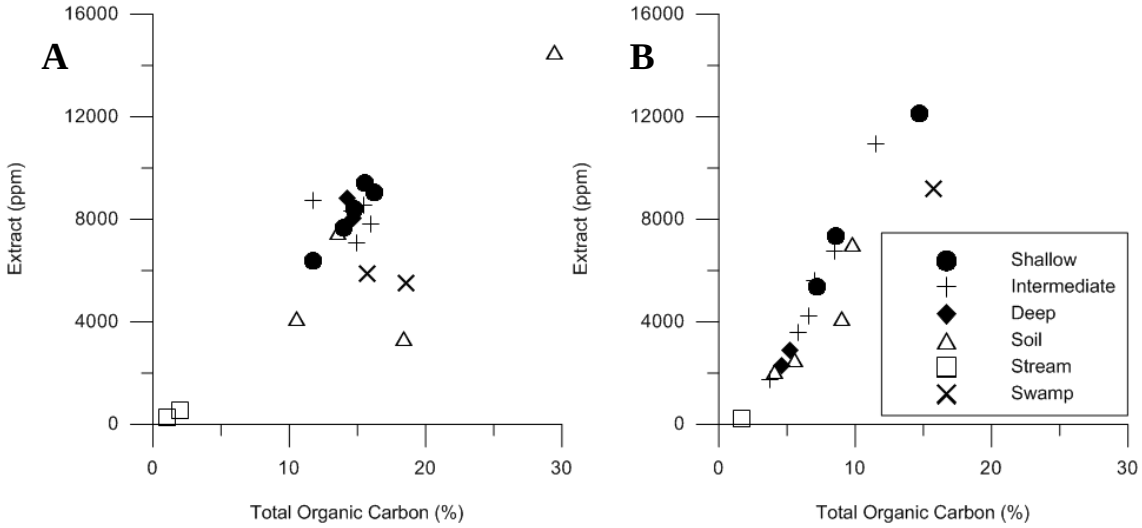

Figure 4.2: mgExt/gTOC plot for Karori Upper Dam (A) and Lake Pounui sediment samples (B). mgExt/gTOC extract yields were similar across sites and environments clustered at the Karori Upper Dam. Extract yields ranged at Lake Pounui and showed no distinct clustering.

Both the Karori Upper Dam and Lake Pounui possess low sulphur concentrations and carbon/sulphur (C/S) ratios with an average C/S ratio of 0.23 and 0.46 for the Karori Upper Dam and Lake Pounui, respectively (Figure 4.3). However, the Karori Upper Dam displayed a weak negative correlation between carbon and sulphur compared to Lake Pounui which has a positive correlation (Figure 4.3).

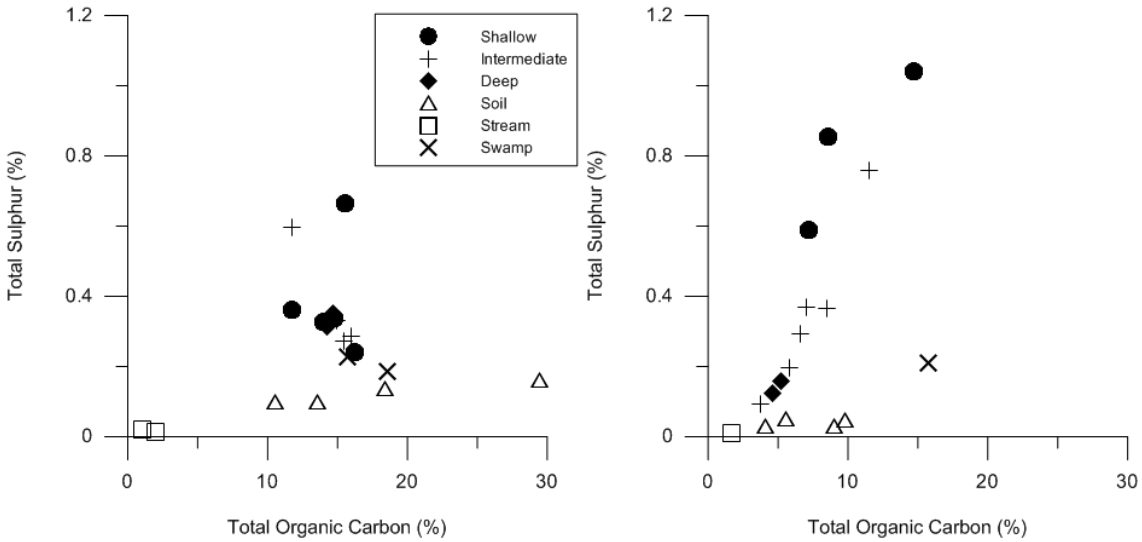

Figure 4.3: Carbon/Sulphur ratio plots for Karori Upper Dam (A) and Lake Pounui sediment samples (B). A weak negative correlation was observed in C/S ratios at the Karori Upper Dam. Comparatively a positive correlation was observed at Lake Pounui.

4.1.2 Bacterial species analysis

Relative proportions and presence/absence data (Appendix 5) were determined from the ARISA fragment lengths using multidimensional scaling (MDS) using PRIMER 6 software (Clarke & Gorley, 2006).

The MDS of ARISA data shows that the bacterial community structure differs between environments at the Karori Upper Dam and Lake Pounui. Terrestrial sediment samples from both sites cluster together and are distinct from the lacustrine samples from both the Karori Upper Dam and Lake Pounui indicating that the bacterial community structure in these environments are different (Figure 4.4).

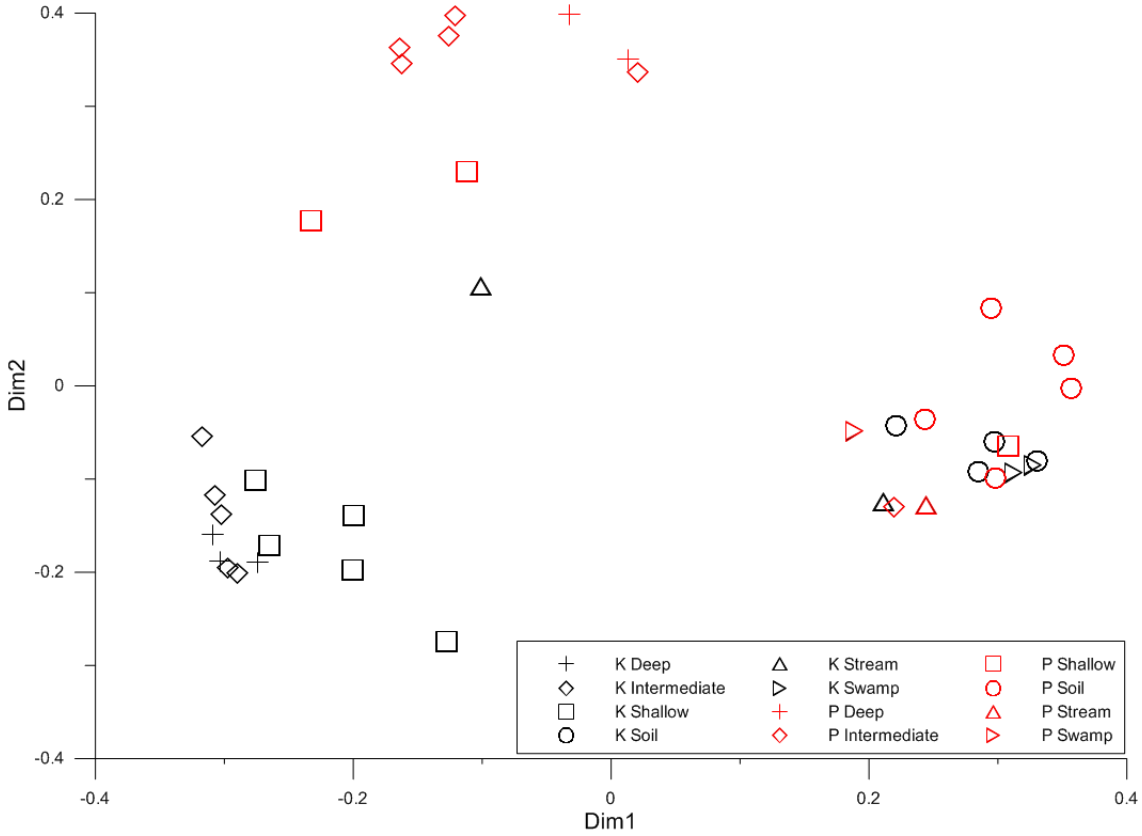

Figure 4.4: Multidimensional scaling plot of sediment sample sites at the Karori Upper Dam (black symbols) and Lake Pounui (red symbols).

Detrended correspondence analysis (DCA) of species and environmental controls for both the Karori Upper Dam and Lake Pounui produced a gradient length of 3.226, indicating that linear methods, such as redundancy analysis (RDA), would be more suitable for analysing this dataset than unimodal methods, such as canonical correlation analysis (CCA).

RDA results indicate that, based on the long vector lengths, temperature and TOC, which are negatively correlated, explain large percentages of the total variance of bacterial communities in lacustrine environments at the Karori Upper Dam and Lake Pounui (X axis, Figure 4.5). Based on vector length and direction, dissolved oxygen (DO) and pH appear positively correlated and explain a similar proportion of variance in bacterial community structure at the Karori Upper Dam and Lake Pounui.

The difference seen within environments at the Karori Upper Dam and Lake Pounui (y axis) appears to be driven by changes in an, as yet, unmeasured variable. It is assumed to be of geochemical origin due to total sulphur (TS) being the only measured variable that explains the only variation along the y-axis (Figure 4.5).

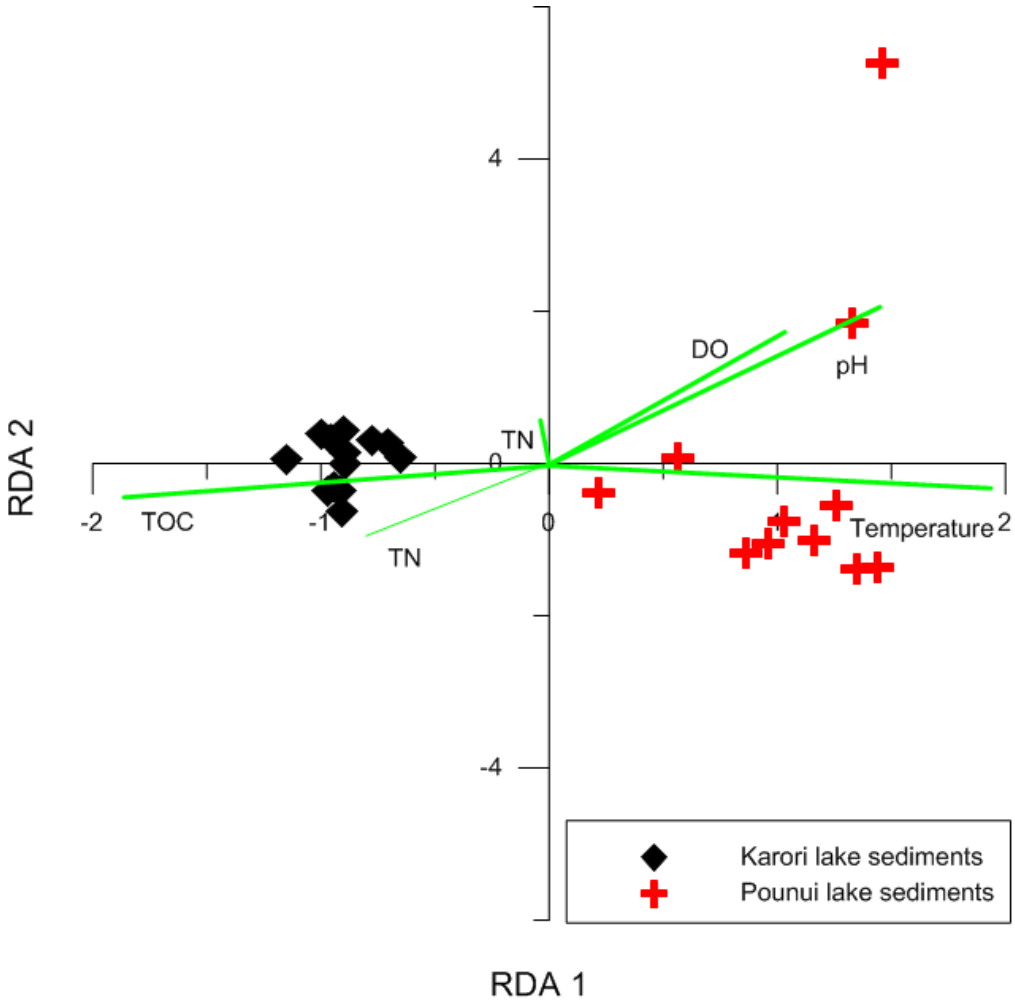

Figure 4.5: Species and environmental controls RDA plot for the Karori Upper Dam and Lake Pounui. Vector lengths (in green) explain the total variance of bacterial communities each environmental controls explains. This RDA shows that the bacterial communities at the Karori Upper Dam and Lake Pounui are different to one another.

Using relative abundance numbers produced by the ARISA data, the origin of bacterial species was determined under the following assumptions:

- species found in soil must be of soil origin,
- species found in streams but not soils must be of stream origin,
- species found in swamps but not soils or streams must be of swamp origin,
- species found in the water column but not present in any catchment environment must originate in the water column, and
- species found in lacustrine sediments but not present in any other environment must be produced in the lake sediments.

Based on these assumptions, Figure 4.6 shows the extent of terrestrial bacterial signatures of the Karori Upper Dam and Lake Pounui.

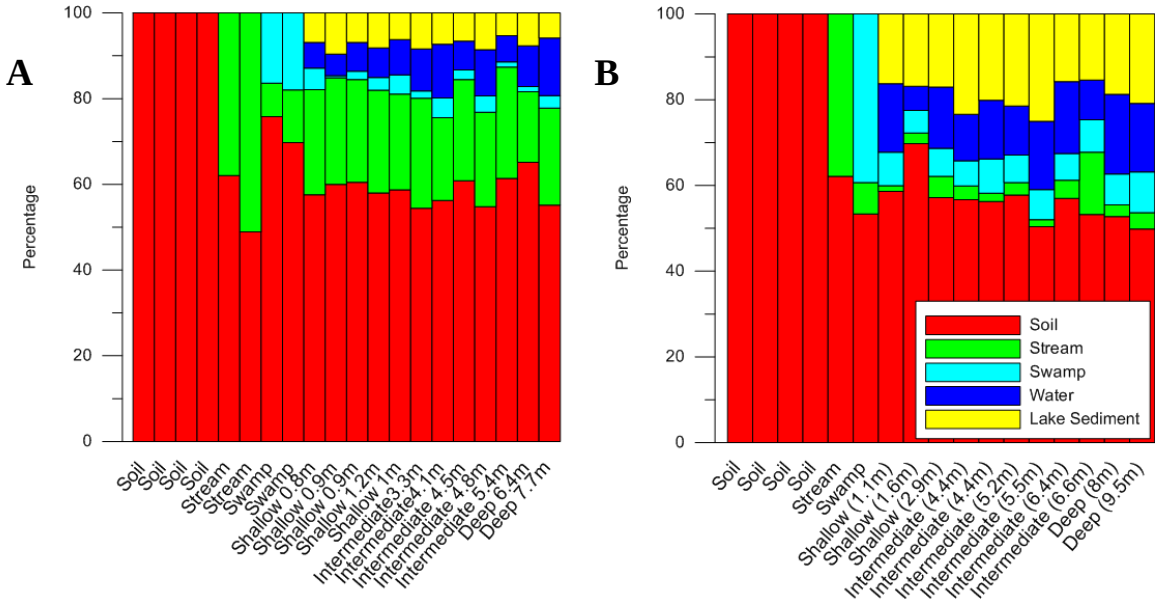

Figure 4.6: Origin of bacterial community structure of sediment samples from the Karori Upper Dam (A) and Lake Pounui catchments (B). All samples had a strong soil-originating bacteria signal.

All lacustrine sites at the Karori Upper Dam had a strong catchment signature: at least 80% of bacterial species found at these sites originated in environments from the catchment. In all lacustrine sediment samples, the majority of bacterial species originated from soil environments with the proportions of soil-originating bacteria ranging from 54.44 to 65.15%. Lake sediments at Lake Pounui also displayed a strong non-lake catchment signature, although not as pronounced as that of the Karori upper Dam. All lacustrine sites had a catchment signature of at least 59%. Similar to the Karori Upper Dam, the majority of bacterial species originated from soil environments with the percentage ranging from 49.81 to 69.78%.

There appears to be no noteworthy difference between the percentages of bacterial species originating from soil environments across bathymetric intervals at the Karori Upper Dam, with soil input ranging from 57.57 to 60.49% in shallow water samples compared to 55.12 to 65.15% in deep water samples (Figure 4.7). In contrast, soil-originating bacterial contributions at Lake Pounui appeared to differ noticeably across

bathymetric changes, with contributions decreasing with depth. The percentage of bacteria species originating from soil environments in shallow sediment samples ranged from 57.15 to 69.78% compared to 49.81 to 52.72% in deep water samples (Figure 4.6 and Figure 4.8).

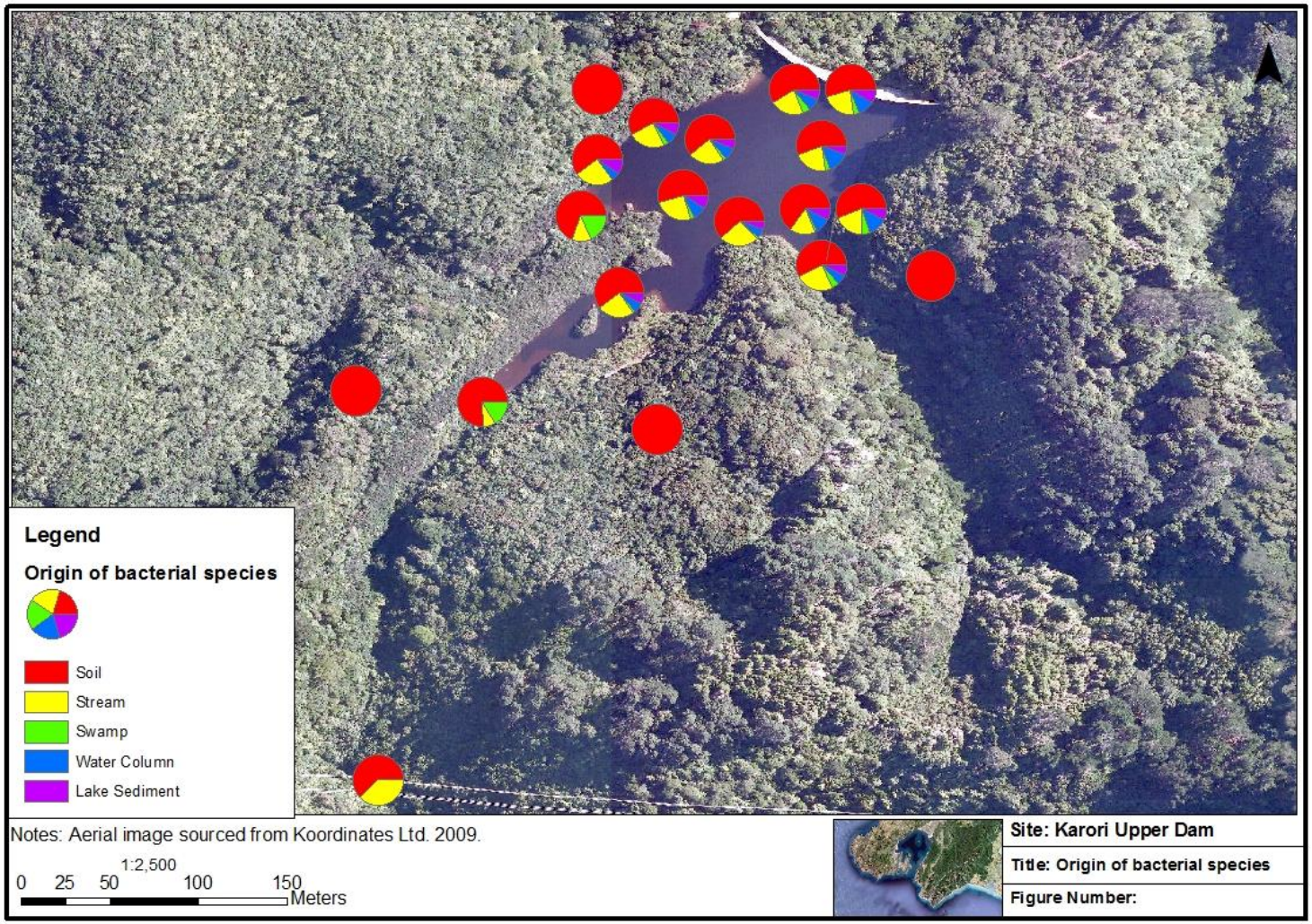

Figure 4.7: Origin of bacterial species at the Karori Upper Dam.

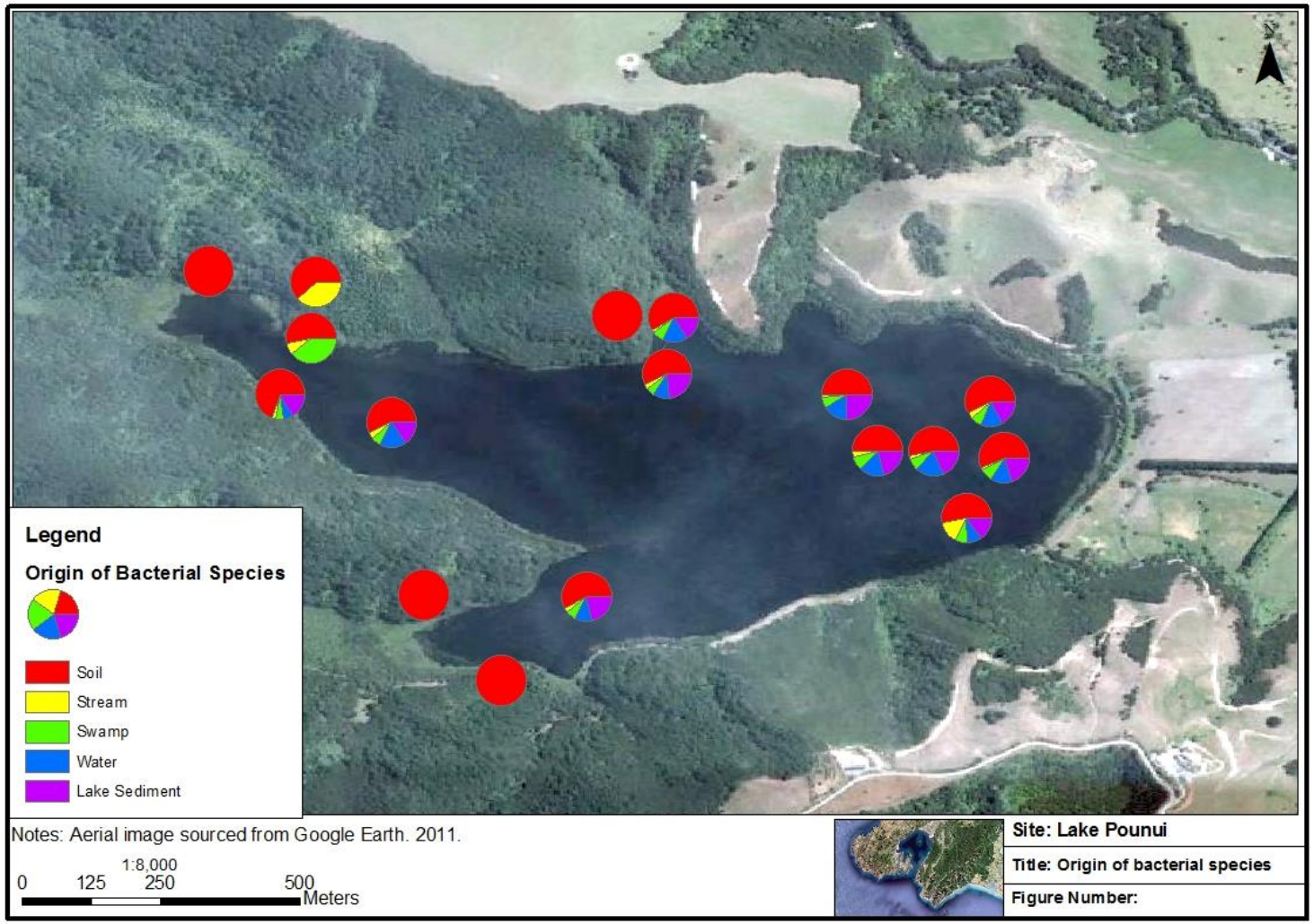

Figure 4.8: Origin of bacterial species at Lake Pounui.

Stream contributions of bacteria to lake sediments were considerably higher at the Karori Upper Dam than at Lake Pounui. Stream contributions at the Karori Upper Dam ranged from 16.46 to 26% compared to 1.33 to 14.51% at Lake Pounui. Stream originating species decreased over depth at the Karori Upper Dam with values ranging from 22.37 to 24.87% in shallow lake sediment samples compared to 16.46 to 22.67% in deep lake sediment samples. Depth appeared to have no effect on stream contribution at Lake Pounui with values ranging from 1.33 to 5% in shallow lake sediments compared to 2.74 to 3.81% in deep lake sediments.

At both the Karori Upper Dam and Lake Pounui, lake sediment samples had minimal swamp input. However, the lake sediment samples from the Karori Upper Dam had noticeably lower swamp contributions, with values ranging from 0.52 to 5.05%, than the Lake Pounui samples, with concentrations from 5.26 to 9.51%. Distance from shore appeared to have little influence on swamp contributions at the Karori Upper Dam with values decreasing minimally between shallow, intermediate and deep lake sediment samples. Swamp contributions at Lake Pounui increased over depth with average concentrations increasing from 6.53% in shallow lake sediments to 8.36% in deep lake sediments.

The Karori Upper Dam had considerably lower concentrations of lacustrine-originating species than Lake Pounui, with concentrations ranging from 5.29 to 9.58% compared to 15.4 to 25.04%, respectively. Depth appeared to have no influence on lake-derived bacteria at the Karori Upper Dam with little difference between concentrations in shallow, intermediate and deep samples (Appendix 6). Lake-derived bacteria concentrations in shallow sediment samples at Lake Pounui were slightly lower than intermediate and deep samples with concentrations ranging from 16.26 to 17.03% in shallow water sediment samples compared to a range of 18.76 to 20.9% in deep water sediment samples.

Species Abundance

The fifteen most abundant bacterial species in each environment were identified for the Karori Upper Dam (Table 4.1) and Lake Pounui (Table 4.2), with the proportion of species listed in brackets. No similarities or unique signatures were seen in the Karori Upper Dam. Three species, 722, 659.3 and 371.6, appeared in both surface water

samples and deep water samples (highlighted in red) however these species were not abundant in lacustrine sediments.

Species proportions varied slightly between environments at the Karori Upper Dam. Terrestrial sediments had lower species abundances compared to water samples and lake sediment samples with the most abundant terrestrial species having a proportion of 0.113 compared to a proportion of 0.183 in lacustrine sediments (Table 4.1). The most abundant deep water species was remarkably more abundant than the most abundant surface water species.

Table 4.1: Numeric codes for the fifteen most abundant bacterial species in each environment at Karori Upper Dam. Numbers in parentheses are percent abundances of species. Highlighted species are present in more than one environment.

Terrestrial Sediments	Surface Water Samples	Deep Water Samples	Lacustrine Sediments
391.3 (0.113)	589.7 (0.181)	700.5 (0.273)	692.5 (0.183)
695.9 (0.101)	722 (0.126)	722 (0.192)	550.5 (0.098)
666.4 (0.084)	659.3 (0.085)	732.7 (0.179)	326.7 (0.094)
393.6 (0.080)	803.5 (0.077)	804.2 (0.092)	254 (0.076)
291 (0.075)	455.9 (0.074)	724 (0.041)	648.4 (0.074)
706.2 (0.075)	590.5 (0.059)	699.4 (0.035)	378.8 (0.069)
274.5 (0.071)	786 (0.057)	371.6 (0.031)	675.3 (0.054)
321.9 (0.058)	371 (0.048)	756.7 (0.029)	530.7 (0.050)
499.3 (0.057)	371.6 (0.045)	507.8 (0.022)	621.4 (0.050)
523.9 (0.054)	768.8 (0.044)	508.5 (0.020)	272.5 (0.044)
675.3 (0.048)	317.3 (0.043)	398.1 (0.019)	260.8 (0.044)
470.4 (0.047)	755.8 (0.043)	588.9 (0.018)	545.7 (0.042)
439.2 (0.046)	689.6 (0.042)	659.3 (0.018)	293.7 (0.041)
110.8 (0.045)	409.1 (0.039)	761.2 (0.015)	517.8 (0.040)
400.1 (0.045)	816.7 (0.037)	803.5 (0.015)	552.7 (0.040)

Species abundances showed a distinct water column *in situ* signal at Lake Pounui (Table 4.2). Eleven of the most abundant species in the surface water column were also present in deep water samples (highlighted in red). However, only one of these species, 378.8, was present in lacustrine sediments. No species abundant in terrestrial sediments were abundant in either the water column or lacustrine sediments.

Whereas species proportions varied markedly across the different environments at the Karori Upper Dam, proportions in environments at Lake Pounui were similar, with a maximum proportion of 0.146 in terrestrial sediments compared to 0.106 in lacustrine sediments. Surface and deep water samples also had similar proportions with a maximum proportion of 0.124 and 0.147 in surface and bottom waters, respectively.

Species 659.9 is the second most abundant species in terrestrial sediments at the Karori Upper Dam and the most abundant species in lacustrine sediments at Lake Pounui.

Table 4.2: Numeric codes for the fifteen most abundant bacterial species in each environment at Lake Pounui. Numbers in parentheses are percent abundances of species. Highlighted species are present in more than one environment.

Terrestrial Sediments	Surface Water Samples	Deep Water Samples	Lacustrine Sediments
393.6 (0.146)	476.1 (0.124)	658.1 (0.147)	695.9 (0.106)
686.6 (0.082)	408.8 (0.101)	372.2 (0.107)	254 (0.095)
358.2 (0.079)	556.2 (0.083)	476.1 (0.104)	621.4 (0.088)
391.3 (0.072)	658.1 (0.077)	409.1 (0.088)	308.9 (0.085)
629.5 (0.066)	378.8 (0.075)	498.5 (0.083)	160.9 (0.082)
685.8 (0.063)	657.7 (0.074)	408.8 (0.079)	317.3 (0.080)
438.1 (0.060)	498.5 (0.071)	378.8 (0.066)	550.5 (0.065)
649.5 (0.059)	755.8 (0.067)	858.2 (0.061)	445.4 (0.058)
402.7 (0.058)	380.3 (0.056)	424.7 (0.051)	571.2 (0.055)
891.9 (0.057)	858.2 (0.051)	648.2 (0.045)	378.8 (0.054)
428.4 (0.055)	908.5 (0.047)	556.2 (0.041)	804.2 (0.051)
440.9 (0.054)	475.6 (0.045)	419 (0.036)	552.7 (0.046)
262.5 (0.052)	409.1 (0.045)	755.8 (0.033)	547.2 (0.046)
278.2 (0.050)	381.2 (0.044)	380.3 (0.030)	555.3 (0.045)
410.5 (0.049)	348.2 (0.041)	856.1 (0.029)	211.2 (0.044)

4.1.3 Spatial variation in GDGTs

4.1.3.1 GDGT Characteristics

Branched and isoprenoid GDGT concentrations were determined for all sediment samples taken from the Karori Upper Dam and Lake Pounui. Isoprenoid GDGT concentrations were low at both sites compared to branched GDGT concentrations (Figure 4.9). Catchment environments at both the Karori Upper Dam and Lake Pounui had consistently lower abundances of isoprenoid GDGTs compared to lacustrine environments. At the Karori Upper Dam isoprenoid GDGT abundances ranged from 2.75 to 12.26% in catchment sediments compared to a range of 14.9 to 17.3% in lake sediments. Similarly, isoprenoid GDGT concentrations in catchment sediments at Lake Pounui ranged from 3 to 8.9% compared to a range of 13.9 to 19.6% in lake sediments.

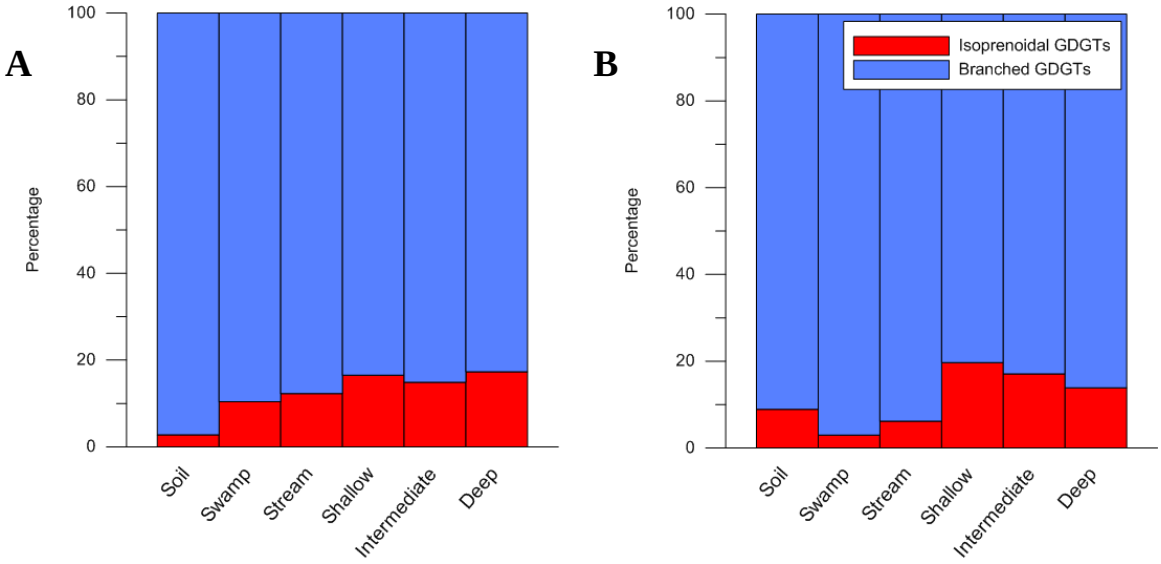

Figure 4.9: Isoprenoid and Branched GDGT concentrations for each environment, Karori Upper Dam (A) and Lake Pounui (B). All samples had distinctly low isoprenoid GDGT concentrations.

Branched and Isoprenoid Tetraether (BIT) indices for both the Karori Upper Dam and Lake Pounui were high (0.89-0.1), further indicating that these sites were dominated by branched GDGT concentrations (Appendix 7, Table 2 and Table 4).

4.1.3.1.1 Isoprenoid GDGTs

Isoprenoid GDGTs were found in all sediment samples (Figure 4.10 and Figure 4.11). Crenarchaeol was the dominant isoprenoid GDGT in soil and swamp sediments with an average concentration of 52.8% (soils), 20.9% (swamps) at the Karori Upper Dam, and 36.5% (soils), and 40.8 (swamps) at Lake Pounui. GDGT-0 was the second most abundant isoprenoid GDGT in these environments at both sites. Average GDGT-0 concentrations in soil and swamp sediments were 20.9 and 32.8% at the Karori Upper Dam, and 37.9 and 35.5% at Lake Pounui.

GDGT-0 was the dominant isoprenoid GDGT in lacustrine sediments at the Karori Upper Dam and Lake Pounui with a relative abundance of at least 67% in all lake sediment samples. All other isoprenoid GDGTs were of little abundance in lacustrine sediments with the abundance of these GDGTs ranging from 0.1 to 13%.

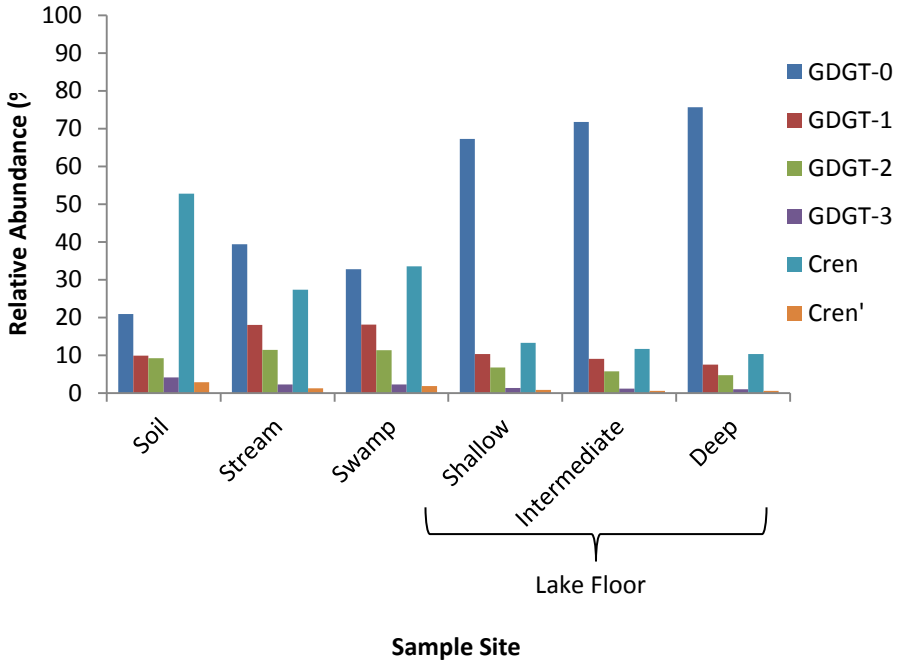

Figure 4.10: Isoprenoid GDGT relative abundances of environment types at the Karori Upper Dam.

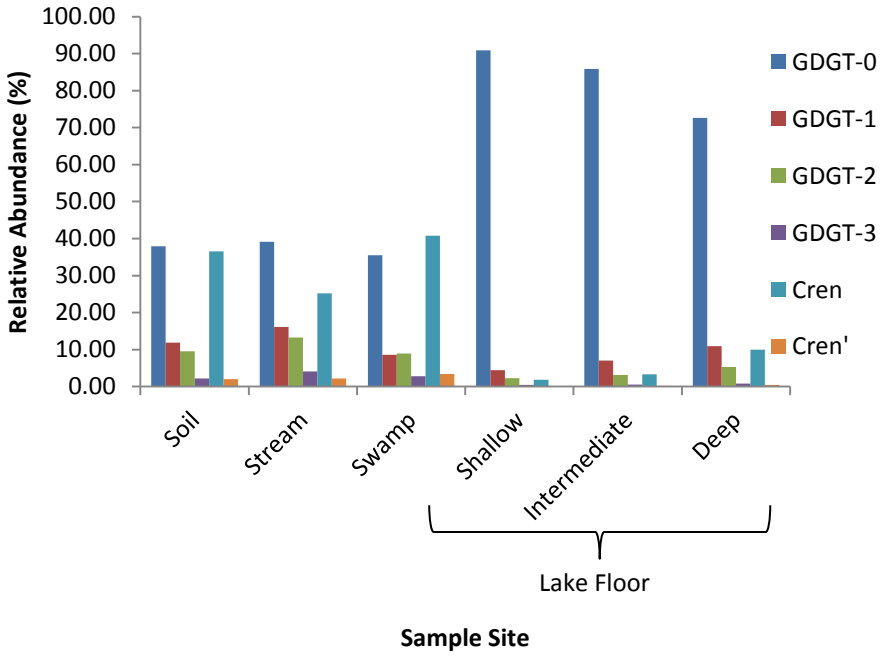

Figure 4.11: Isoprenoid GDGT relative abundances of environment types at Lake Pounui.

4.1.3.1.2 Branched GDGTs

Branched GDGTs were found in all sediment samples from the Karori Upper Dam and Lake Pounui, with GDGTs without cyclopentyl moieties most abundant in all environments (series "a"; Figure 4.12 and Figure 4.13).

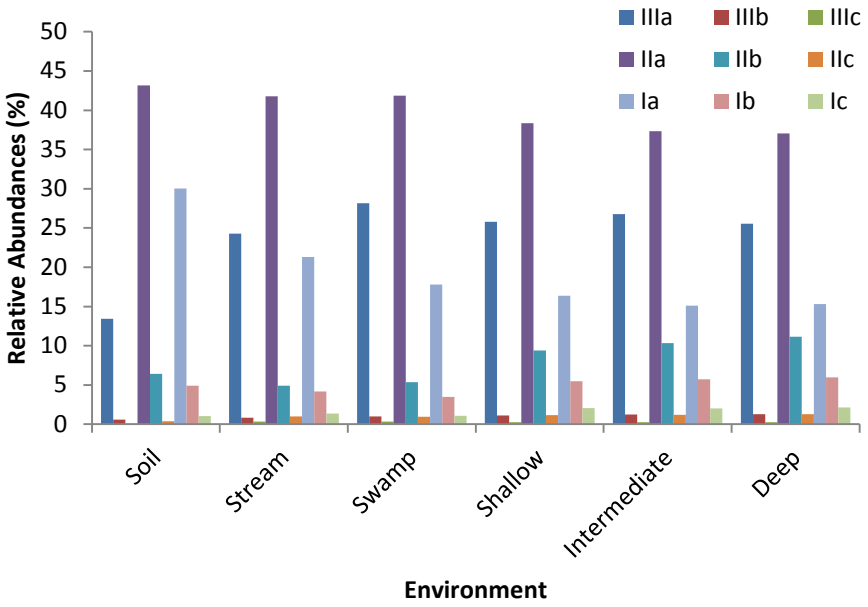

Figure 4.12: Branched GDGT relative abundances of environment types at the Karori Upper Dam.

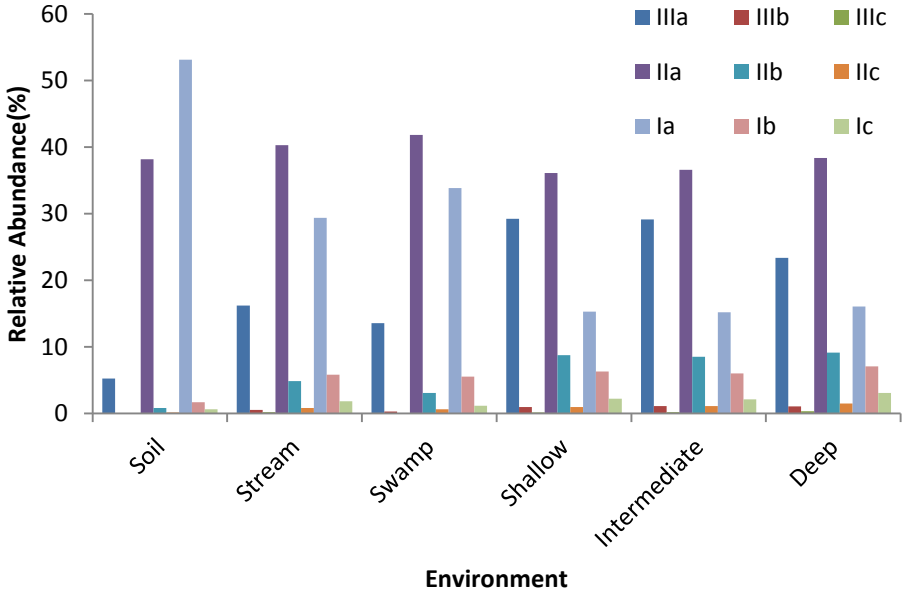

Figure 4.13: Branched GDGT relative abundances of environment types at Lake Pounui.

Relative abundances of GDGTs IIIb, IIIc, IIb, IIc, Ib and Ic were low in all sediment samples at both the Karori Upper Dam and Lake Pounui (Appendix 7, Table 1 and Table 2).

The most abundance branched GDGT in all soil sediments at the Karori Upper Dam, excluding one soil sample, was GDGT IIa with abundances ranging from 39% to 46%. Comparatively GDGT Ia was the most abundant GDGT in soils at Lake Pounui with concentrations ranging from 48.9% to 61.8%. Although still of low abundance at both the Karori Upper Dam and Lake Pounui, GDGTs Ib, IIb and IIIc were considerably more abundant in Karori Upper Dam soils compared to the soils at Lake Pounui (Table 4.3).

Table 4.3: The average percent of GDGTs Ib, IIb and IIIa in soil sediments at the Karori Upper Dam and Lake Pounui.

	Average %
Karori Upper Dam	
GDGT Ib	4.89
GDGT IIb	6.42
GDGT IIIa	13.43
Lake Pounui	
GDGT Ib	1.71
GDGT IIb	0.83
GDGT IIIa	5.24

GDGT IIa was the most abundant lipid in swamp and stream environments at both the Karori Upper Dam Lake Pounui with an average concentration of 42% and 41% at the respective sites. The second and third most abundant GDGTs in stream and swamp environments at the Karori Upper Dam were GDGT IIIa with an average concentration of 26.2% and GDGT Ia with an average concentration of 19.7%. All other branched GDGTs had low concentrations in these environments with average concentrations ranging from 0.39% (GDGT IIIc) to 5.1% (GDGT IIb).

Stream and swamp environments had similar branched GDGT abundances at Lake Pounui. GDGTs Ia and IIIa were the second and third most abundant branched GDGT in both environment with an average concentration of 31.6% and 14.9% respectively Similarly to the Karori Upper Dam, all other branched GDGTs at Lake Pounui were of

low abundance with concentrations ranging from 0.13% (GDGT IIa) to 5.7% (GDGT Ib).

GDGTs Ic, IIc, IIIb and IIIc accounted for less than 1.8% of the relative abundance of branched GDGTs in terrestrial sediments at both sites.

GDGT IIa and IIIa were the most abundant lipids in lacustrine sediments at both the Karori Upper Dam and Lake Pounui (Figure 4.12 and Figure 4.13). Abundances appear consistent across the lake floor with little variation seen between shallow, intermediate and deep lake sediments (Table 4.4).

Table 4.4: Average GDGT relative abundance in lacustrine sediments at the Karori Upper Dam and Lake Pounui.

	IIa	IIIa
Karori Upper Dam		
Shallow	38.36	25.79
Intermediate	37.15	26.85
Deep	37.08	25.55
Lake Pounui		
Shallow	36.19	29.46
Intermediate	38.28	23.43
Deep	36.55	29.18

GDGTs Ib, Ic, and IIb were consistently more abundant in lacustrine sediments compared to soil sediments at the Karori Upper Dam and Lake Pounui with deep sediments consistently having the most abundant concentration of each listed GDGT (Figure 4.14). GDGTs Ib, Ic and IIb were consistently higher in soils at the Karori Upper Dam compared to concentrations in soils at Lake Pounui.

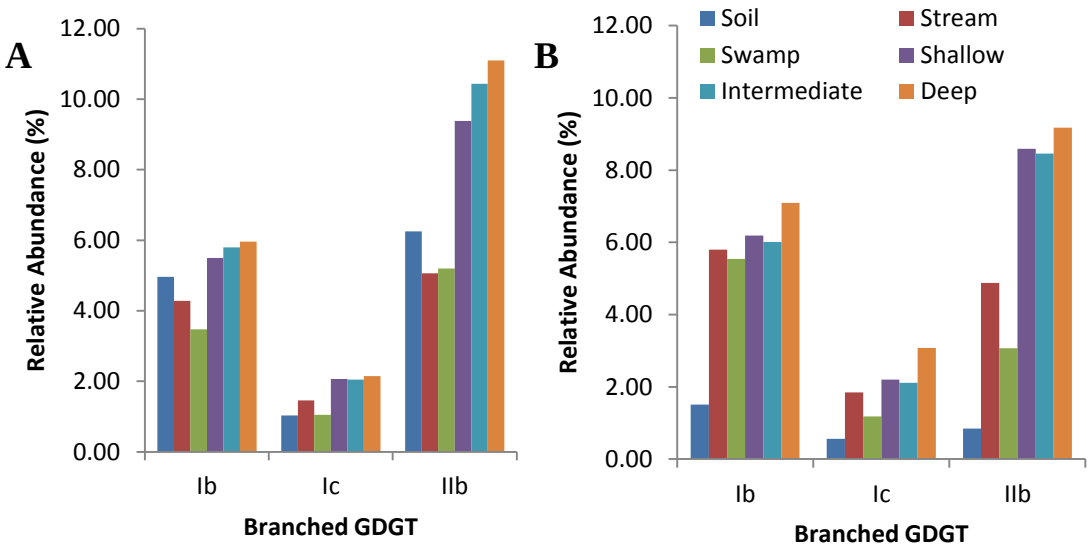

Figure 4.14: Comparisons of branched GDGTs Ib, Ic and IIb in lacustrine and terrestrial sediments at the Karori Upper Dam (A) and Lake Pounui (B) as a proportion of total GDGTs. GDGTs Ib, Ic and IIb were all consistently higher in soils compared to other environments.

4.1.3.2 Spatial Variation

To further assess the spatial variation of branched GDGTs across environments, MBT/CBT values were calculated for both sites (Figure 4.15).

MBT/CBT values calculated for the Karori Upper Dam showed little difference between lacustrine and soil environments with an average value of 0.47 in deep lake sediments compared to an average value of 0.45 in soils. Stream and swamp environments, however, displayed a unique signal with an average value of 0.33 and 0.27 respectively. Due to the similarity between values, there was no distinct clustering between environments at the Karori Upper Dam (Figure 4.15A).

Comparatively, MBT/CBT values calculated for Lake Pounui showed a clear distinction between branched GDGTs found in soil environments and those found in lacustrine sediments (Figure 4.15 B). The stream and swamp samples clustered between these two environments.

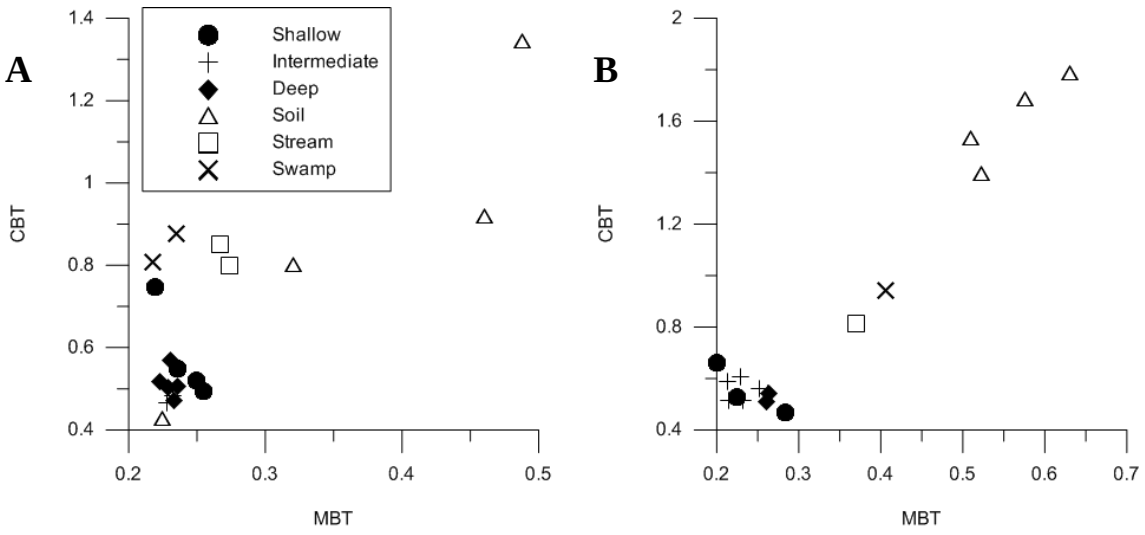

Figure 4.15: MBT versus CBT for the Karori Upper Dam (A) and Lake Pounui (B). MBT versus CBT values clustered distinctly between environments at Lake Pounui. No similar pattern was observed for samples taken from the Karori Upper Dam.

Depth appeared to have no influence at either site on MBT or CBT values at Lake Pounui with calculations consistent regardless of bathymetry (Appendix 7, Table 2 and Table 4).

4.1.3.3 Relationship between branched GDGT abundances and environmental controls

This section will explore the relationship between environmental controls and branched GDGT abundances in lacustrine sediments at the Karori Upper Dam and Lake Pounui. To determine this relationship, statistical analysis was undertaken on temperature, pH, DO and conductivity, environmental variables that previously have been deemed important to consider when using the MBT/CBT proxy (Pearson et al, 2011).

Pearson's correlation analysis indicated that DO and conductivity were highly correlated with temperature and pH. DO and conductivity therefore were excluded from analysis at the Karori Upper Dam and Lake Pounui. Analysis of variance (ANOVA) was used to determine the unique variance of temperature and pH and the significance on branched GDGT concentrations at the Karori Upper Dam. Results from the ANOVA indicated that at the Karori Upper Dam, temperature explained a unique and significant portion of variance; pH, however did not (Figure 4.16A). Comparatively, although both temperature and pH explained unique variance, neither of these variables explained a significant portion of variance in GDGT abundances at Lake Pounui (Figure 4.16B).

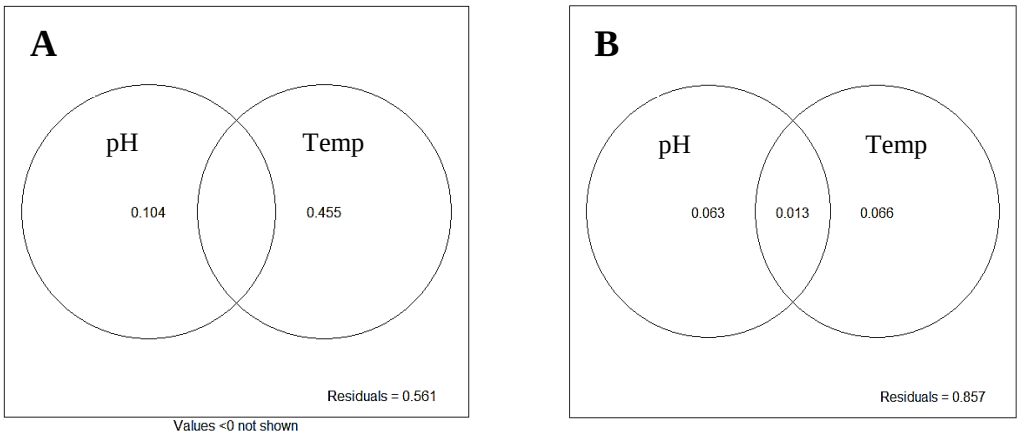

Figure 4.16: Branched GDGT variance explained by pH and temperature at the Karori Upper Dam (A) and Lake Pounui (B). The number in each circle represents the variance explained by the pH and Temperature. The middle number refers to the variance explained by both pH and Temperature. Temperature explained a unique and significant portion of variance at the Karori Upper Dam.

4.1.4 Bacterial Community and GDGT Linkages

Statistical analysis using forward selection was carried out to determine the bacterial species that are significant in relation to branched GDGT abundances in lacustrine sediments (Table 4.5).

All species at the Karori Upper Dam and Lake Pounui had different variance inflation factors lower than 20 (Table 4.5), indicating that each significant species in the lake sediments explained unique variation in branched GDGT abundances (ter Braak & Šmilauer, 2002)

Table 4.5: Summary of the statistically significant bacteria at the Karori Upper Dam and Lake Pounui and the variance explained by each species.

Karori Upper Dam		Lake Pounui	
Species	Variance	Species	Variance
245.2	4.03	391.3	2.21
666.4	1.43	722	1.58
465.6	1.675	390	2.69
731.8	2.44	344.8	1.41
479.3	5.08	581.6	2.92
387.6	2.27	521.8	2.57
786	4.1	343.7	1.84
480.8	4.93		

4.1.4.1 Karori Upper Dam

Species 245.2 was only found in lacustrine sediments suggesting it only lives in this environment. Species 731.8 was also only identified in lake sediments suggesting that this is also l within this environment. Species 786 was only identified in water samples and lacustrine sediments suggesting that this species only lives within the water column. Section 4.2.2 will elaborate this further. Species 666.4 was identified in soils, streams and swamps but only one intermediate lake sample, suggesting that this species is living in the catchment surrounding the lake. All other species were identified in both the lake and surrounding catchment and therefore further research would need to be undertaken in order to determine their provenance.

Forward selection identified that species 666.4 appears to have a strong relationship with GDGT IIa and GDGT Ia at the Karori Upper Dam (Figure 4.17). Species 465.9 appears to explain variation in the relative abundances of GDGTs Ib and Ic in lake sediments at this site.

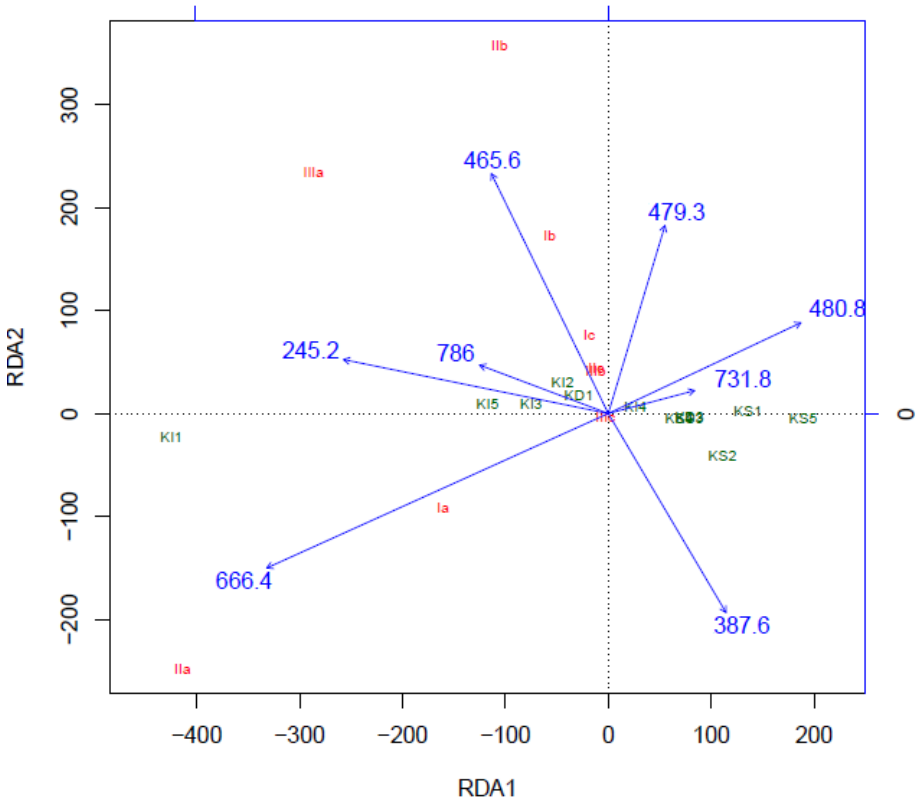

Figure 4.17: Forward selection model for the Karori Upper Dam. Species 666.4 has a strong relationship with GDGT IIa and GDGT Ia. Species 465.6 aligned with GDGTs Ib and Ic at this site. Green labels refer to the sampling sites as defined in Appendix 4. Red labels refer to the branched GDGTs.

4.1.4.2 Lake Pounui

Species 390, which explained 2.69% of branched GDGT variance, was only found in lacustrine sediments suggesting it is produced in this environment. Species 521.8, with a variance of 2.51%, was also only identified in lake sediments suggesting that this is also produced within this environment. Species 581.6 was only identified in water samples and lacustrine sediments suggesting that this species is produced within the water

column. Section 4.2.2 will elaborate on this further. All other species were identified in both the lake and surrounding catchment and therefore further research would need to be undertaken in order to determine their provenance.

Forward selection identified that species 722 appears to have a strong relationship with GDGT Ic (Figure 4.18).

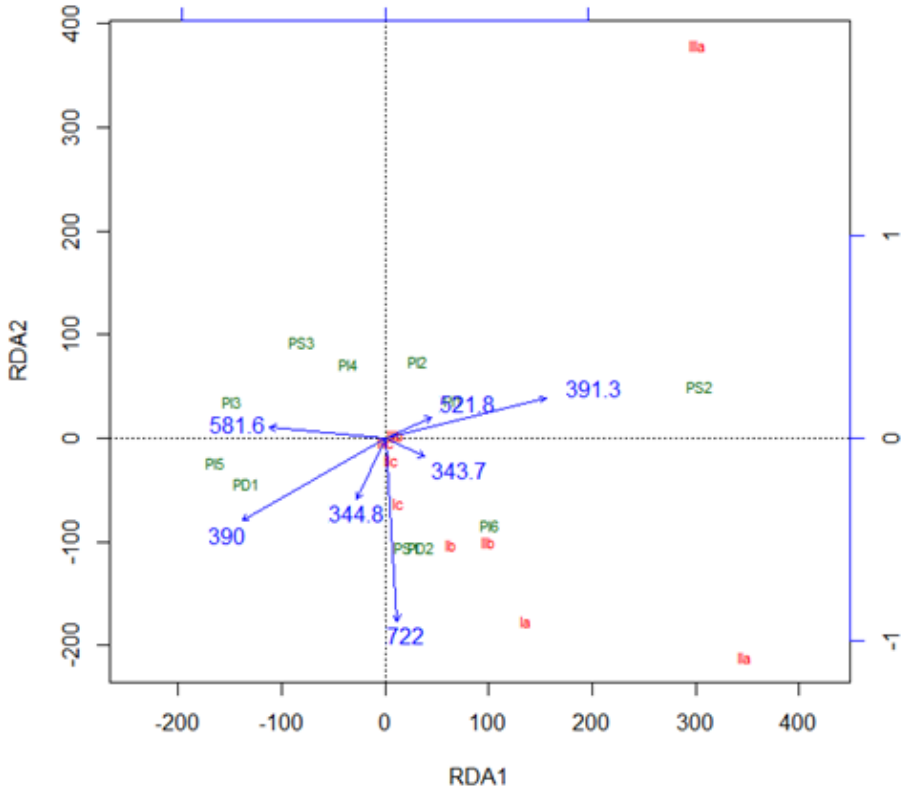

Figure 4.18: Forward selection model for Lake Pounui. Species 722 aligned with GDGT Ic at this site. Green labels refer to the sampling sites as defined in Appendix 4. Red labels refer to the branched GDGTs.

4.2 Seasonal variations of lake water bacteria and bacterial lipids and their relationship with environmental controls

Environmental controls within the water column were measured monthly in order to ascertain any seasonal patterns that could influence the abundance and structure of bacterial communities and concentrations of branched GDGTs within lacustrine environments. This section will explore the seasonal fluctuations of environmental controls and bacterial species within the water column.

4.2.1 Seasonal Fluctuations of Environmental Controls

4.2.1.1 Temperature

Monitoring of the Karori Upper Dam was undertaken over a 22 month period, March 2012 to December 2013. Temperature data retrieved from the water column showed strong seasonal variations. The water column at the Karori Upper Dam was strongly stratified, with a strong thermocline present, in summer months with temperatures ranging from 16.8°C to 18.55°C at a depth of 1m, compared to a temperature range of 12.3°C to 14.2°C at a depth of 8m (Figure 4.19A, Appendix 8).

Temperature depth profiles, however, indicated little temperature variation throughout the water column during winter months, June to August, with a temperature range of 7.6°C to 9.6°C at a depth of 1m, compared to a temperature range of 7.3°C to 9.0°C at a depth of 8m.

Monitoring at Lake Pounui was undertaken for a 13 month period, commencing from November 2012 to December 2013. Temperature logger data retrieved at 1m intervals in the water column, and monthly monitoring data at Lake Pounui recorded vast temperature variations between summer and winter months. However, no substantial thermocline appears present with little variation in temperatures throughout the water column with a summer temperature range of 18.8°C to 21.3°C at a depth of 1m compared to a range of 17.3°C to 20.2°C at a depth of 9m (Figure 4.19B).

Water temperatures were consistently cooler in winter months; however, as was the case in summer months, there was limited temperature variation throughout the water column. Winter temperature ranged from 9.2°C to 11.2°C at a depth of 1m compared to a range of 9.2°C to 11.1°C in bottom waters. The similarity in temperature values

throughout the water column indicates that Lake Pounui is well-mixed throughout both summer and winter months.

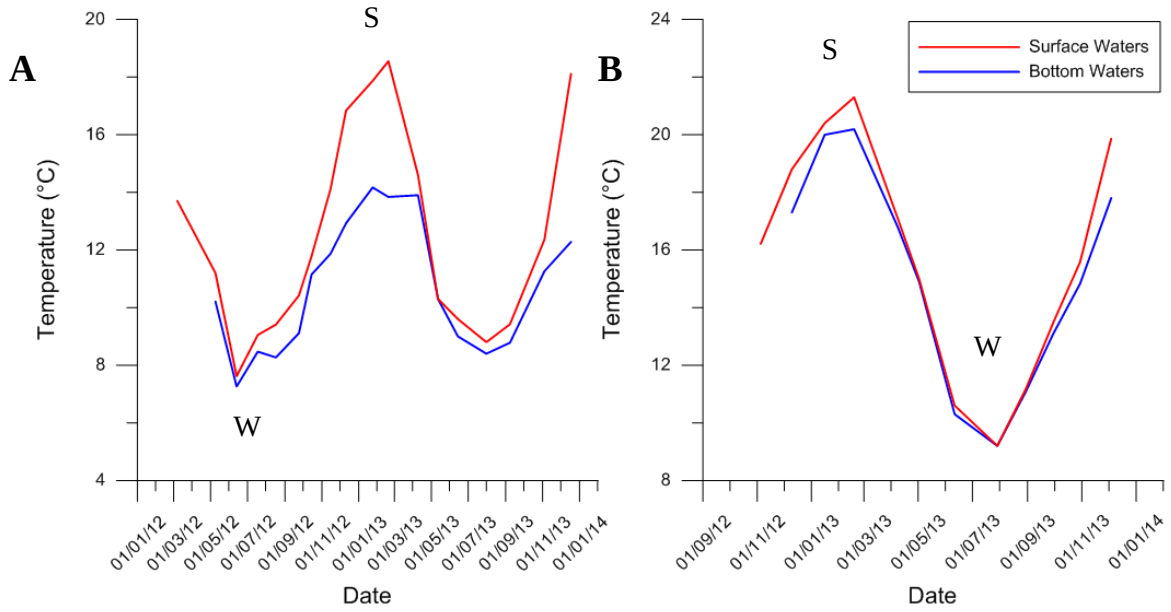

Figure 4.19: Surface and bottom water temperature at the Karori Upper Dam (A) and Lake Pounui (B) over the monitoring period. Surface waters refer to a measurement taken at a depth of 1m, bottom waters refer to a measurement taken at a depth of 8m at the Karori Upper Dam and 9m at Lake Pounui. Measurements showed strong seasonal fluctuations over the monitoring period at both sites. "S" refers to summer peak measurements, "W" refers to minimum winter measurements.

4.2.1.2 pH

Due to missing data[2], pH values will only be considered from samples taken between November 2012 and the end of the monitoring period at the Karori Upper Dam (Figure 4.20A). pH values varied both seasonally and throughout the water column at the Karori Upper Dam. pH values were consistently higher in summer months, December to February, with stratification present with a pH range of 7.6 to 8.98 and 6.42 to 6.8 at a depth of 1m and 8m, respectively.

pH values were generally lower in winter months, June to August, at the Karori Upper Dam. pH values ranged from 4.9 to 8.23 at a depth of 1m, and 4 to 7.41 at a depth of 8m.

[2] pH probe was not working on the water meter for this time period.

pH levels for samples from Lake Pounui will only be considered from 10/12/2012 to 4/12/2013, again as a result of missing data[3]. pH values varied during the monitoring period both seasonally and throughout the water column (Figure 4.20B). The maximum recorded pH value at a depth of 1m was 9.53, recorded on 18/02/2013, compared to the minimum value of 7.01, recorded on 30/08/2013.

At a depth of 9m the maximum and minimum pH values were 7.4 (29/07/2013) and 6.58 (18/02/2013), respectively.

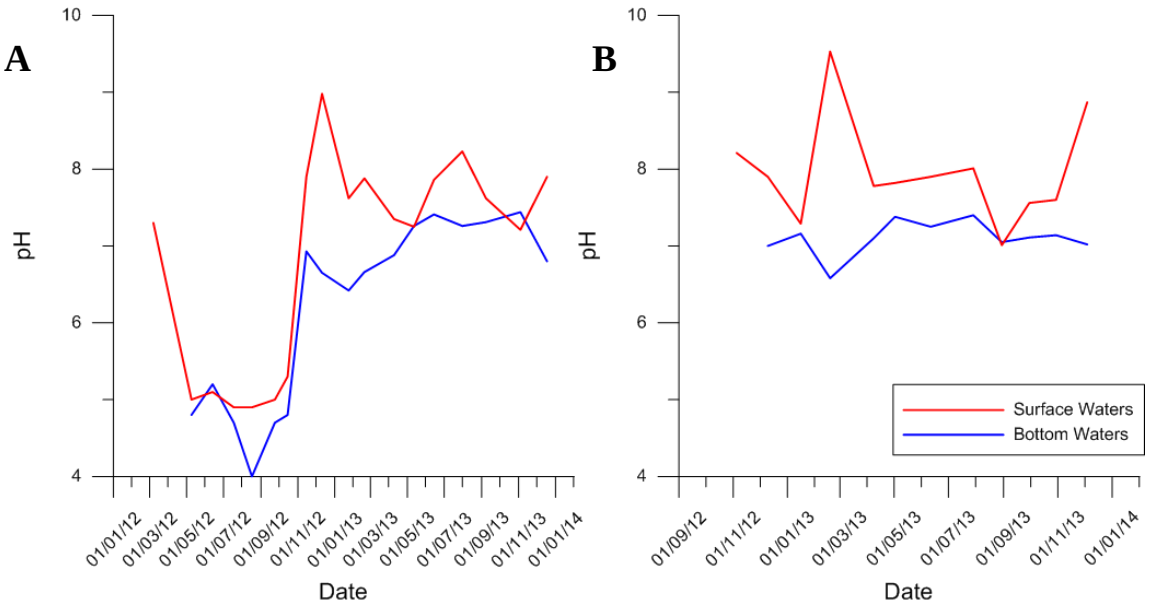

Figure 4.20: Surface and bottom water pH at the Karori Upper Dam (A) and Lake Pounui (B) over the monitoring period. Surface waters refer to a measurement taken at a depth of 1m, bottom waters refer to a measurement taken at a depth of 8 and 9m at the Karori Upper Dam and Lake Pounui. Measurements showed fluctuations over the monitoring period at both sites.

4.2.1.3 Dissolved Oxygen

Dissolved oxygen (DO) levels had strong variations both seasonally and throughout the water column at the Karori Upper Dam and Lake Pounui (Figure 4.21).

DO concentrations were consistently higher in surface waters compared to bottom waters at the Karori Upper Dam. Summer DO levels ranged from 5.33 to 8.33 mg/l at a

depth of 1m. Anoxic conditions were recorded in all summer months (December to February 2012 and December 2013) in the lower water column with these conditions being met a minimum depth of 4m on 18/02/2013 and a maximum depth of 8m on 11/12/2012 (Appendix 8: See attached CD). Winter DO levels ranged from 0.33 to 11.4 mg/l at a depth of 1m compared to 0.18 to 9.65 mg/l at a depth of 8m. Anoxic conditions were not met during winter months.

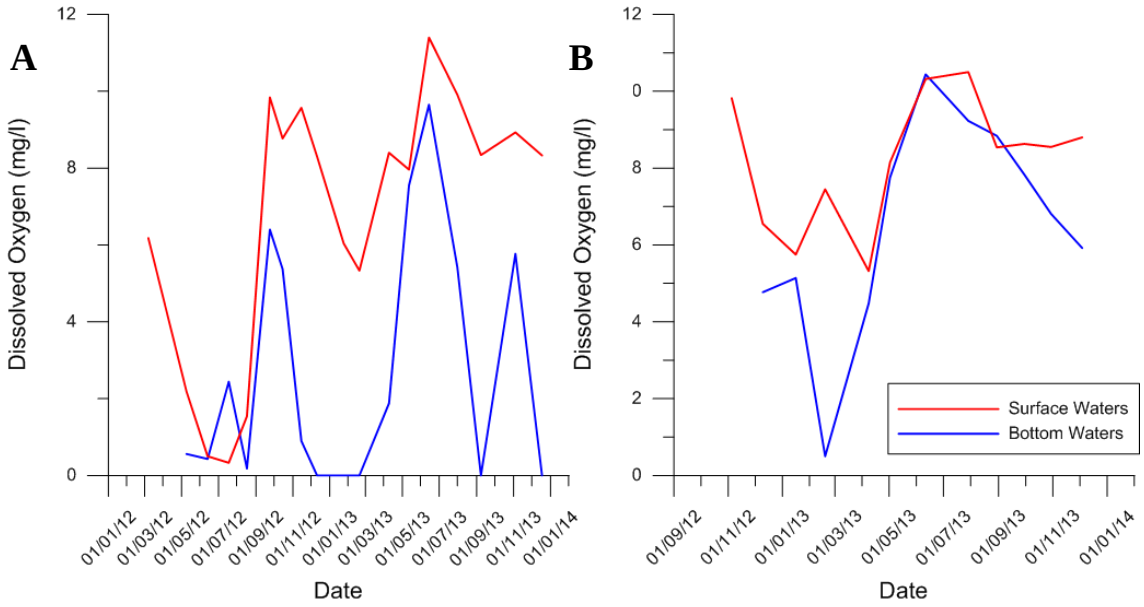

Figure 4.21: Surface and bottom water DO measurements at the Karori Upper Dam (A) and Lake Pounui (B) taken over the monitoring period. Surface waters refer to a measurement taken at a depth of 1m, bottom waters refer to a measurement taken at a depth of 8 and 9m at the Karori Upper Dam and Lake Pounui respectively.

Dissolved oxygen (DO) levels at Lake Pounui varied significantly both seasonally and throughout the water column during the monitoring period. The maximum recorded DO level was 10.5 mg/l, at 1m 29/07/2013, compared to a minimum recorded DO level of 0.5 mg/l, 9m 18/02/2013 (Appendix 9: See attached CD).

Anoxic conditions were recorded once during the monitoring period, at a depth of 9m on 18/02/2013. February saw the most variation throughout the water column in dissolved oxygen readings with a maximum value of 7.45mg/l at a depth of 1m. Values consistently decreased throughout the water column with a value of 2.86 mg/l at a depth of 5m before reaching the previously mentioned value of 0.5 mg/l at a depth of 9m.

4.2.1.4 Conductivity

Conductivity levels showed strong variation during the monitoring period with the maximum levels ranging from 306 to 137µS/cm. However, there does not appear to be strong seasonal variation with conductivity levels ranging from 179 to 234µS/cm in summer compared to values ranging from 143 to 236.4µS/cm in winter at a depth of 1m (Figure 4.22A).

Conductivity levels varied seasonally at Lake Pounui. However, there was generally minimal variation throughout the water column. Conductivity levels were generally lower in warmer months (November to February) before increasing from May to June (Figure 4.22B). The average summer, November to February, conductivity level at a depth of 1m was 166.13 µS/cm compared to a value of 174.82 in µS/cm cooler months, May to August. Average conductivity levels at a depth of 9m were 166.50 and 173.73 µS/cm in warmer and cooler months respectively (Appendix 9: See attached CD).

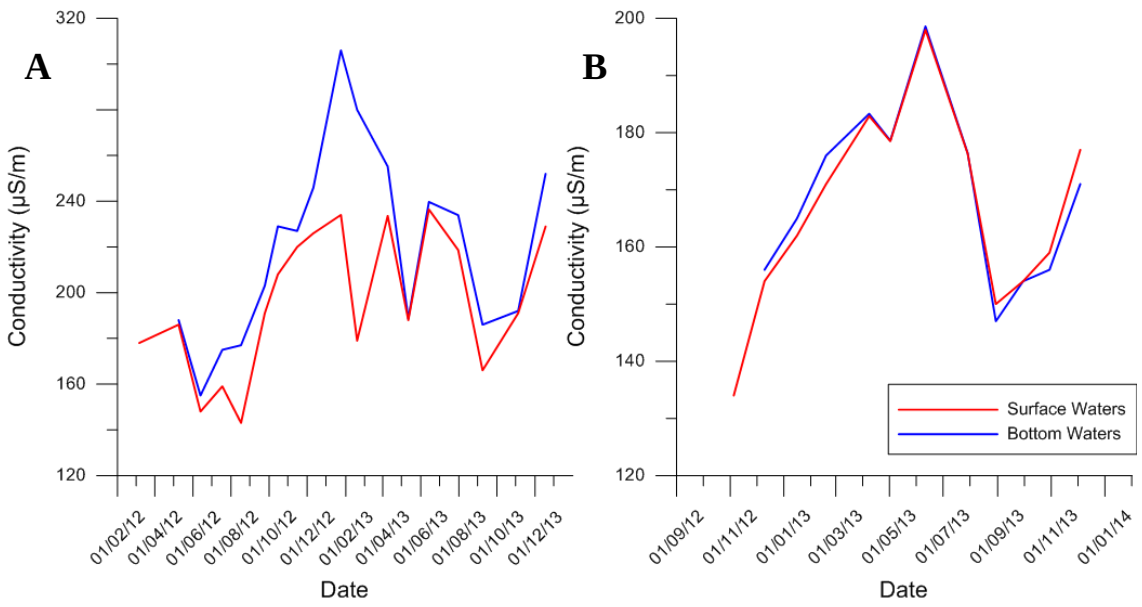

Figure 4.22: Surface and bottom water conductivity levels at the Karori Upper Dam (A) and Lake Pounui (B) over the monitoring period. Surface waters refer to a measurement taken at a depth of 1m, bottom waters refer to a measurement taken at a depth of 8 and 9m at the Karori Upper Dam and Lake Pounui respectively. Seasonal variations of water conductivity were observed at both sites.

4.2.1.5 Other Environmental Controls

Other measured environmental variables (listed in Section 3.4.1) showed little significant change over the monitoring period. This section will therefore briefly explain the main trends shown by these environmental controls. The relevant figures for each environmental control are attached in Appendix 10.

Total dissolved solids (TDS) values at the Karori Upper Dam ranged from 98 to 194ppm over the monitoring period. Almost all TDS depth profiles showed the same pattern with TDS values increasing over depth (Appendix 10, Figure 1A). There appears to be no seasonal variation in TDS values at the Karori Upper Dam.

TDS values were fairly consistent at Lake Pounui over the thirteen month monitoring period. The minimum recorded TDS was 81ppm, measured at 1m on 5/11/2012, with a maximum recorded value of 129.35ppm, at a depth of 7 and 9m on 11/06/2013. Average TDS values were consistently lower throughout the water column in summer months compared to winter months (Appendix 10, Figure 1B).

Oxygen reduction potential (ORP) values varied greatly throughout the monitoring period at both the Karori Upper Dam and Lake Pounui. Due to missing values[3], only ORP measurements from March 2012 to February 2013 and August 2013 to December 2013, will be considered at the Karori Upper Dam and Lake Pounui respectively.

ORP values were consistently lower in surface waters, at a depth of 1m, compared to a depth of 8m at the Karori Upper Dam (Appendix 10, Figure 2A). ORP values decreased in summer months with values ranging from -298.2to 34.2mV in surface waters compared to a range of 23.5to 96.1mV in winter months. This trend was also observed in bottom waters, at a depth of 8m, with summer ORP values ranging from -417.7 to -184.1mV compared to values ranging from 73.3to 101.5mV in winter months.

ORP values had strong seasonal variation at Lake Pounui with an average value of -203.08mV in summer months compared to a value of 18.05mV in winter at a shallow water depth of 1-2m. This variation was consistent throughout the water column with an

[3] Probe measuring ORP was not working for this time period.

ORP value of -212.89mV in summer compared to a winter value of 32.77mV at a bottom water depth of 7-9m (Appendix 10, Figure 2B).

Salinity remained fairly stable over the monitoring period at both the Karori Upper Dam and Lake Pounui. Salinity levels were constant throughout the water column with less than 0.03ppt of variation between sampling points with a maximum level of 0.19ppt and a minimum of 0.09ppt at the Karori Upper Dam (Appendix 10, Figure 3A). These results suggest that no halocline was present at the Karori Upper Dam. Salinity values at Lake Pounui ranged from 0.08 to 0.1ppt. Average salinity levels were 0.09ppt throughout the water column in both summer and winter (Appendix 10, Figure 3B). A weak halocline was present from November 2012 to January 2013.

4.2.2 Temporal variations in bacterial communities

Both bacteria community structure and the abundance of bacterial species experienced seasonal variations in the water column at the Karori Upper Dam and Lake Pounui throughout the monitoring period.

Using relative abundance numbers produced by the ARISA data, the origin of bacterial species in the water column was determined under the following hypotheses:

- species found in soil must be of soil origin,
- species found in streams but not soils must be of stream origin,
- species found in swamps but not soils or streams must be of swamp origin, and
- species found in the water column but not present in any other environment must be have originated from the water column.

Based on these hypotheses the origin of bacterial species present during monthly monitoring at the Karori Upper Dam and Lake Pounui was determined (Figure 4.23 and Figure 4.24).

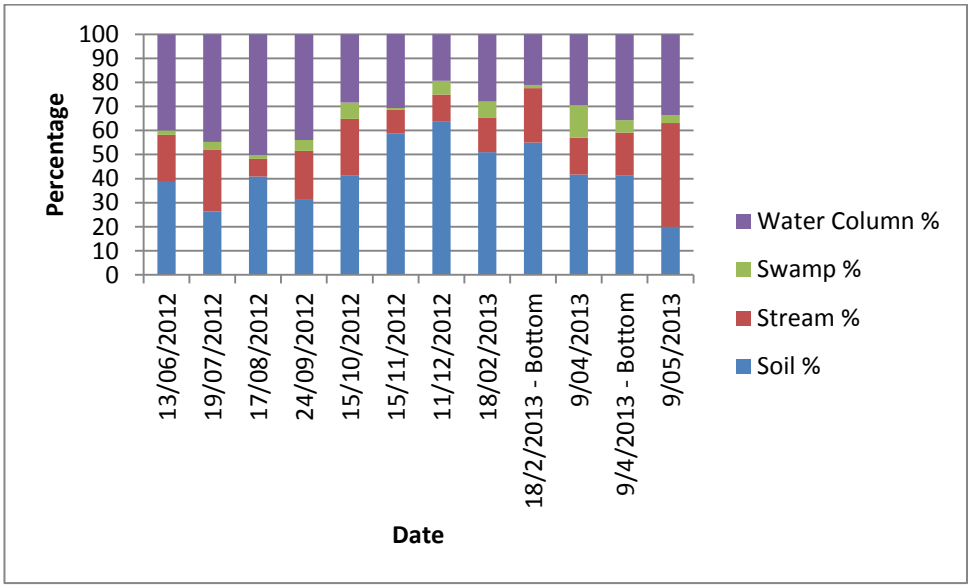

Figure 4.23: Origin of bacterial species in the water column at the Karori Upper Dam. Unless stated, samples were taken at a depth of 1m. Bottom samples refer to samples taken at a depth of 8m.

The bacteria communities for all of the water samples taken from the Karori Upper Dam, bar the sample taken on 17/8/2012, had the greatest proportion of bacteria

originating from catchment environments with percentages ranging from 55%, (19/07/2012) to 80% (11/12/2012). The sample taken on the 17/08/2012 had catchment input of 49% (Appendix 11, Table 1).

Contributions of bacterial species originating from soils in the surrounding catchment varied greatly with concentrations ranging from 20% (9/05/2013) to 63% (11/12/2012). There appears to be seasonal variation in the proportion of soil originating bacteria present in the water column at the Karori Upper Dam. Warmer months, November to February, recorded soil inputs ranging from 51 to 63.6% compared to 20 to 40.8% recorded in cooler months, May to August.

All samples taken from the water column at Lake Pounui had at least 40% of the bacterial community source represented by bacterial of a terrestrial origin (Figure 4.24). Bacterial communities originating from soils in the surrounding catchment comprised between 32%, (16/01/2013) and 66% (5/11/2012) of the bacterial population present in the water samples (Appendix 11, Table 2). No seasonal trend was observed.

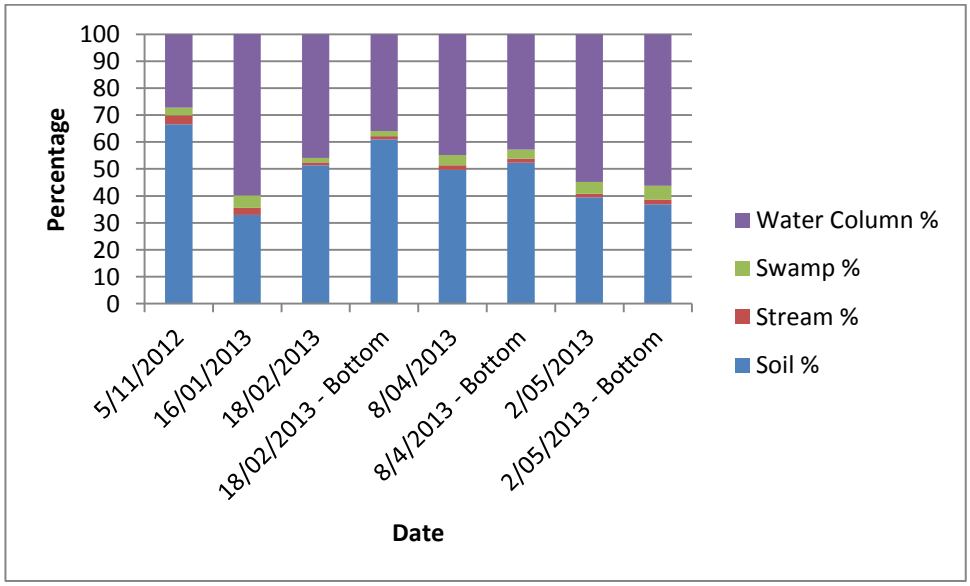

Figure 4.24: Origin of bacterial species in the water column at Lake Pounui. Unless stated, samples were taken at a depth of 1m. Bottom samples refer to samples taken at a depth of 9m.

The concentrations of stream-originating bacteria present within the water column also varied greatly at the Karori Upper Dam. Concentrations ranged from 7.34% (17/08/12)

to 43.06% (9/05/13). There appears to be minor seasonal variation in the concentrations of stream originating bacteria in the water colum. Stream contributions were generally low in warmer months, December to February, with concentrations ranging from 9.7 to 14.2%. Contrary to the Karori Upper Dam, bacteria originating from streams varied little at Lake Pounui with concentrations ranging from 1.6% (18/02/13) to 3.4% (5/11/12). No seasonal pattern was observed.

The concentration of bacterial species originating from swamp environments appear to be minimal at the Karori Upper Dam with concentrations ranging from 0.79%, (15/11/2012), to 13.51%, (9/04/2013). There appear to be no seasonal flucutations in bacterial species originating from swamp environments at the Karori Upper Dam. Bacterial species originating in swamps also had minimal input to the water column at Lake Pounui with proportions ranging between 1.6 and 4.6%. No seasonal trends were observed.

Lake water bacteria concentrations ranged from 19.38% (11/12/2012), to 50.25% (17/08/2012) with an average value of 33.8%. Relative abundances of species originating from within the water column exhibited seasonal variation at the Karori Upper Dam. Maximum quantities of bacteria produced in the water column were measured in cold months, May to August, with proportions ranging from 33.7 to 50.3%. Comparatively, the lowest contribution of water column originating bacteria was present in the warmer months of December and February with values of 19.4 and 28% respectively. Water column *in situ* produced bacterial species made up between 27% (5/11/2012), and 59% (16/01/2013), of the bacterial species present within the water column at Lake Pounui. Again, there was no observed seasonal variation.

There appears to be no substantial difference between water samples taken from a depth of 1m and 9m with both depths having a similar percentage of bacteria from a terrestrial origin and bacteria produced within the water column. This pattern occurs in both lakes (Appendix 11, Table 1 and Table 2).

4.2.2.1 Species abundance and richness

Species abundance in the water column at Lake Pounui strongly indicates the production of *in situ* bacteria (Table 4.2). As discussed in Section 4.1.2, eleven of the fifteen most abundant surface water species were also abundant in bottom water samples.

Figure 4.25 and Figure 4.26 show the fluctuations of the eleven bacteria abundant in both surface and bottom water from summer to autumn months. The most abundant species, 476.1, was present in three samples taken on 1/11/12, 18/02/13 (surface sample) and 2/5/13 (both surface and bottom samples).

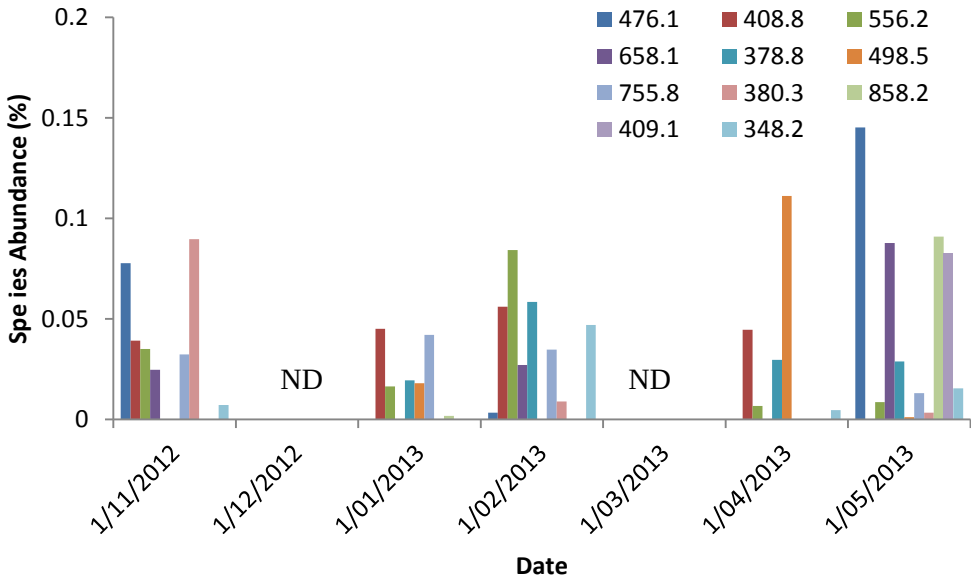

Figure 4.25: Summer to autumn variation of in situ produced bacterial species in surface waters at Lake Pounui. Note sampling did not occur in January 2012 or March 2013 hence no species data are available for these months (ND).

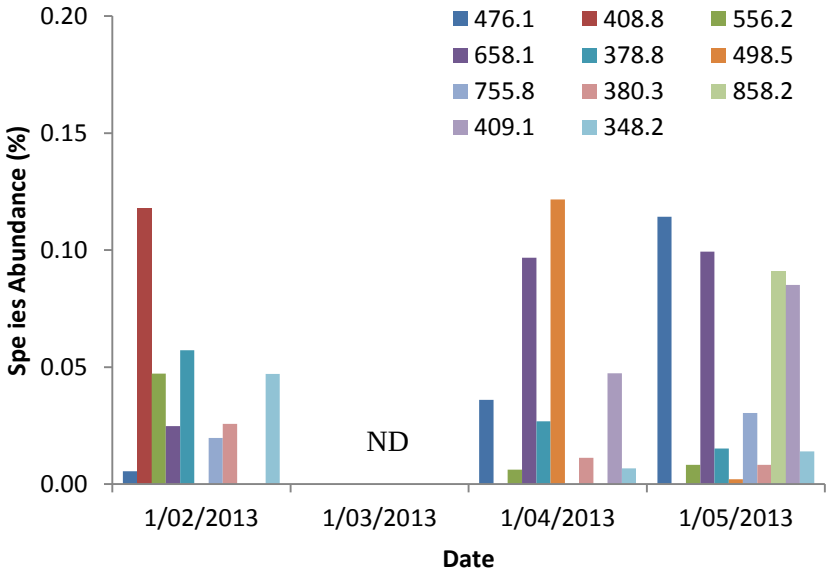

Figure 4.26: Summer to autumn variation of in situ produced bacterial species in bottom waters at Lake Pounui. Note sampling did not occur in March 2013 hence no species data is available for this month (ND).

Species 476.1 was identified in all water samples excluding two surface samples (16/01/13, 8/04/13). No seasonal variation was observed: however, this species was most abundant in both 2/05/13 surface and bottom water samples with a proportion of 0.15 and 0.11 in surface and bottom waters respectively (Appendix 12, Table 1 and Table 2). The relative abundances of species 408.8 may indicate seasonal variability with this species being most abundant in both surface (relative proportion of 0.056) and bottom water samples (relative proportion of 0.12) taken on the 18/02/13. Relative abundances then noticably decreased in cooler months (Appendix 12, Table 1 and Table 2). Species 556.2 was identified in all water samples, however, no seasonal variation of the abundances of this species was observed (Appendix 12, Table 1 and Table 2). This species was most abundant in both surface and bottom samples taken on 18/02/13 but was noticably more abundant in surface waters (0.056) compared to bottom water samples (0.047). No seasonal variation of species 658.1 was observed in either surface or bottom water samples (Appendix 12, Table 1 and Table 2).

Species 378.8 was identified in all water samples excluding the 5/11/12. Relative abundances of this species showed similar patterns in surface and bottom waters with the maximum abundances being measured on 18/02/13. Species abundance noticably decreased after this peak indicating that this species may be more abundant in warmer

months compared to cooler months. Species 498.5 was only present in water samples taken on 16/01/13, 8/04/13 and 2/05/13, including bottom water samples. Species abundance shows similar patterns in surface and bottom waters with the maximum abundance recorded on 8/04/13. Species 755.8 was present in all surface and bottom water samples excluding samples taken on 8/01/13. Relative abundances of this species suggests seasonal variability in surface waters, with proportions peaking on 16/01/13 (Appendix 12, Table 1 and Table 2). No seasonal variability was observed in bottom water samples. Species 380.3 was present in three out of five surface samples and all bottom water samples. No seasonal trend was observed (Appendix 12, Table 1 and Table 2). Species 858.2 was present in two surface samples and one bottom water sample, however, no seasonal pattern was observed. Species 409.1 was only observed in one surface sample and two bottom water samples. Species abundances peaked on the 2/05/13. This may indicate that this is a species that thrives in conditions that occur in cooler months. Species 348.2 was identified in all water samples excluding the 16/01/13 surface water samples. Species abundance showed a similar pattern in both surface and bottom samples with the maximum abundance being recorded on 18/02/13.

The Shannon diversity index was used to calculate species richness and diversity in water column species at Lake Pounui (Figure 4.27 and Figure 4.28). Due to previous results indicating no distinct *in situ* signal of bacteria produced within the water column, this statistical test was not applied to the Karori Upper Dam.

There appears to be no observed correlation between temperature, conductivity or pH with species richness in surface water samples at Lake Pounui (Figure 4.27). There does appear to be a negative correlation between surface dissolved oxygen levels and species diversity of surface waters (Figure 4.27B).

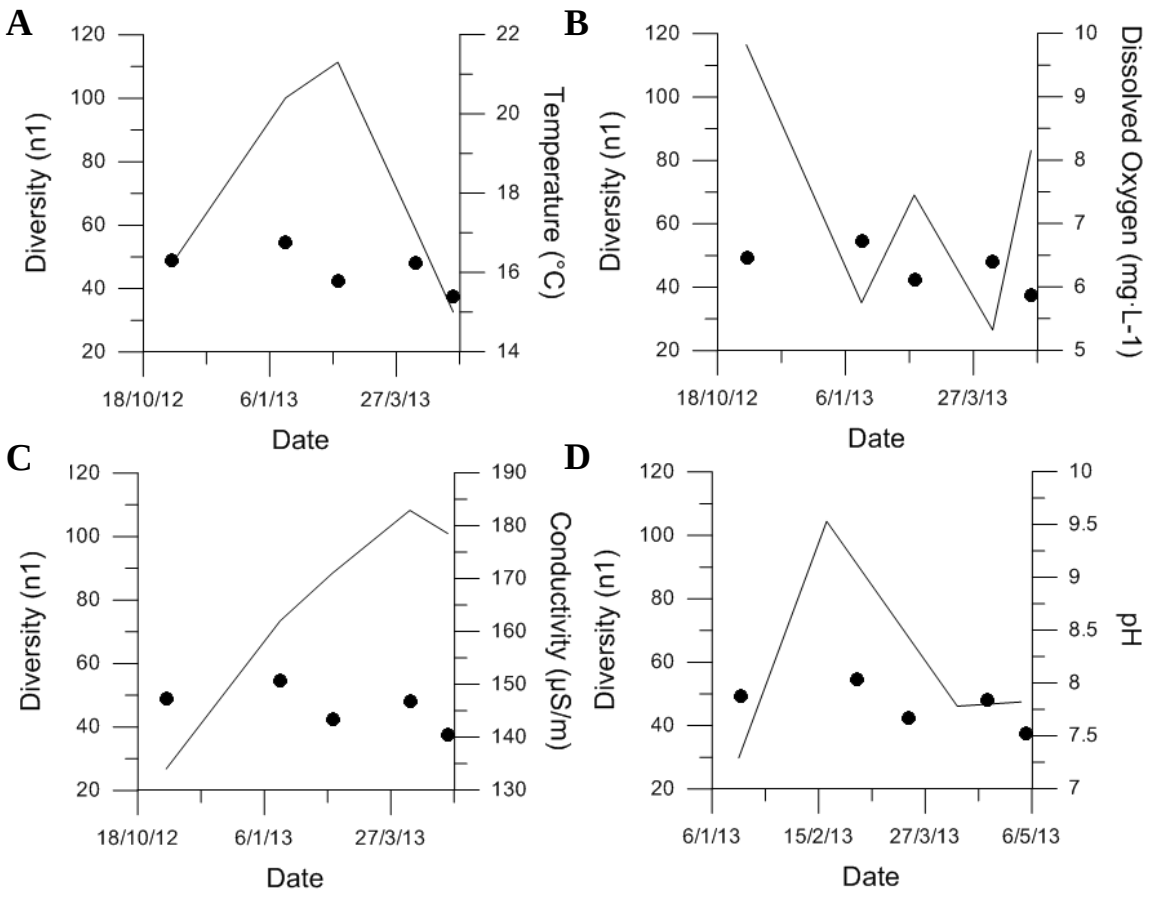

Figure 4.27: Species richness determined by the Shannon diversity index plotted against temperature (A), dissolved oxygen (B), conductivity (C), and pH (D) for surface waters, a depth of 1m, at Lake Pounui. The circles reflect species diversity. The lines represent the measured environmental control.

No correlation was observed between water temperature, DO or pH and bacterial species in bottom water samples at Lake Pounui (Figure 4.28). There is a positive correlation between bottom water species richness and water conductivity and a negative correlation with dissolved oxygen and pH (Figure 4.28). However, since only three samples were obtained, further monitoring will have to be undertaken to better determine this relationship.

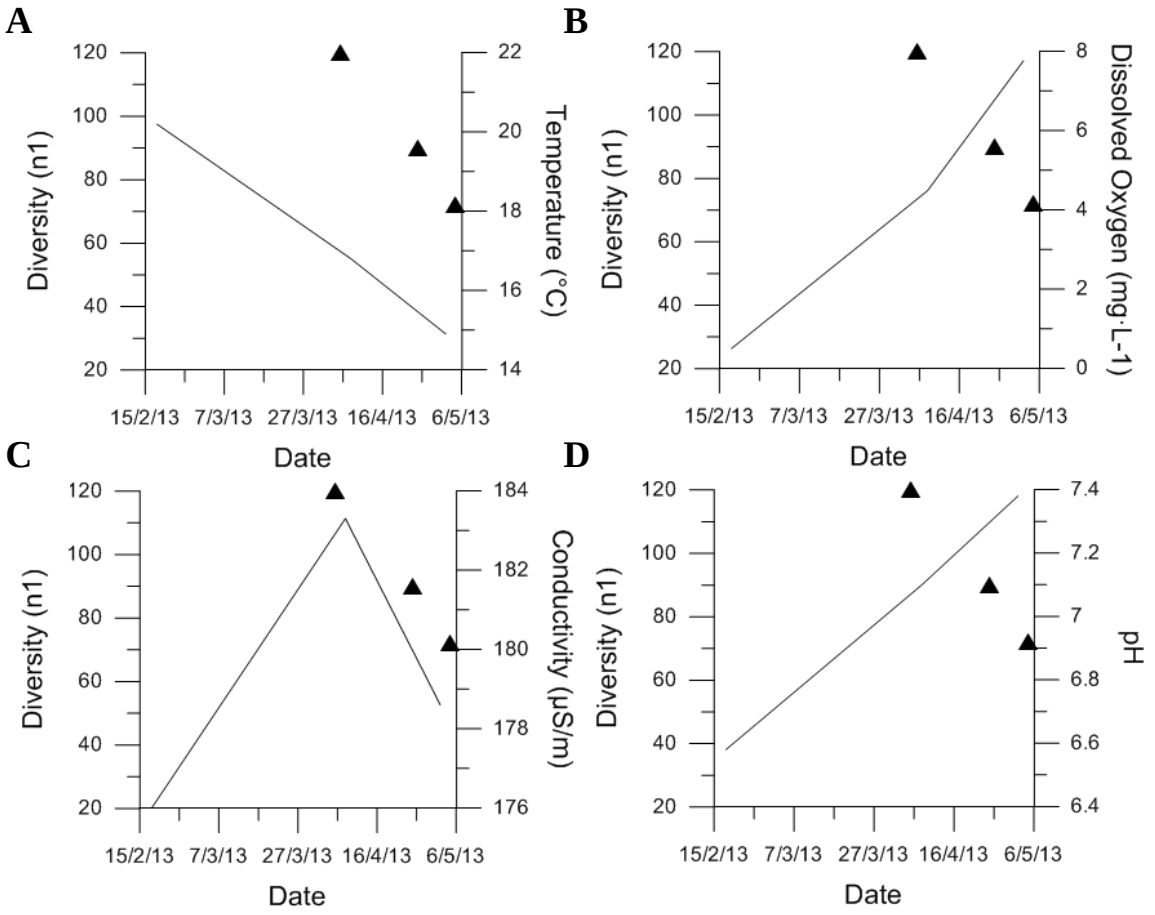

Figure 4.28: Species richness determined by the Shannon diversity index plotted against temperature (A), dissolved oxygen (B), conductivity (C), and pH (D) for water at a depth of 9m at Lake Pounui. The lines represent the measured environmental control.

4.2.2.2 Bacterial communities and GDGT linkages

Forward selection of species and GDGT data was undertaken to determine species that are significant to GDGT abundances in lake sediments (Table 4.5, Section 4.1.4). As stated in Section 4.1.4 only species 786 was identified as being produced in the water column at the Karori Upper Dam (Table 4.5). This species explains 4.1% of the variance in branched GDGT abundances in lacustrine sediments (Table 4.5) and was the seventh most abundant species in surface waters at the Karori Upper Dam (Table 4.5).

To determine the relationship between the relative abundance of species 786 and measured environmental controls, Pearson's correlation analysis was undertaken. No correlation was determined (Table 4.6).

Table 4.6: Pearson's correlation coefficients for environmental variables and species 786 at the Karori Upper Dam. Note that due to little fluctuation of other environmental controls only DO, pH, temperature and conductivity were used for this analysis. This test was only carried out on surface water (depth of 1m) samples as species 786 was only present in one bottom water sample.

	DO	pH	Temperature	Conductivity	786
DO	1.00	0.51	0.45	0.79	0.18
pH	0.51	1.00	0.84	0.70	0.03
Temperature	0.45	0.84	1.00	0.70	0.29
Conductivity	0.79	0.70	0.70	1.00	0.17
786	0.18	0.03	0.29	0.17	1.00

No seasonal trend was observed in relation to relative abundances of this species (Figure 4.29).

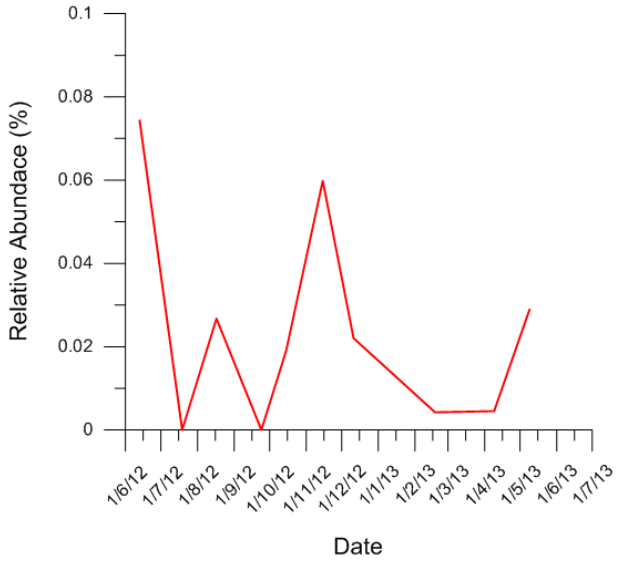

Figure 4.29: Relative abundances of species 786 throughout the monitoring period at the Karori Upper Dam. No seasonal pattern was observed.

Forward selection of species and GDGT data was undertaken to determine species that are significant to GDGT abundances in lake sediments (Table 4.5, Section 4.1.4). As stated in Section 4.1.4 only species 581.6 was identified as being produced in the water column at Lake Pounui (Table 4.5). This species explains 2.92% of the variance in branched GDGT abundances in lacustrine sediments. To determine the relationship between the relative abundance of species 581.6 and measured environmental controls, Pearson's correlation analysis was undertaken. A positive correlation was determined between species abundance and water pH (Table 4.7).

Table 4.7: Pearson's correlation coefficients for environmental variables and species 581.6 at Lake Pounui. Note that due to little fluctuation of other environmental controls only DO, pH, temperature and conductivity were used for this analysis. The highlighted numbers correspond to the positive correlation between pH and species abundance of species 581.6

	DO	pH	Temperature	Conductivity	581.6
DO	1.00	0.65	0.35	0.13	0.52
pH	0.65	1.00	0.33	0.15	0.95
Temperature	0.35	0.33	1.00	0.60	0.22
Conductivity	0.13	0.15	0.60	1.00	0.15
581.6	0.52	0.95	0.22	0.15	1.00

This species was present in January and February surface water samples and February and May bottom water samples. Due to the small number of samples it was not possible to determine if there were any seasonal trends in the relative abundance of this species.

4.3 Summary

This section described the results of the research aims set out in Section 1. The spatial variation of bacterial communities and branched GDGTs in lake catchments was first explored before stating the results of temporal variations of bacterial communities in lacustrine environments. Chapter 5 will go on to discuss the significance of these results and compare the results of this thesis to previous studies before discussing future pathway options.

5 Discussion

5.1 Introduction

This chapter will first discuss the spatial variability of environmental controls and bacterial species before inferring branched GDGT linkages. This chapter will then discuss the temporal variations of environmental controls and bacterial species. Inferences will then be made to identify the species likely producing branched GDGTs found in lacustrine sediments, where they are produced and what environmental controls they most likely respond to.

This chapter will conclude by considering the utility of the MBT/CBT paleoproxy in small lake catchments before discussing potential future work.

5.2 Spatial variation of bacterial communities and branched GDGTs in two New Zealand lake catchments

This section will address the first research question: what is the spatial variation of bacterial communities and branched GDGTs in two New Zealand lake catchments? It will first discuss the spatial variation of geochemical parameters and the necessity of understanding this to determine the origin of organic matter present in lacustrine sediments. This section will then go on to discuss the distribution of bacterial communities at the Karori Upper Dam and Lake Pounui before discussing and comparing the distribution and abundances of branched GDGTs within these catchments to international literature.

5.2.1 Geochemical variation

Geochemical analysis can determine the nature and origin of organic matter present in lake sediments (Hakanson & Jansson, 1983; Meyers & Teranes, 2001). This is important for this research as it will indicate whether the lacustrine sediment is dominated by allochthonous or authochthonous organic material. This will further aid the understanding of spatial variation and origin of bacterial communities and branched GDGTs at the Karori Upper Dam and Lake Pounui. Geochemical results based on elemental compositions at both the Kaori Upper Dam and Lake Pounui suggest that the lake sediments of the two catchments are strongly characterised by differences in the origin of their organic matter.

C/N ratios calculated for the Karori Upper Dam were all high regardless of sample site (Figure 4.1A, Section 4.1). The similarity in high C/N ratios in terrestrial and lacustrine environments at the Karori Upper Dam indicates that there is little autochthonous organic matter present in lacustrine sediments, indicating that it is mainly terrestrial in origin and that the terrestrial signature extending into the lake is not limited to the lake bed surrounding the catchment banks.

Contrary to the results from Karori Upper Dam, the C/N ratios calculated at Lake Pounui showed distinct differences between lacustrine and terrestrial environments. C/N ratios were low for all lacustrine sediments, including those in close proximity to the catchment banks, indicating that Lake Pounui has a large component of autochthonous organic matter (Figure 4.1B, Section 4.1).

This is significant as it implies that the limnetic systems of the Karori Upper Dam and Lake Pounui are distinctly different and therefore it can be expected that the abundances and distribution of branched GDGTs at these sites will be influenced strongly by different processes.

The difference in the TOC normalised extract yields between sites supports this observation. In this thesis, as stated in Section 3.2.2, TOC is equivalent to total carbon as no inorganic carbon was present in any sediment samples. TOC enters lacustrine sediments from authochthonous (e.g. carbon uptake through photosynthesis by aquatic organisms) and allochthonous sources (e.g. atmospheric deposition, erosion of leaf litter) (Cohen, 2003). TOC measurements are vital to understand the nature and abundance of organic matter in lacustrine environments (Meyers & Teranes, 2001).

The high normalised extract yields of TOC at Lake Pounui suggests that there are high concentrations of easily degradable *in situ* produced organic material (Appendix 4, Table 2) (O'Sullivan, 2004). Comparatively, the Karori Upper Dam lacustrine sediments had low TOC normalised extract yields, indicating a large proportion of allochthonous material, most likely insoluble lignin and organic matter (O'Sullivan, 2004). This is supported by the distinct differences seen in the average proportion of TOC present in soils at the Karori Upper Dam and Lake Pounui (Figure 4.2, Section 4.1).

As could be expected from the rain-fed lacustrine environments of the Karori Upper Dam and Lake Pounui, sulphur concentrations were low. Although this appears to have no impact on the spatial variation of organic matter or branched GDGTs at either site (Figure 4.3, Section 4.1.1) it can be expected to have an impact on the structure of bacterial communities in lacustrine environments and therefore will be further discussed in Section 5.3.1 (Golterman, 1975). The similarity of low sulphur concentrations of the Karori Upper Dam and Lake Pounui suggests that although the latter site is in close proximity to the coast, it is not influenced by coastal processes.

5.2.2 Distribution of bacterial communities

As discussed above, geochemical analysis indicated that the origin of organic matter in lacustrine sediments strongly differ at the Karori Upper Dam and Lake Pounui suggesting that two sites are controlled by different processes. The abundance and distributions of bacterial communities at these sites reinforces this observation.

All sediment samples at the Karori Upper Dam had large proportions of bacteria originating from terrestrial environments, specifically soil environments (Figure 4.6A, Section 4.1.2). No strong unique signature was present in the water column at the Karori Upper Dam. This lack of signal is significant as it is assumed that a terrestrial bacterial signal in lacustrine sediments would create bias towards an abundance of terrestrial derived GDGTs. Bendle et al. (2010) noted that temperature reconstructions using the MBT/CBT paleoproxy would be biased towards the area and environment which supplied the largest concentrations of organic matter to the sample site. Therefore, under this assumption, the substantial terrestrial signature of the Karori Upper Dam would limit the accuracy and applicability of the MBT/CBT as a lacustrine paleotemperature proxy for this site.

Similar to the Karori Upper Dam, all lake sediment samples from Lake Pounui had large proportions of bacteria originating from soil environments. Regardless of similarities in what appears to be a terrestrial bacteria signal in lacustrine sediments, species abundances indicated that the bacterial communities at the Karori Upper Dam and Lake Pounui have unique structures. Species abundance at Lake Pounui indicates a strong production of bacteria within the water column (Figure 4.6, Section 4.1.2). These results are significant as it is likely that bacterial communities within the water column and bacterial species inhabiting lacustrine sediments will respond to different environmental controls compared to those in the surrounding catchment (Tierney & Russell, 2009).

The RDA of bacterial species and environmental controls indicated that bacterial communities in lacustrine sediments, as discussed above, differed significantly between the Karori Upper Dam and Lake Pounui (Figure 4.5). The RDA also indicated that there was significant variation in bacterial community structure between samples at both sites. The RDA suggested that the cause of variation seen between sites is due to a yet

unmeasured variable or variables. It is assumed to be a variable of geochemical origin as total sulphur was the only measured variable that explained variation between sites (Figure 4.5, Section 4.1.2).

Although it is thought to be a geochemical variable within sediment which drives spatial variation between sites, the RDA of bacterial species and environmental controls suggested that temperature and TOC were the main drivers of variability within environments at both the Karori Upper Dam and Lake Pounui (Figure 4.5, Section 4.1.2). This implies that although the Karori Upper Dam and Lake Pounui possess distinctly different catchments and unique bacterial community structures, temperature still appears to be an important driver for both sites.

This appears consistent with previous research. Research undertaken by Loomis et al. (2014) looking at the effects of environmental controls on the distribution of branched GDGTs in lacustrine sediments in east African Lakes found that the total concentrations of branched GDGTs correlated highly with TOC concentrations. This is significant because current studies suggest that the lipid producing bacteria are most likely heterotrophic (Weijers et al, 2006; Weijers et al, 2010). As discussed in Section 2.2.2, heterotrophic bacteria rely on the production of energy caused by the oxidation of organic carbon.

It is important to note, that based on the abundance of GDGT-0 in sediments and the cren/cal ratio (Appendix 7), it was determined that methanogenisis occurred at Lake Pounui, indicating the presence of methanogenic archaea. Methanogenesis is a metabolic process which occurs in anoxic conditions and is the main anaerobic process that occurs in lake sediments (Blaga et al, 2009). It occurs when there is increased primary production within the water column or an influx of vegetation from the surrounding catchment which causes organic matter to rapidly sink causing lake sediments to become anoxic. In some circumstances bottom waters can also reach anoxic conditions causing methanogenis to occur within the water column (Sinnginghe-Damste et al, 2012). It is a process that can involve either methnogenic archaea or sulphate reducing bacteria (Blumenberg et al, 2004). Naeher et al. (2014) also identified the presence of methanogenic Archaea at Lake Rotsee, Switzerland. The suggested presence of methanogenisis and methanogenic archaea indicates a change in

environmental conditions of the lake system and may identify an additional source of lipid producing bacteria not being driven by temperature change alone.

5.2.3 Branched GDGTs

5.2.3.1 Distribution and abundances of GDGTs

Isoprenoid and branched GDGTs were identified in all sediment samples at both sites. The low abundance of Archaea derived isoprenoid GDGTs at both the Karori Upper Dam and Lake Pounui indicates that the use of isoprenoid GDGTs would not be reliable and thus the TEX_{86} temperature proxy would not be an accurate paleoproxy at either site. This conclusion has been made for many other lake studies conducted globally where isoprenoid GDGTs have been found in low abundance and thus the utility of the TEX_{86} temperature proxy has been limited (Powers et al, 2004; Tierney et al, 2009; Powers et al, 2010 Tierney et al, 2010; Woltering et al, 2011; Berke et al, 2012; Blaga et al, 2013). Therefore this thesis will disregard the TEX_{86} temperature proxy.

Branched GDGTs, however, were abundant at both sites suggesting that the use of these lipids would be appropriate for this research. Consistent with previous research (Weijers et al, 2007; Blaga et al, 2010), abundant quantities of branched GDGTs without cyclopentyl moieties were found in all sediment samples at both sites. Branched GDGTs with cyclopentyl moieties were of negligible abundance in sediment samples, consistent with previous research undertaken by Weijers et al. (2007). This is important, as Weijers et al. (2007) determined that there is a significant correlation between the number of cyclopentyl moieties and soil pH. This relationship is used to reconstruct past pH levels (CBT equation in Section 2.2).

Although there were similarities in GDGT abundances at the Karori Upper Dam and Lake Pounui, as implied by both geochemical analysis and analysis of bacterial communities, there were unique signatures in branched GDGT distributions between sites.

5.2.3.2 Branched GDGT Structure

The most abundant bacterial lipids in all sediment samples excluding one sample at the Karori Upper Dam were GDGT IIa and IIIa (Section 4.1.3). GDGT abundances were similar regardless of environment type with no distinct difference in GDGT abundances

between lacustrine sediments and sediments from the surrounding catchment. The similarity of GDGT abundances in both terrestrial and lacustrine environments indicates that there is insignificant production of *in situ* branched GDGTs from either the water column or lacustrine sediments in the Karori Upper Dam and that a dominant terrestrial GDGT signature extends throughout the lake bed.

In their investigation of the spatial variation of branched GDGTs at Lake Cadagno, Switzerland, Niemann et al. (2012) found similar GDGT abundances in all sediment samples. No significant distinction between GDGT abundances between sample sites was identified, with GDGT IIa and GDGT IIIa being the most abundant branched GDGTs in all sites apart from two samples from meadow environments with irregular soils. This suggests that the provenance of branched GDGTs in lacustrine sediments and catchment soils at Lake Cadagno are the same. Application of the lacustrine calibrated MBT/CBT (Zink et al, 2010; Tierney et al, 2010) paleotemperature proxy to Lake Cadagno created inaccurate reconstructions with temperatures estimated 6-9°C warmer than measured. However, application of the soil derived MBT/CBT proxy was accurate at this site suggesting that sites dominated by a terrestrial lipid can still be used for accurate temperature reconstructions, provided that the terrestrial lipid-temperature relationship has been accurately constrained.

In contrast, GDGT characteristics at Lake Pounui were distinctly different between environments as shown by the results of geochemical analyses and bacterial community identification. All lake sediment samples at Lake Pounui were characterised by a high abundance of GDGT IIa and GDGT IIIa. Tierney et al. (2010) and Sun et al. (2011) also showed that these GDGTs were most abundant in tropical lakes in Africa and China, respectively.

Concentrations of GDGT IIIa were distinctly lower in soil environments compared to lake environments at both the Karori Upper Dam and Lake Pounui (Figure 4.12 and Figure 4.13). Research undertaken by Weijers et al. (2007) on branched GDGTs in soils found that at some sites this GDGT had one of the highest concentrations whereas at other sites the concentrations were below detection limit indicating that site may influence the distribution of this lipid. GDGT IIb and to a lesser extent GDGT Ic were more abundant in lakes than soils, stream or swamps at both the Karori Upper Dam and

Lake Pounui (Figure 4.12 and Figure 4.13). This suggests that these lipids may primarily be produced in lake environments. GDGT Ia was highly abundant in soils (Figure 4.12 and Figure 4.13). Abundances of this GDGT were distinctly lower in streams, swamps and lake sediments indicating that this GDGT may originate in soil environments and is washed into lakes via streams that are partially filtered in swamps. Further research will need to be undertaken to determine if this relationship exists.

As discussed above, the relative abundances of branched GDGTs at each site differs significantly. This does not appear to be a result of discrepancies between datasets but, as Wang et al. (2012) suggest, evidence which supports the notion that a variety of branched GDGTs can originate from various environments due to different bacterial community structures.

Results from this thesis also suggest that bacterial species producing lipids in soil environments are different from the lipid-producing species in lacustrine environments. As stated in Section 4.1.4.1 statistical analysis identified species 666.4 as having a strong relationship with GDGT IIa and GDGT Ia at the Karori Upper Dam. The abundance of these species was greatest in soils environments suggesting that this lipid originates in soils. In comparison species 465.9, which is most abundant in lake sediments, had a relationship with GDGTs Ib and Ic indicating that this species and the corresponding lipids predominantly originate in lake systems.

5.2.3.3 BIT Index

The Branched and Isoprenoid Tetraether (BIT) index is based on the ratio of isoprenoid GDGTs to branched GDTGs in lake sediments and can be used to determine the origin of organic matter (Blaga et al, 2009). A high BIT ratio indicates a high proportion of branched GDGTs within a sample and (Blaga et al, 2009) suggested that this indicates organic matter of a terrestrial nature. On the other hand, a low BIT value is said to indicate a high proportion of isoprenoid GDGTs compared to branched GDGT quantities and is thought to indicate a strong presence of organic matter from a lacustrine environment (Blaga et al, 2009).

Gunther et al. (2014) and Blaga et al. (2009) suggested that the application of the BIT index to lacustrine sediments may help to identify the impact of terrestrial derived

GDGTs in lake sediments. These researchers identified that at their sites there appeared to be little indication that an *in situ* source of GDGTs was present in lacustrine sediments. BIT values from various sample sites within the catchment furthered this suggestion with BIT values decreasing between river and water column samples, and between water column and lake sediments. Gunther et al. (2014) suggest that this decrease in BIT values is due to an increase in archaea production in lacustrine environments.

However, this does not appear consistent with findings from the Karori Upper Dam. As discussed above the origin of branched GDGTs in lacustrine sediments at this site appear to be mainly terrestrial in origin with also low archaea components. Based on Gunther et al.'s (2014) suggestion, a decrease in BIT values should have been observed across sample sites. However, there was no significant decrease in BIT values at the Karori Upper Dam suggesting that this may not be a true indicator of the origin of branched GDGTs found in lacustrine sediments.

BIT values were similar for all sediment samples at Lake Pounui (Appendix 7, Table 4) which implies an *in situ* production of branched GDGTs either within the water column or lake sediments, according to Gunther et al. (2014). However, as demonstrated by the Karori Upper Dam this is not necessarily an accurate indicator of the origin of organic matter present within a lake.

5.2.3.4 MBT/CBT ratios

As previously stated in Section 2.2 Weijers et al. (2007) found that the degree of methylation and cyclisation of bacterial branched GDGTs in soils responded to changes in air temperature and soil pH respectively. Section 2.2 states the equations used to calculate both MBT and CBT ratios. A high MBT ratio of branched GDGTs indicates a decrease in methylation and therefore an increase in MBT values represents an increase in temperatures. The CBT ratio is based on the relationship between the relative abundance of cyclopentyl moieties and sediment pH. An increase in the CBT ratio indicates a decrease in sediment pH (Weijers et al, 2007). Soil pH was not measured for this thesis and therefore this observation cannot be verified here.

MBT/CBT ratios calculated for the Karori Upper Dam reinforced the observation that the lacustrine sediments at this lake were heavily influenced by a terrestrial signature. Samples were clustered together regardless of environment type and did not show a distinct individual signal. Niemann et al. (2012) reported similar results at Lake Cadagno, which they attributed to the small size of that site, which made it more susceptible to soil erosion from the surrounding catchment. As a result the flux of allochthonous branched GDGT signal may have over-ridden any autochthonous GDGT production in the lake system. As discussed in Section 3.1 the Karori Upper Dam is also a small lake suggesting that lake size may be a critical factor in the applicability of a lake derived MBT/CBT paleotemperature proxy.

In contrast to the Karori Upper Dam, MBT/CBT ratios calculated for Lake Pounui exhibited strong branched GDGT signatures unique to each specific habitat. Lacustrine sediment samples clustered together, with a distinct separation from soil samples. Swamps and streams had unique signals, clustering between lacustrine and soil samples (Figure 4.1.5, Section 4.1.3.2). These results were in good agreement with research undertaken by Tierney and Russell (2009) at Lake Towuti, Indonesia. At this site, soil, river and lake samples clustered into distinct environments. MBT/CBT values calculated for Lake Qinghai, China, also showed similar results to Lake Pounui (Wang et al, 2012). Offshore sediments clustered together with higher MBT/CBT ratios while MBT/CBT ratios in catchment soils were consistently lower. Similar to the clustering of stream and swamp samples at Lake Pounui, nearshore samples at Lake Qinghai clustered between soil and offshore samples (Wang et al, 2012).

The results from Pounui, Qinghai and Tuwiti lakes indicate that unique GDGT signatures can be obtained for specific environments of a lake and catchment and that, there may be strong *in situ* production of branched GDGTs within certain lake environments. Lake Towuti (area = 561.1 km^2) and Lake Qinghai (area = 4,186 km^2) are large lakes, further indicating that size is an important factor when determining the utility of the MBT/CBT paleotemperature proxy for lake sites.

5.3 Identification of the lipid producing bacteria and GDGT linkages

This section will address the relationship between bacterial species and environmental controls. As discussed in Section 5.2 results from this thesis suggest that any unique *in*

situ lake GDGT produced signal is dominated by a significant terrestrial GDGT signal at the Karori Upper Dam. Therefore, this section will focus on the identification of the lipid producing bacteria and habitat at Lake Pounui.

Results from this thesis (Section 4.2.2) suggest a strong *in situ* lake production of bacterial species and therefore *in situ* GDGTs originating in both the water column and lacustrine sediments at Lake Pounui. This is consistent with previous studies in this field (Blaga et al, 2009; Betchel et al, 2010; Tierney et al, 2010). Research undertaken by Schoon et al. (2013) looking at the effect of pH and alkalinity on the distribution and abundances of both core branched lipids and intact polar lipids in eutrophic lakes in Iowa and meso-oligotrophic lakes in Minnesota also indicated the presence of an *in situ* source of branched GDGTs within the water column. These researchers sampled suspended particulate matter within lakes to determine the abundance of core, dead fossilised lipids and intact polar, living lipids. Their results recorded a high proportion of intact polar GDGTs, 18% to 45%, in the sediment particulate matter indicating the presence of branched GDGTs originating from within the water column. Underestimation of temperature by reconstructions using the soil calibrated MBT-CBT proxy using both core and intact polar lipids reinforced this observation further.

The lipid producing bacteria within the water column appears to be a different species of bacteria compared to the branched GDGT producers in soils as research undertaken by Weijers et al. (2007) first developing the MBT/CBT paleoproxy in soils found that the branched GDGTs were most abundant in soils with a pH less than six. Water column pH at Lake Pounui did not decrease below 6.58 (Figure 4.20B, Section 4.2.1) indicating that it may not be the same species across environments. However, due to the diversity of the acidobacteria phylum it may still be a part of this phylum.

5.3.1 Relationship between species richness and environmental controls through the seasonal cycle

This section will briefly reiterate the difference between species richness in surface and bottom waters at Lake Pounui and the suggested relationship between species richness and environmental controls before comparing these results to literature to determine the habitat in which the bacteria thrive in. This section will then try to identify the environmental controls in which they respond to, thus determining the habitat and season in which these bacteria are most abundant. Determining seasonal variation of lipid producing bacteria is fundamental. If the lipid producing bacteria primarily lives or grows in one season it will be expected that the GDGT signal would be biased towards this season (Anderson et al, 2014). My research indicated that the most influential factor affecting GDGT distribution was in fact the abundance of bacterial species. Based on this assumption the following section will make linkages between the distribution of bacterial communities within the water column at Lake Pounui and branched GDGT distributions from previous studies.

5.3.1.1 Environmental Controls

The relationship between environmental controls and species abundance has already been discussed in Section 5.2.2. Therefore this section will focus on the relationship between environmental controls and species within the water column at Lake Pounui by looking at species richness.

As stated in Section 4.2.2 species richness differed between surface and bottom waters at Lake Pounui. Species richness appeared to have a negative correlation with dissolved oxygen in both surface and bottom waters (Figure 4.27B, Figure 4.28B, Section 4.2.2). Species richness in bottom waters also appears to possibly have a positive correlation with conductivity and a negative correlation with pH, however, due to the limited sample size, further research would need to be undertaken to determine the significance of these relationships. Therefore this section will just focus on dissolved oxygen.

As stated above, this research determined that species richness was negatively correlated with dissolved oxygen in both surface and bottom waters at Lake Pounui. This is consistent with research undertaken by Sinninghe Damste et al. (2009) on Lake

Challa, Africa which determined that the production of GDGTs within the water column provided a significant contribution to the abundance of branched GDGTs present in sediments at this lake. Branched GDGTs in the water column at Lake Challa appeared significantly more abundant in permanently anoxic conditions, and in water depths of greater than 45m, compared to GDGT abundances in surface waters. This suggests that the lipid producing bacteria may live in habitats which are low in oxygen. This would appear reasonable if, as previously suggested by Weijers et al. (2007) the lipid producing bacteria are of anaerobic origin.

However, Tierney et al. (2010) found that dissolved oxygen did not explain significant variance in branched GDGT distribution at their sites. This suggests that branched GDGTs may be produced by different species which respond to different environmental controls. In order to determine the relationship between dissolved oxygen and species abundance at Lake Pounui future monitoring will need to take place.

5.3.1.2 Seasonal Variation

Due to limited water samples at Lake Pounui the seasonal relationship of significant species cannot be determined.

Based on the seasonal fluctuation of measured environmental variables (Section 4.2.1), however, it can be assumed that the structure of bacterial communities within the water column would change seasonally. As discussed above, dissolved oxygen had a negative correlation with species richness in both surface and bottom waters at Lake Pounui. As discussed in Section 4.2.1 dissolved oxygen levels fluctuated over the monitoring period with levels being distinctly lower in summer months compared to winter months. Based on the negative correlation with dissolved oxygen, species richness would be expected to be much greater during warmer months. Sun et al. (2011) and Zink et al. (2010) both suggested that MBT/CBT values are biased towards months of increased bioproduction suggesting that reconstructions at Lake Pounui will be biased towards summer temperatures.

Previous studies also indicate that the latitude of a site determines if seasonal variation in GDGT abundances occurs. Loomis et al. (2012) who undertook research on 111 of East African lakes located in close proximity to the equator with minimal latitudinal

variation found that there was minimal seasonal variation in branched GDGT distributions at these sites. This is consistent with research undertaken by Anderson et al. (2014) who determined that soils in Colombia, South America which experience minimal seasonal variation would more accurately reconstruct past temperatures. Thus sites which have little seasonal temperature fluctuations, the MBT/CBT proxy will most likely reconstruct mean annual temperatures as the production of branched GDGTs remains constant throughout the year. It can be therefore be expected, that sites like Lake Pounui that have strong seasonal fluctuations would be biased towards the month in which species production and therefore branched GDGT production is greatest.

5.3.2 Relationship between bacterial species, branched GDGTs and environmental controls in the water column at Lake Pounui

Temperature and pH appear to be the most influential environmental controls on branched GDGT abundances in lacustrine sediments at both the Karori Upper Dam and Lake Pounui (Section 4.1.3). pH was also identified as having a positive correlation with species 581.6, a species identified as having being significant in branched GDGT abundances in lacustrine sediments at Lake Pounui (Figure 4.18). This supports recent studies which found temperature and pH are the main influence on branched GDGT distribution (Weijers et al, 2007; Pearson et al, 2011).

Tierney et al. (2010) found that temperature and pH were strongly correlated with the abundance of branched GDGTs identified in lacustrine sediments in East African lakes. Similarly, Loomis et al. (2012) determined that the relative abundance of branched GDGTs in tropical lakes in East Africa were strongly correlated with mean annual air temperature, but shows that environmental variables including pH explained large portions of variance and in some circumstances it was not possible to determine the unique influence of any one variable. Thus, although temperature still appears to be the main driver on the abundances of branched GDGTs, other variables may also play an important role. Therefore future calibrations of the MBT/CBT paleotemperature proxy are needed to be undertaken to ensure paleoclimate reconstructions are based independently on temperature.

Research undertaken by Gunther et al. (2014) on lakes within the Tibetan region found that water pH and salinity levels were the environmental controls that had the largest impact on the abundance and distribution of branched GDGTs in lacustrine sediments. Research undertaken by Yang et al. (2014) identified the presence of branched GDGTs in alkaline soils (pH of up to 9), showing that the species producing branched GDGTs can thrive in strongly alkaline environments. Nevertheless branched GDGTs were more abundant in acidic and neutral soils (Yang et al, 2014) which is supported by the relationship between branched GDGT abundance and pH observed in this research.

The distinct differences in the origin of organic matter, bacterial communities and branched GDGTs in the lake sediments of the Karori Upper Dam and Lake Pounui are consistent with other research into the applicability of the MBT/CBT paleotemperature

proxy. The utility of the lacustrine calibrated MBT/CBT proxy appears to be influenced heavily by location.

5.4 Evaluation of the MBT/CBT paleoproxy in small lakes systems in New Zealand

This section will address the utility of the MBT/CBT paleotemperature proxy in small lake systems in New Zealand by comparing results from the present study with research undertaken globally.

This research determined that the lake calibrated MBT/CBT method would not be an accurate paleotemperature proxy at the Karori Upper Dam. This is due to the predominantly terrestrial origin of organic matter, bacterial communities and branched GDGTs within the lake system representing a soil bias at this site. Other researchers (Betchel et al, 2010; Niemann et al, 2012) have also found that the lake calibrated MBT/CBT paleoproxy will not be suitable in environments which have a bias towards terrestrially-derived branched GDGTs. Research undertaken by Betchel et al. (2010) on Lake Brienz, an oligotrophic lake in Switzerland found that branched GDGTs were most abundant in surface waters, with concentrations significantly decreasing with water depth. This GDGT signal is suggested to represent an influx of branched GDGTs from the surrounding catchment into this lake as branched GDGT concentrations peaked in spring surface waters, indicating an increase in organic matter from soils due to increases in sediment transport influenced by the melting of snow (Betchel et al, 2010). However, other studies have successfully applied the soil calibrated MBT/CBT proxy to sites dominated by terrestrial-originating GDGTs suggesting that this may be an appropriate proxy for the Karori Upper Dam (Fawcett et al, 2011; Kumar Das et al, 2012; Niemann et al, 2012).

In contrast to the Karori Upper Dam, this research determined that the lake derived MBT/CBT paleotemperature proxy would be an accurate proxy at Lake Pounui. Lake Pounui had a strong autochthonous lake GDGT signal. Lake Lugano, a eutrophic lake in Switzerland, also identified a strong *in situ* production of branched GDGTs within the water column (Betchel et al, 2010). Branched GDGTs were 1.5 times more abundant in the water column at Lake Lugano compared to Lake Brienz and did not decrease throughout the water column indicating an *in situ* production of branched GDGTs within the water column. This may suggest that nutrient status (oligotrophic versus eutrophic) is an important distinguishing factor

Wu et al. (2014) undertook research on the Yellow river, China, to identify the spatial variability of branched and isoprenoid GDGTs. Their research indicated no distinct difference between branched GDGTs found in the head and mouth of the river, suggesting that is little to no production of branched GDGTs within the river itself. This is important as it implies that only some sites display an *in situ* production of branched GDGTs and therefore a water calibrated MBT/CBT paleotemperature proxy will only be viable at certain sites.

Blaga et al. (2009) sampled both within the water column and the lake sediment of 47 lakes following a longitudinal transect. Their results indicated that branched GDGT concentrations of lakes sampled in the north were most abundant compared to isoprenoid GDGTs. In comparison, samples taken from the south were most abundant in isoprenoid GDGTs. These researchers suggest that the distinct differences of GDGT abundances is due to different catchment variables including topography, climate variables and soil transportation (Blaga et al, 2009). This appears consistent with the research from this thesis. The Karori Upper Dam is much smaller than Lake Pounui with two tributaries directly feeding the lake. This site has minor swamp areas surrounding the entrance of the tributaries to the lake which appear insignificant and do not appear to act as a buffer to terrestrial material entering the Karori Upper Dam. In contrast, no tributaries directly feed Lake Pounui, with streams being buffered by the large swamp areas which surround the lake. This is likely to limit terrestrial material and terrestrial originating branched GDGTs from entering Lake Pounui.

This research has shown that the lake calibrated MBT/CBT paleotemperature proxy will only be applicable to certain sites in New Zealand, but not all. Therefore, in order to ensure accurate reconstructions with the MBT/CBT paleotemperature proxy in lake systems, the origin of branched GDGTs must be determined.

5.5 Future work

In order to continue developing the MBT/CBT paleotemperature proxy into a reliable reconstruction tool in lacustrine sediments future work should be focussed on the following areas:

1. Positive identification of the branched GDGT producing bacteria in the water column. Identification of the bacterial communities within the water column will enable understanding of the environment and environmental controls they respond to. This will help identify viable sites where the MBT/CBT paleoproxy will be applicable.

2. Temporal monitoring of lake sites over at least two annual cycles to confirm the relationship between bacterial community structures and environmental controls. This will enable identification of the seasonal controls on the production of branched GDGTs, such as the relative importance of mean annual temperature and mean summer temperature.

3. Application of this research to other sites to ensure the results of this thesis are consistent and align with international results. This will enable a more accurate approach to determining the viability of lacustrine sites which will be appropriate for the application of the MBT/CBT paleotemperature proxy.

4. Once the range of possible GDGT producing bacteria has been adequately narrowed down (from results of studies similar to the research undertaken in this thesis) then more targeted cultivation of bacteria could take place. Cultivation of the lipid producing bacteria is necessary in order to accurately work out the environmental control they respond to in a controlled environment. This work will help to identify the likely habitat of these bacteria.

6. Conclusion

This thesis set out to (1) determine the spatial variation of bacterial communities and branched GDGTs produced in two lake systems; and (2) determine the seasonal cycle of environmental controls on the composition of bacterial communities and corresponding lipids in two New Zealand lakes. These aims will help determine the viability and utility of using bacterial lipids as a paleotemperature proxy in small New Zealand lacustrine systems.

The results of this thesis indicated that the lake calibrated MBT/CBT paleotemperature proxy would create accurate reconstructions at Lake Pounui; however, it does not appear to be an accurate paleoproxy for the Karori Upper Dam. Although research suggests that at sites similar to the Karori Upper Dam the soil derived MBT/CBT proxy may be applicable.

Spatial Variation

Isoprenoid and branched GDGTs were identified in all samples from both the Karori Upper Dam and Lake Pounui. However, archaea-derived GDGT abundances were low indicating that the TEX_{86} temperature proxy would not be an accurate paleoproxy at these sites.

Lacustrine sediments from both the Karori Upper Dam and Lake Pounui showed signals of organic matter, bacterial communities, and branched GDGTs derived from the surrounding catchment. These catchment fluxes were much more prominent at the Karori Upper Dam compared to Lake Pounui. Previous research has suggested that sites similar to the Karori Upper Dam are heavily influenced by soil transportation from the surrounding catchment, over riding any autochthonous GDGT signal. This is thought to limit the reliability of using a lacustrine-derived branched GDGT paleoproxy at such sites. However, previous research also suggests that at sites similar to these, the soil calibrated MBT/CBT paleotemperature proxy may be viable, if it can be accurately constrained. In contrast to the Karori Upper Dam, Lake Pounui had a strong autochthonous branched GDGT signal within the lake system that was not disturbed by terrestrial input from the surrounding catchment.

This research determined that it is most likely a geochemical variable in the sediment resulting in the spatial variation between sites. However, redundancy analysis

determined that temperature and TOC were the main drivers of variability within environments at both the Karori Upper Dam and Lake Pounui suggesting that temperature is an important environmental control regardless of site choice.

Temporal Variations

Results from this study identified bacterial species originating from both within the water column and in lacustrine sediments at Lake Pounui. Bacterial communities within the water column varied both seasonally and throughout the water column at Lake Pounui, as did measured environmental controls. Bacterial communities had greater diversity in summer months, with diversity becoming limited in cooler months. Species richness had a negative correlation with dissolved oxygen suggesting that this seasonal pattern is driven by seasonal fluctuations in dissolved oxygen levels. Surface water bacterial species and quantities differed strongly compared to bottom water bacterial species suggesting that surface and bottom water communities are structured differently.

Bacterial richness and diversity in both the surface water and bottom waters of Lake Pounui had a negative correlation with dissolved oxygen suggesting that the bacterial species producing branched GDGTs are anaerobic. However, due to limited sampling this will need to be further researched. Forward selection of bacterial species and GDGT data suggest that the bacterial species most likely producing the branched GDGTs in the water column at Lake Pounui is species 581.6. Species abundance of 581.6 appeared positively correlated with water pH. Due to limited water samples, the season in which this species is produced could not be determined.

In conclusion, this thesis has shown that a lake calibrated MBT/CBT paleoproxy should enable accurate paleoreconstructions of the sedimentary record at Lake Pounui. Further work into the environmental controls, seasonal cycle and identification of bacteria-producing lipids will improve the accuracy and reliability of these reconstructions.

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Appendix 2: Mo Bio Laboratories protocol manual

1. Approximately 25g of the sample was placed within powerbead tubes and then mixed briefly. This begins the process of breaking down unwanted material and preserving DNA matter.
2. 60 µl of solution C1 containing agents was added to the sample. The solution was mixed briefly enabling the agents to begin to degrade the fatty acids of organisms present within the sample.
3. Samples then underwent beadbeating for two minutes to ensure that samples were homogenised. This process breaks open the cells of organisms within the sample prepping for the extraction of DNA.
4. The supernatant was removed from the powerbead tubes and transferred into collection tubes.
5. 250 µl of Solution C2 was added to the collection tubes. This helps separate DNA and non DNA material. Samples were then placed in ice for five minutes.
6. Samples were centrifuged and the supernatant was then transferred to new collection tubes. The pellet was discarded.
7. 200 µl of solution C3 was added to the clean collection tubes. This solution also helps separate DNA and non DNA material. Samples were then mixed and 750 µl of the supernatant was transferred into new collection tubes.
8. 1.2 ml of solution C4 was added to the new collection tubes. This solution is high in salt and enables DNA to bind to it, allowing the DNA material to be preserved.
9. 675 µl of this mix was added to a spin filter and then centrifuged for one minute. The flow through was discarded. This step was repeated three times. This process is removing non DNA material that does not bind to solution C4.
10. 500 µ of solution C5 was added, and then samples were centrifuged for 30 seconds. This solution is ethanol based and cleans the DNA that was bound to solution C4 and removes any left other non DNA matter. The supernatant was discarded.
11. The samples were then centrifuged for one minute removing any trace of solution C5.
12. 100 µl was then added to the sample. This allows the DNA that was bound to solution C4 to be released. Samples were centrifuged for 30 seconds. The spin filter was then removed. DNA was then stored at -20°C until further use.

Appendix 3: Next generation sequencing

Due to time constraints 25 of 37 sediment samples, 14 and 11 from the Karori Upper Dam and Lake Pounui, respectively, were chosen to undergo next generation sequencing. Samples were chosen to be representative of each specific environment at each site. Therefore any samples that appeared anomalous from the ARISA results were excluded from next generation sequencing.

To determine the bacterial community and diversity at each sample site the DNA samples previously extracted to undergo ARISA were amplified by PCR using Bacterial primers 5'-GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GGG ACT ACH VGG GTW TCT AAT -3' and 5'-TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG GTG CCA GCM GCC GCG GTA A -3'.

The PCR samples consisted of 50uM samples made up of 45uM Plat Super Master Mix, 10uM of each primer and 10-20ng of extracted DNA. The PCR samples were held at a temperature of 94°C for 2 minutes before running for 27 cycles at intervals of, 30 seconds at 94°C, 30 seconds at 54°C, 45 seconds fat 68°C, and 5 minutes at 68°C. Samples were then held at a temperature of 10°C forever.

In order to discount any contamination that may have occurred during the PCR, samples were then run through an argose gel. Samples then underwent PCR clean up using a magnetic plate in accordance with the Agencourt protocol. Cleaned up samples were then quantified using the Qubit 2.0 to ensure that the samples had at least 10 ug of DNA to undergo next generation sequencing.

The samples were then sent to the University of Waikato to undergo next generation sequencing. This method amplifies the V3 and V4 regions of the 16s rRNA gene within DNA in order to identify bacterial communities within a sample, using the following methods.

The same methods were used for water samples.

Appendix 4: Geochemical Analysis

Table 1: Geochemical analysis for Karori Upper Dam sediments.

	Depth (m)	Name	TOC (%)	TN (%)	TS (%)	TOC/TN	TOC/TS	Extract [ppm]	mgExt/gTOC
K6 Kaori	Shallow (0.8m)	KS1	15.6	1.07	0.66	14.6	23	9399	60
K11 Kaori	Shallow (0.9m)	KS2	16.3	0.95	0.24	17.1	68	9065	56
K12 Kaori	Shallow (009m)	KS3	14.8	1.10	0.34	13.5	44	8396	57
K10 Kaori	Shallow (1.2m)	KS4	14.0	0.94	0.33	15.0	43	7692	55
K1 Kaori	Shallow (1m)	KS5	11.7	0.86	0.36	13.6	32	6393	55
K8 Kaori	Intermediate (3.4m)	KI1	14.9	0.92	0.33	16.3	45	7065	47
K5 Kaori	Intermediate (4.1m)	KI2	11.7	0.98	0.60	12.0	20	8733	75
K9 Kaori	Intermediate (4.5m)	KI3	16.0	1.01	0.29	15.9	56	7808	49
K2 Kaori	Intermediate (4.8m)	KI4	14.6	1.04	0.33	14.1	45	8317	57
K7 Kaori	Intermediate (5.4m)	KI5	15.5	1.02	0.27	15.1	57	8540	55
K3 Kaori	Deep (7.7m)	KD3	14.2	1.03	0.31	13.8	45	8802	62
K4 Kaori	Deep (6.4m)	KD1	14.7	1.01	0.35	14.5	42	8048	55
K15 Kaori	Soil	KSo1	10.5	0.80	0.10	13.2	106	4144	39
K16 Kaori	Soil	KSo2	29.5	1.34	0.16	22.0	182	14519	49
K19 Kaori	Soil	KSo3	13.5	0.86	0.10	15.8	134	7480	55
K20 Kaori	Soil	KSo4	18.4	1.18	0.14	15.6	134	3328	18
K17 Kaori	Stream	KSt1	2.0	0.15	0.01	13.4	143	542	27
K18 Kaori	Stream	KSt2	1.0	0.08	0.02	12.0	50	251	25
K13 Kaori	Swamp	KSw1	18.6	1.13	0.19	16.4	100	5503	30
K14 Kaori	Swamp	KSw2	15.7	0.81	0.23	19.5	69	5860	37

Table 2: Geochemical analysis for Lake Pounui sediments.

	Depth (m)	Name	TOC (%)	TN (%)	TS (%)	TOC/TN	TOC/TS	Extract [ppm]	mgExt/gTOC
P1 Pounui	Shallow (1.1m)	PS1	14.7	1.51	1.04	9.7	14	12139	82
P11 Pounui	Shallow (1.6m)	PS2	8.6	0.95	0.85	9.1	10	7332	85
P7 Pounui	Shallow (2.9m)	PS3	7.2	0.92	0.59	7.8	12	5390	75
P2 Pounui	Intermediate (4.4m)	PI1	11.5	1.36	0.76	8.4	15	10961	95
P8 Pounui	Intermediate (4.4m)	PI2	6.6	0.86	0.29	7.6	22	4242	65
P3 Pounui	Intermediate (5.2m)	PI3	7.0	0.84	0.37	8.4	19	5589	80
P10 Pounui	Intermediate (6.4m)	PI5	5.8	0.68	0.20	8.5	29	3575	62
P9 Pounui	Intermediate (6.6m)	PI6	3.7	0.47	0.09	7.9	40	1744	47
P4 Pounui	Deep (8m)	PD1	5.2	0.65	0.16	8.0	33	2904	56
P5 Pounui	Deep (9.5m)	PD2	4.6	0.57	0.12	8.0	38	2302	50
P6 Pounui	Intermediate (5.5m)	PI4	8.5	1.11	0.37	7.6	23	6755	80
P17 Pounui	Soil	PSo1	5.6	0.37	0.05	15.0	109	2525	45
P18 Pounui	Soil	PSo2	4.1	0.31	0.03	13.4	131	2067	50
P19 Pounui	Soil	PSo3	9.8	0.41	0.05	24.2	202	7008	71
P20 Pounui	Soil	PSo4	9.0	0.63	0.03	14.3	303	4139	46
P21 Pounui	Stream	PSt1	1.7	0.21	0.01	8.2	165	219	13
P22 Pounui	Swamp	PSw1	15.7	1.17	0.21	13.5	74	9182	58

Appendix 6: Origin of bacteria in sediments

Table 1: Origin of bacteria present in catchment and lake sediments at the Karori Upper Dam.

	Shallow 0.8m	Shallow 0.9m	Shallow 0.9m	Shallow 1.2m	Shallow 1m	Intermediate 3.3m	Intermediate 4.1m	Intermediate 4.5m	Intermediate 4.8m	Intermediate 5.4m
Soil %	57.57	59.98	60.49	58.02	58.71	54.44	56.24	60.85	54.81	61.38
Stream %	24.49	24.87	23.96	23.93	22.37	25.66	19.29	23.58	22.04	26
Swamp %	5.05	0.52	1.96	2.96	4.47	1.7	4.61	2.24	3.79	1.19
Water %	6	5.04	6.72	6.9	8.22	9.77	12.53	6.76	10.78	6.14
Shallow %	6.89	9.58	6.87	8.18	6.23	5.91	6.11	5.34	5.77	3.69
Intermediate %	0	0	0	0	0	2.51	1.21	1.23	2.8	1.6
Deep %	0	0	0	0	0	0	0	0	0	0
Lake Sediment	6.89	9.58	6.87	8.18	6.23	8.42	7.32	6.57	8.57	5.29
Terrestrial	87.11	85.37	86.41	84.91	85.55	81.8	80.14	86.67	80.64	88.57
Lacustrine	12.89	14.62	13.59	15.08	14.45	18.19	19.85	13.33	19.35	11.43

	Deep 6.4m	Deep 7.7m	Soil	Soil	Soil	Soil	Stream	Stream	Swamp	Swamp
Soil %	65.15	55.12	100	100	100	100	62.03	48.9	75.77	69.76
Stream %	16.46	22.67	0	0	0	0	37.97	51.1	7.84	12.28
Swamp %	1.23	2.92	0	0	0	0	0	0	16.38	17.96
Water %	9.46	13.43	0	0	0	0	0	0	0	0
Shallow %	5.71	4.59	0	0	0	0	0	0	0	0
Intermediate %	1.99	0.75	0	0	0	0	0	0	0	0
Deep %	0	0.53	0	0	0	0	0	0	0	0
Lake Sediment	7.7	5.87	0	0	0	0	0	0	0	0
Terrestrial	82.84	80.71	100	100	100	100	100	100	99.99	100
Lacustrine	17.16	19.3	0	0	0	0	0	0	0	0

Table 2: Origin of bacteria present in catchment and lake sediments at Lake Pounui.

	Shallow (1.1m)	Shallow (1.6m)	Shallow (2.9m)	Intermediate (4.4m)	Intermediate (4.4m)	Intermediate (5.2m)	Intermediate (5.5m)	Intermediate (6.4m)	Intermediate (6.6m)
Soil %	58.6	69.78	57.15	56.7	56.23	57.78	50.35	56.98	53.25
Stream %	1.33	2.44	5	3.19	1.97	2.91	1.65	4.23	14.51
Swamp %	7.8	5.26	6.53	5.89	7.98	6.4	7.01	6.24	7.57
Water %	16.01	5.64	14.3	10.8	13.68	11.44	15.95	16.76	9.27
Shallow %	16.26	16.89	17.03	18.37	16.14	16.6	19.23	10.99	5.19
Intermediate %	0	0	0	5.04	4	4.87	5.81	4.82	10.21
Deep %	0	0	0	0	0	0	0	0	0
Terrestrial %	67.73	77.48	68.68	65.78	66.18	67.09	59.01	67.45	75.33
Lacustrine %	32.27	22.53	31.33	34.21	33.82	32.91	40.99	32.57	24.67

	Deep (8m)	Deep (9.5m)	Soil	Soil	Soil	Soil	Stream	Swamp
Soil %	52.72	49.81	100	100	100	100	62.14	53.35
Stream %	2.74	3.81	0	0	0	0	37.86	7.32
Swamp %	7.22	9.51	0	0	0	0	0	39.33
Water %	18.56	15.98	0	0	0	0	0	0
Shallow %	13.56	13.5	0	0	0	0	0	0
Intermediate %	4.03	5.11	0	0	0	0	0	0
Deep %	1.17	2.29	0	0	0	0	0	0
Terrestrial %	62.68	63.13	100	100	100	100	100	100
Lacustrine %	37.32	36.88	0	0	0	0	0	0

Appendix 7: GDGT analysis

Table 1: Relative abundances of branched and isoprenoid GDGTs at the Karori Upper Dam

		Isoprenoid GDGTs						Branched GDGTs								
Label	Environment	1302	1300	1298	1296	1292	1292'	1050	1048	1046	1036	1034	1032	1022	1020	1018
KS1	Shallow	60451	9724	6893	1383	13431	1011	121793	6217	1646	171073	44603	6244	74248	24893	9229
KS2	Shallow	24662	4729	3099	678	7331	301	128366	4049	1204	192636	30114	3491	77826	18330	4844
KS3	Shallow	44084	7196	4500	943	8443	421	129573	5505	1299	202770	55195	6756	88149	32477	13188
KS4	Shallow	48665	6354	4285	879	8151	536	139129	6777	1309	202868	51401	6167	84940	30122	10882
KS5	Shallow	37665	5148	3116	639	5341	455	101702	4382	834	154386	44900	5593	68581	26653	11549
KI1	Intermediate	63962	8808	6266	1367	13858	584	274747	11403	2770	411535	100481	11663	168496	55931	20088
KI2	Intermediate	73447	8841	5500	858	8788	439	192516	8541	1171	236638	70886	8450	101398	40478	14828
KI3	Intermediate	50564	6761	4483	902	10049	505	186704	8712	1611	259373	70083	8208	101430	39274	12605
KI4	Intermediate	49753	6453	3572	784	7641	401	155087	7557	1519	216752	67486	7670	85371	35785	13514
KI5	Intermediate	70949	8244	4875	1133	10013	555	195575	9772	2096	277307	78198	9354	110863	43895	15103
KD1	Deep	62564	6802	3986	742	9146	462	167886	8229	1669	242131	75493	8849	98840	39959	14646
KD3	Deep	62573	5624	3915	1023	7938	548	136987	6921	1586	200086	57415	6547	83783	31326	11061
KSo1	Soil	4547	1809	1561	848	12407	561	80960	2756	204	275702	38432	2160	152285	29612	6790
KSo2	Soil	594	0	0	0	448	0	17807	810	101	91001	8603	689	87798	12224	2064
KSo3	Soil	553	84	209	0	0	0	25895	124	0	152795	5308	604	165494	9080	1918
KSo4	Soil	1607	1566	1468	599	5545	458	78263	5224	383	133101	44626	2265	48230	23049	5103
KSt1	Stream	12962	10433	6716	1595	16397	974	117974	5898	2744	191847	25457	4001	102137	21246	7698
KSt2	Stream	75746	30294	19085	3626	45297	1903	507137	15196	6475	883746	100710	21462	446192	86217	27477
KSw1	Swamp	15859	11429	6849	1401	21627	1008	215136	8107	3058	313074	42910	7450	129450	26095	8317
Ksw2	Swamp	12716	4364	3036	602	7665	639	102357	2999	996	159123	17602	3130	71246	13007	3722

Table 2: GDGT indices calculated for the Karori Upper Dam.

Label	Environment	BIT	MBT	MBTm	CBT	CR	MBT/CBT	Cal/Cren	soil_pH	MAT (°C)	MAT (°C)	MAT Tierney (°C)	Tex86	SST (°C)	SST (°C)
KS1	Shallow	0.96	0.236	0.282	0.55	0.28	0.43	4.50	7.32	0.6	6.9	13.5	0.49	13	13
KS2	Shallow	0.98	0.219	0.290	0.75	0.18	0.29	3.36	6.80	-2.1	6.0	10.7	0.46	11	12
KS3	Shallow	0.98	0.250	0.255	0.52	0.30	0.48	5.22	7.39	1.5	7.7	14.8	0.45	10	11
KS4	Shallow	0.98	0.236	0.276	0.55	0.28	0.43	5.97	7.32	0.6	6.9	13.6	0.47	12	12
KS5	Shallow	0.98	0.255	0.255	0.49	0.32	0.52	7.05	7.47	2.0	8.0	15.1	0.45	10	11
KI1	Intermediate	0.98	0.230	0.276	0.57	0.27	0.41	4.61	7.27	0.1	6.6	13.3	0.48	13	13
KI2	Intermediate	0.98	0.232	0.300	0.48	0.33	0.48	8.36	7.49	1.0	6.7	13.0	0.43	9	10
KI3	Intermediate	0.98	0.223	0.286	0.52	0.30	0.43	5.03	7.40	0.2	6.2	13.3	0.47	11	12
KI4	Intermediate	0.98	0.228	0.278	0.47	0.34	0.49	6.51	7.54	0.9	6.5	14.4	0.42	8	9
KI5	Intermediate	0.98	0.229	0.280	0.50	0.31	0.46	7.09	7.44	0.6	6.5	13.9	0.44	10	10
KD1	Deep	0.98	0.233	0.270	0.47	0.34	0.50	6.82	7.53	1.2	6.8	14.7	0.43	9	10
KD3	Deep	0.98	0.236	0.272	0.50	0.31	0.47	7.88	7.43	1.0	6.9	14.3	0.49	14	14
KSo1	Soil	0.98	0.320	0.143	0.80	0.16	0.40	0.37	6.65	2.4	11.6	16.5	0.62	24	22
Kso2	Soil	1.00	0.460	0.085	0.92	0.12	0.50		6.35	8.3	19.3	17.8			
KSo3	Soil	1.00	0.488	0.072	1.35	0.05	0.36		5.22	5.7	20.8	15.9	0.71	32	28
KSo4	Soil	0.98	0.224	0.247	0.43	0.37	0.52	0.29	7.63	1.1	6.3	16.1	0.62	24	22
KSt1	Stream	0.96	0.274	0.264	0.80	0.16	0.34	0.79	6.66	0.1	9.0	12.1	0.47	12	12
KSt2	Stream	0.98	0.267	0.253	0.85	0.14	0.31	1.67	6.52	-0.7	8.6	11.8	0.45	10	11
KSw1	Swamp	0.97	0.217	0.300	0.81	0.16	0.27	0.73	6.64	-2.8	5.9	10.2	0.45	10	11
KSw2	Swamp	0.98	0.235	0.284	0.88	0.13	0.27	1.66	6.46	-2.5	6.9	10.1	0.49	14	14

Table 3: Relative abundances of branched and isoprenoid GDGTs at the Lake Pounui

		Isoprenoid GDGTs						Branched GDGTs								
Label	Environment	1302	1300	1298	1296	1292	1292'	1050	1048	1046	1036	1034	1032	1022	1020	1018
PD1	Deep	80709	11695	5210	815	9979	494	166353	7157	2503	260427	64918	10560	108432	49737	22272
PD2	Deep	102863	15953	8094	1160	15150	704	199185	9416	2879	339763	78328	12760	143256	61060	25652
PI1	Intermediate	169174	9438	4090	801	3475	290	261830	9815	1518	307466	78772	8876	129346	54849	16776
PI2	Intermediate	135160	8365	3658	670	3437	226	262114	9788	1670	296615	63332	7550	116296	42975	14303
PI3	Intermediate	141734	9864	4636	989	3563	191	196134	6615	1347	237540	49139	6059	100002	34727	12992
PI4	Intermediate	139314	8726	4387	940	4041	155	248523	9800	1747	271473	68457	9082	103396	46693	16079
PI5	Intermediate	107700	13681	6058	878	8292	385	171580	7165	1580	244057	56705	8777	112442	45240	16712
PI6	Intermediate	127508	16727	7064	1200	8514	424	223901	10007	2139	355765	81760	10657	151413	57405	21740
PS1	Shallow	197317	8792	4294	908	2939	308	198596	7548	1315	317584	91083	9770	151733	69205	27683
PS2	Shallow	243854	10431	5541	1211	5403	374	339188	11285	1431	390704	96039	10008	157785	67424	20362
PS3	Shallow	149156	9795	4868	926	3482	241	238823	7405	1429	251834	45230	5857	96453	31020	10920
PSo1	Soil	2052	29	89	22	39	78	23796	149	262	183617	2642	420	279077	6883	2055
PSo2	Soil	1881	38	61	35	0	0	38251	129	87	248920	6387	717	301555	14960	6114
PSo3	Soil	7717	0	80	0	15	16	7265	365	0	81197	1706	425	151694	1942	813
PSo4	Soil	28829	12594	9945	2260	38934	2080	12750	0	80	84295	2260	747	99697	2976	1050
PSt1	Stream	8480	3497	2883	884	5465	464	53598	1701	670	133242	16121	2734	97188	19171	6091
PSw1	Swamp	4213	1024	1062	331	4836	398	52315	1060	183	161113	11814	2389	130359	21343	4548

Table 4: Table 2: GDGT indices calculated for Lake Pounui.

Label	Environment	BIT	MBT	MBTm	CBT	ratio	MBT/CBT	Cal/Cren	soil_pH	MAT (°C)	MAT (°C)	MAT Tierney (°C)	Tex86	SST (°C)	SST (°C)
PS1	Shallow	1.00	0.28	0.24	0.47	0.34	0.61	67.1	7.54	3.8	9.6	16.1	0.39	4.8	6.4
PS2	Shallow	0.99	0.22	0.32	0.53	0.30	0.43	45.1	7.38	0.2	6.3	11.2	0.41	6.5	7.7
PS3	Shallow	0.99	0.20	0.36	0.66	0.22	0.30	42.8	7.03	-2.2	5.0	8.3	0.38	4.5	6.1
PI1	Intermediate	1.00	0.23	0.31	0.51	0.31	0.45	48.7	7.41	0.7	6.7	11.8	0.35	2.3	4.3
PI2	Intermediate	0.99	0.21	0.34	0.59	0.26	0.36	39.3	7.21	-1.0	5.7	10.1	0.35	2.1	4.2
PI3	Intermediate	0.99	0.23	0.32	0.60	0.25	0.38	39.8	7.17	-0.3	6.6	10.9	0.37	3.6	5.4
PI4	Intermediate	0.99	0.21	0.34	0.51	0.31	0.42	34.5	7.41	-0.2	5.7	11.0	0.39	4.8	6.4
PI5	Intermediate	0.98	0.26	0.27	0.54	0.29	0.48	13.0	7.33	1.9	8.4	13.8	0.35	1.8	3.9
PI6	Intermediate	0.99	0.25	0.26	0.56	0.27	0.45	15.0	7.29	1.3	7.8	14.3	0.34	1.2	3.4
PD1	Deep	0.98	0.26	0.25	0.51	0.31	0.51	8.1	7.43	2.2	8.3	15.3	0.36	2.6	4.5
PD2	Deep	0.98	0.26	0.24	0.54	0.29	0.49	6.8	7.34	2.0	8.4	15.5	0.38	4.8	6.3
PSo1	Soil	1.00	0.58	0.05	1.69	0.02	0.34	163.5	4.33	7.0	25.7	15.9	0.77	36.3	32.1
PSo2	Soil	1.00	0.52	0.06	1.40	0.04	0.37		5.09	7.0	22.7	16.2	0.71	31.6	28.3
PSo3	Soil	1.00	0.63	0.03	1.79	0.02	0.35	65.3	4.06	8.7	28.6	16.3	1.00	54.9	47.3
PSo4	Soil	0.84	0.51	0.06	1.53	0.03	0.33	0.7	4.73	5.1	22.0	15.8	0.52	16.1	15.6
PSt1	Stream	0.98	0.37	0.17	0.81	0.15	0.45	1.6	6.62	4.8	14.3	15.5	0.55	18.0	17.2
PSw1	Swamp	0.99	0.41	0.14	0.94	0.11	0.43	0.9	6.28	5.4	16.3	15.4	0.64	25.3	23.1

Appendix 10: Seasonal variation of other measured environmental controls

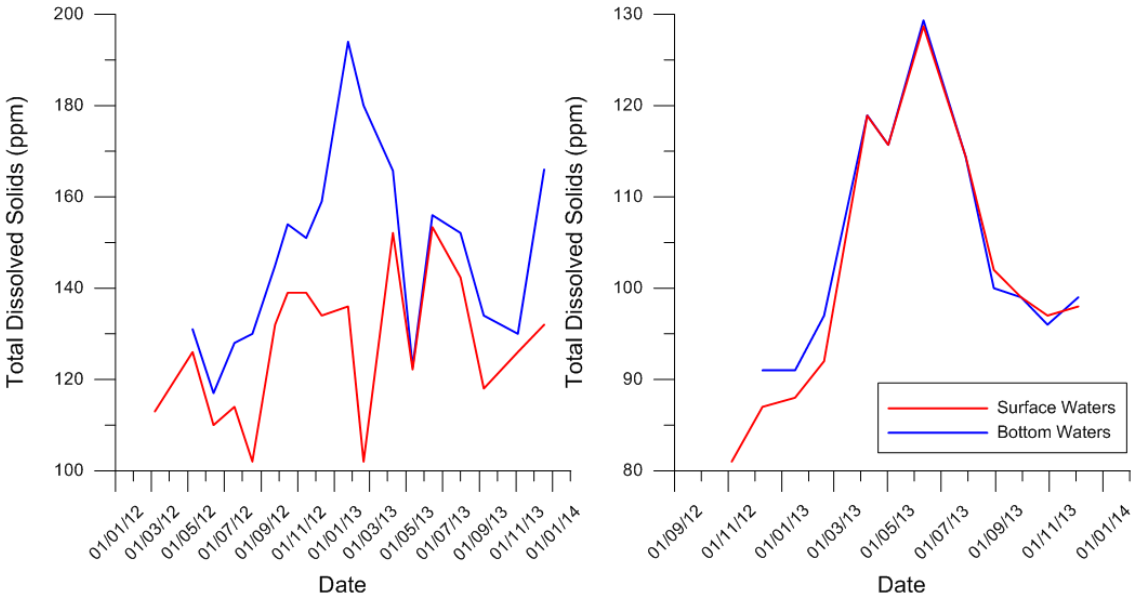

Figure 1: *Surface and bottom water conductivity levels at the Karori Upper Dam (A) and Lake Pounui (B) over the monitoring period. Surface waters refer to a measurement taken at a depth of 1m, bottom waters refer to a measurement taken at a depth of 8m and 9m at the Karori Upper Dam and Lake Pounui respectively.*

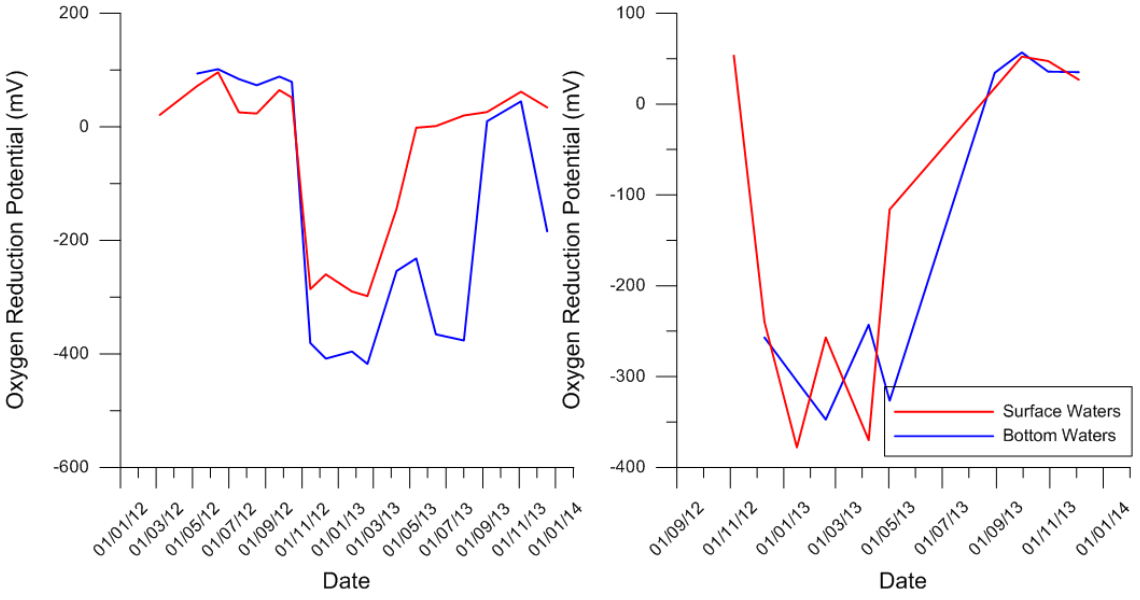

Figure 2: *Surface and bottom water conductivity levels at the Karori Upper Dam (A) and Lake Pounui (B) over the monitoring period. Surface waters refer to a*

measurement taken at a depth of 1m, bottom waters refer to a measurement taken at a depth of 8m and 9m at the Karori Upper Dam and Lake Pounui respectively

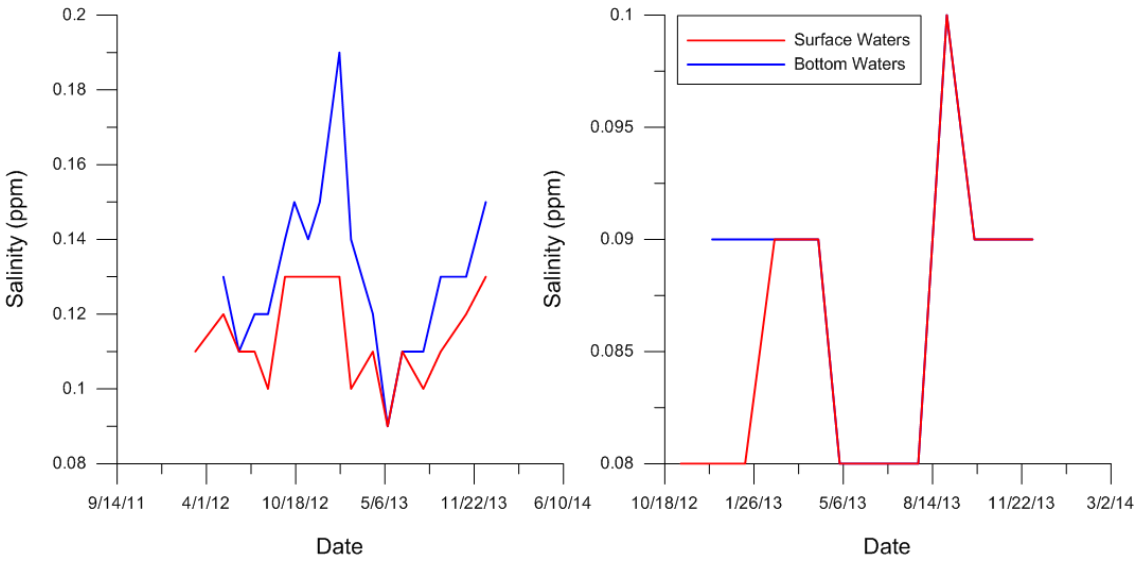

Figure 3: *Surface and bottom water conductivity levels at the Karori Upper Dam (A) and Lake Pounui (B) over the monitoring period. Surface waters refer to a measurement taken at a depth of 1m, bottom waters refer to a measurement taken at a depth of 8m and 9m at the Karori Upper Dam and Lake Pounui respectively*

Appendix 11: Origin of bacteria within the water column

Table 1: Origin of bacteria within the water column at the Karori Upper Dam. Samples are all surface water samples (taken at a depth of 1m) unless the date is preceded by a 'B'. 'B' water samples refer to samples taken from bottom waters (taken at a depth of 8m).

	13/06/12	19/07/12	17/08/12	24/09/12	15/10/12	15/11/12	11/12/12	18/02/13	B 18/2/13	9/04/13	B 9/04/13	9/05/13
Soil %	38.68	26.34	40.84	31.2	41.4	58.85	63.59	51.03	54.92	41.7	41.42	20.06
Stream %	19.49	25.64	7.34	20.35	23.48	9.7	11.39	14.19	22.62	15.32	17.63	43.06
Swamp %	1.89	3.32	1.57	4.49	6.69	0.79	5.63	6.82	1.43	13.51	5.25	3.13
Water %	39.94	44.7	50.25	43.96	28.43	30.67	19.38	27.96	21.03	29.47	35.7	33.74
Shallow %	0	0	0	0	0	0	0	0	0	0	0	0
Intermediate %	0	0	0	0	0	0	0	0	0	0	0	0
Deep %	0	0	0	0	0	0	0	0	0	0	0	0
Terrestrial %	60.06	55.3	49.75	56.04	71.57	69.33	80.62	72.04	78.97	70.53	64.3	66.26
Lacustrine %	39.94	44.7	50.25	43.96	28.43	30.67	19.38	27.96	21.03	29.47	35.7	33.74

Table 2: Origin of bacteria within the water column at Lake Pounui. Samples are all surface water samples (taken at a depth of 1m) unless the date is preceded by a 'B'. 'B' water samples refer to samples taken from bottom waters (taken at a depth of 8m).

	5/11/12	16/01/13	18/02/13	B 18/02/13	8/04/2013	B 8/04/13	2/05/13	B 2/05/13
Soil %	66.53	32.97	51.34	60.94	49.73	52.26	39.5	36.86
Stream %	3.4	2.65	1.12	1.2	1.64	1.55	1.33	1.75
Swamp %	2.86	4.6	1.64	1.88	3.81	3.32	4.41	5.13
Water %	27.21	59.79	45.9	35.98	44.83	42.87	54.76	56.25
Lake Sediment %	0	0	0	0	0	0	0	0
Shallow %	0	0	0	0	0	0	0	0
Intermediate %	0	0	0	0	0	0	0	0
Deep %	0	0	0	0	0	0	0	0
Terrestrial	72.79	40.21	54.1	64.02	55.17	57.13	45.24	43.75
Lacustrine	27.21	59.79	45.9	35.98	44.83	42.87	54.76	56.25

Appendix 12: Bacteria concentrations

Table 1: Concentrations of the most abundant bacteria within the surface waters at Lake Pounui.

Species	5/11/2012 (proportion)	16/01/2013 (proportion)	18/02/2013 (proportion)	8/04/2013 (proportion)	2/05/2013 (proportion)
476.1	0.07772	0.00000	0.00342	0.00000	0.14531
408.8	0.03924	0.04509	0.05618	0.04469	0.00000
556.2	0.03515	0.01649	0.08427	0.00674	0.00866
658.1	0.02469	0.00000	0.02709	0.00000	0.08779
378.8	0.00000	0.01943	0.05849	0.02962	0.02896
498.5	0.00000	0.01803	0.00000	0.11123	0.00111
755.8	0.03238	0.04218	0.03479	0.00000	0.01314
380.3	0.08973	0.00000	0.00893	0.00000	0.00346
858.2	0.00000	0.00177	0.00000	0.00000	0.09102
409.1	0.00000	0.00000	0.00000	0.00000	0.08252
348.2	0.00729	0.00000	0.04703	0.00466	0.01549

Table 2: Concentrations of the most abundant bacteria within the bottom waters at Lake Pounui.

Species	18/02/2013 (proportion)	8/04/2013 (proportion)	2/05/2013 (proportion)
476.1	0.00553	0.03604	0.11423
408.8	0.11784	0.00000	0.00000
556.2	0.04720	0.00622	0.00823
658.1	0.02475	0.09672	0.09932
378.8	0.05724	0.02688	0.01524
498.5	0.00000	0.12160	0.00212
755.8	0.01970	0.00000	0.03039
380.3	0.02568	0.01125	0.00815
858.2	0.00000	0.00000	0.09093
409.1	0.00000	0.04734	0.08506
348.2	0.04703	0.00665	0.01401