Low-intensity land use in grassland catchments: Effects on a large, oligotrophic lake

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Abstract

In southern New Zealand, many upland streams drain into large oligotrophic lakes surrounded by native grassland, low-intensity farming, and small urban centers. Little work has been undertaken to determine the impact low-intensity development has on nutrient dynamics and microbial activity in these large lake systems. Lake Wanaka, Central Otago, was chosen as a study site since the recent appearance of nuisance organic aggregates and changes in phytoplankton community structure suggest the lake is not in a steady state. Research undertaken for this project included intensive sampling of tributaries to the lake during different seasons and hydrological conditions, following the path of two tributaries out into the lake, and laboratory-based experiments.

In the Wanaka catchment, pasture cover correlated positively with stream dissolved organic carbon (DOC), total nitrogen (TN) and nitrate-nitrogen (NO_3-N) concentrations. Nitrogen concentrations were not influenced by weather-related variables, but temperature and soil moisture mitigated the influence of pasture cover on surface water DOC concentration under very dry or wet conditions. Neither land use nor weather-related conditions correlated with total phosphorus (TP) or dissolved phosphorus (DRP) concentrations in streams, possibly reflecting good P-binding in soils, low-intensity agriculture in the catchment and/or lack of sampling during high flow events. Amending lake water with stream water in the laboratory did not influence the production of sticky polysaccharides (i.e. transparent exopolymer particles (TEP)), but enriching treatments with high concentrations of N and P increased TEP 1.7 to 9.3 times over unamended treatments. Phytoplankton cell numbers, diatom abundance, and chl *a* also increased in response to nutrient-enrichment, and organic aggregates were visible in nutrient-enriched treatments within 6 days.

In the field, the intermixing depth of a main river inflow varied under stratified and un-stratified conditions, affecting where catchment-derived material was delivered in the Lake. Nutrient and DOC concentrations in the Matukituki River were within range of the Lake, and the river plume was capable of stimulating phytoplankton growth in nearshore waters. Despite similar bulk DOC concentrations, dissolved organic matter (DOM) character and lability differed between the River and the Lake. DOM from

deep-sourced lake water contained more aromatic, refractory structures than shallower lake water or river water. The river had almost double the number of organic sulphur compounds than the lake, including potential sulfonates. The source of the S is unknown, but may be geologic in origin or reflect agricultural activity in the River catchment.

In the laboratory, riverine bacterial communities could break down a diverse array of organic substances regardless of season, suggesting a consistent labile supply of DOM. In contrast, organic substrate use patterns in the lake were seasonal, and varied by depth. Lake water amended with Matukituki River water stimulated bacterial respiration and uptake of DOC and P, but did not affect bacterial productivity, which may reflect limitations of the experimental design.

My results indicate low intensity land use in grassland catchments affects nutrient flux and microbial processes in Lake Wanaka. These data provide a foundation for future research on land development and microbial dynamics in similar large, oligotrophic lake systems.

Acknowledgements

List of Acronyms

DO: Dissolved oxygen

DOC: Dissolved organic carbon

DOM: Dissolved organic matter

LMW DOM: Low molecular weight dissolved organic matter

tDOM: Terrestrially-derived dissolved organic matter

OM: Organic matter

POC: Particulate organic carbon

TOC: Total organic carbon

DIC: Dissolved inorganic carbon

TDN: Total dissolved nutrients

P: Phosphorus

TP: Total phosphorus

DRP: Dissolved reactive phosphorus

N: Nitrogen

TN: Total nitrogen

NO_3-N: Nitrate-nitrogen

DIN: Dissolved inorganic nitrogen (NO_3-N + ammonium-N (NH_4-N))

DON: Dissolved organic nitrogen

DOP: Dissolved organic phosphorus

cDOC: Chromophoric dissolved organic carbon

TEP: Transparent exopolymer particles

chl *a*:   Chlorophyll *a*

TSS: Total suspended solids

SSC: Suspended solid concentration

CHO: Formulae containing only carbon, hydrogen, oxygen

CHO-N: Formulae containing carbon, hydrogen, oxygen and nitrogen

CHO-S: Formulae containing carbon, hydrogen, oxygen and sulphur

CHO-P: Formulae containing carbon, hydrogen, oxygen and phosphorus

FT-ICR-MS: Fourier transform ion cyclotron resonance mass spectrometry

SPE: Solid phase extraction

AI: Aromaticity index

DBE: Double bond equivalents

DBE/C: Double bond equivalents normalised to the number of carbons

SBR: Specific bacterial respiration

BP: Bacterial productivity

BA: Bacterial abundance

BR: Bacterial respiration

BGE: Bacterial growth efficiency

BPD: Bacterial physiological diversity

BOD: Biological oxygen demand

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1 Introduction

Fresh water habitats provide a source of food, drinking water and energy. Yet many anthropogenic activities that use freshwater sources also pollute waterways, impair water quality, degrade habitats and decrease biodiversity (Ongley 1996). Over the past 40 years, gains have been made in controlling point-source discharges of pollutants into streams and lakes (US-EPA 2000, Davies−Colley 2013, Malaj *et al.* 2014) but diffuse input of nutrients, contaminants, and sediments through runoff or subsurface flow remains a significant problem (Edmondson 1994, Carpenter *et al.* 1998, Correll *et al.* 1999, Beeton 2002).

Nitrogen (N) and phosphorus (P) are the most widely recognised nutrients in waterways, (Uchida 2000), and the dissolved inorganic components are the most bioavailable forms for plants (Reynolds and Davies 2001, Rabalais 2002). In undisturbed systems, N and P are scarce and are tightly cycled (Gundersen and Bashkin 1994, Moss *et al.* 2013). However, excessive inputs of N or P can disrupt the balance between nutrient inputs and nutrient cycling in aquatic systems (Lavelle *et al.* 2005), leading to eutrophication of streams and downstream water bodies. Eutrophication prolongs and intensifies aquatic weed growth and can reduce habitat availability, cause volatility in dissolved oxygen levels, alter food webs and lower species diversity (Carpenter *et al.* 1998, Dauer *et al.* 2000). Fertilizers and manure can facilitate eutrophication by creating a surplus of macronutrients in soils above what is necessary for plant growth (Carpenter *et al.* 1998). This surplus is then available for input into water bodies via runoff, leaching, and infiltration to ground water.

Like N and P, carbon is an essential nutrient present in all living organisms. Both autotrophic and heterotrophic organisms require carbon (either as CO_2 for autotrophs or in organic form for heterotrophs) for biosynthesis. Organic carbon is frequently distinguished as either particulate (POC) or dissolved (DOC), where DOC is capable of passing through a 0.45- to 0.7-μm pore size filter. The majority of organic carbon exported downstream is comprised of DOC, particularly in small, undisturbed catchments (Hope *et al.* 1994). Dissolved organic carbon is derived from external (allochthonous) and internal (autochthonous) sources, yet the character of naturally

occurring DOC remains largely unknown, as its molecular composition is extremely complex (Kim *et al.* 2003, Gonsior *et al.* 2011, Mead *et al.* 2013). In general, aquatic DOM sources, such as algal exudate, often contain high concentrations of freshly-produced carbohydrates (Biddanda and Benner 1997), while terrestrially-derived DOM (tDOM) spans a continuum from relatively unaltered easily identifiable plant residues to strongly altered plant and animal material (Biddanda and Benner 1997). tDOM tends to contain a higher proportion of polymerised humic substances such as lignins and tannins (Benner 2004) or their soluble microbial degradation products. These humic substances are synthesised through decomposition and humification processes, and tend to be enriched in aromatic structures that are relatively recalcitrant (Johnson *et al.* 2009, Wagner *et al.* 2015). In aquatic systems, allochthonous DOM can either be incorporated into the food web via the microbial loop (Pace *et al.* 2007, Solomon *et al.* 2011), or mineralised, resulting in the release of nutrients and CO_2 into the water column, stimulating primary production (Solomon *et al.* 2015).

1.1 Factors affecting the movement of nutrients and organic matter to downstream waterbodies

1.1.1 Landscape characteristics

Landscape characteristics such as soil conditions, vegetation cover and catchment slope influence the movement of nutrients and DOC into waterways (Farley *et al.* 2004, Mark and Dickinson 2008, Wilson and Xenopoulos 2008). Soil conditions such as drainage ability, mineral content, grain size, porosity, composition and compaction (Letey and Vaughan 2013), influence the ability of the soil to absorb water and adsorb nutrients and organic material. High mineral content in soils leads to the formation of organo-mineral complexes that are resistant to microbial degradation, thereby reducing the amount of DOC available for export to waterways (Post and Kwon 2000, Bass *et al.* 2011). Finely textured clay soils can strongly adsorb phosphate ions, while coarsely textured sandy soils are more inert and are less able to retain P (Busman *et al.* 2002). Nitrogen leaching rates are also influenced by soil texture and soil moisture levels, with sandy soils particularly vulnerable to leaching due to large pore sizes and poor water retention (USDA 2001, Letey and Vaughan 2013).

Overlying vegetation cover influences nutrient and organic matter concentrations in soils. While soils beneath undisturbed grasslands and many forested systems (Davidson *et al.* 2004, Bond 2008) tend to be nutrient-poor, fertilizers, animal waste

and high plant productivity can increase the amount of N and P beneath pastures or crops. Soils and subsoils can become C-enriched through increased plant productivity (Lambert *et al.* 2000, Hedley *et al.* 2009) or the build up of leaf litter (Beets *et al.* 2002, Wilson and Xenopoulos 2008). Poor soil management practices on farms, or root openings in forested systems, can improve flow paths, facilitating the movement of DOC into streams (Dalva and Moore 1991, Moore 2003, Wilson and Xenopoulos 2008).

Catchment slope also influences the movement of nutrients and DOC into waterways. Concentrations of DOC tend to increase on flat or gently sloping agricultural land where fecal and detrital material can be retained in the soil, while steeper slopes tend to have less soil organic matter (Lambert *et al.* 2000). Steeper slopes also shorten the contact time between moving water and the upper soil horizons, reducing the movement of DOC into streams (Moore 1989, Lambert *et al.* 2000, Wilson and Xenopoulos 2008). In contrast, in undisturbed catchments, nitrate (NO_3) concentrations have been shown to increase in downstream water bodies with increasing slope (D'Arcy and Carignan 1997). This is potentially because shallower slopes tend to have a higher potential for denitrification (Martin *et al.* 2004), which can reduce the amount of NO_3 available for leaching into streams.

1.1.2 Hydrological connectivity of the landscape

Weather-related factors that affect the hydrological connectivity of the landscape also influence the movement of nutrients and DOM into streams. The hydrological connectivity of the landscape is described as the water-mediated movement of material from terrestrial systems to downstream water bodies (Stieglitz *et al.* 2003, Freeman *et al.* 2007). Nutrients and organic matter are transported to streams and lakes via runoff, subsurface flow and groundwater, and weather-related factors such as rainfall (Bass *et al.* 2011), soil moisture capacity (Wilson and Xenopoulos 2008) and snowmelt (Ågren *et al.* 2010) affect the movement of water through the catchment. During dry periods, water may primarily move vertically from surface soils to subsoils, with lateral surface or subsurface flow occurring after significant rain events (Stieglitz *et al.* 2003). The lateral flow of water through the catchment can increase NO_3 and P concentrations in streams (Arheimer and Lidén 2000) as subsurface flow leaches N and soluble P from the soil, and surface runoff mobilises clay particles and metal hydroxides that can strongly adsorb P (Gustafsson *et al.*

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2012). High discharge events associated with rainfall can also increase DOC export significantly (Bass *et al.* 2011), although prolonged spells of high rainfall and runoff can result in hysteresis as water from the upper catchment dilutes downstream DOC concentrations (Meyer and Tate 1983).

Small headwater streams play an important role in maintaining the hydrological connectivity of the landscape (Freeman *et al.* 2007, Wipfli *et al.* 2007). In addition to controlling the quantity of water delivered downstream (Freeman *et al.* 2007, Mark and Dickinson 2008), these streams are small and shallow, resulting in a high benthic surface area to water volume ratio that allows for rapid in-stream uptake of nutrients (Dodds and Oakes 2008, Johnson 2008). While nutrient concentrations tend to be very low in small streams draining undisturbed grassland catchments (Riley *et al.* 2003, Niyogi *et al.* 2007), increasing in-stream nutrient concentrations related to anthropogenic activity can saturate the biota involved in nutrient uptake and processing (O'Brien *et al.* 2007) resulting in increased nutrient export downstream (Johnson 2008). Anthropogenic development in headwater catchments can also influence the export of DOC to downstream water bodies (Quinn and Stroud 2002, Bass *et al.* 2011) by altering drainage patterns (Wilson and Xenopoulos 2008) and soil organic matter content (Lambert *et al.* 2000, Hedley *et al.* 2009).

1.2 Nutrient enrichment in large lakes

Downstream water bodies such as lakes act as sinks for incoming nutrients and organic matter from the catchment. Over time, a lake naturally progresses from oligotrophic (low-productivity) to eutrophic (high productivity) as nutrients and other matter from the catchment are discharged into the lake. Lake trophic status is determined by nutrient concentrations, primary productivity and water clarity (McColl 1972). Oligotrophic lakes are characterised by low nutrient concentrations, low rates of planktonic productivity and low sedimentation rates. These lakes are clear and well-oxygenated, and exhibit 'bottom-up' control, with microbial nutrient-cycling playing a significant role in the flow of nutrients and organic material into the food web (Søndergaard *et al.* 1988). Overall productivity and functioning in oligotrophic systems tend to be driven by benthic primary productivity (Vadeboncoeur *et al.* 2002), the replenishment of carbon or nutrients from the hypolimnion back to the euphotic zone (Schallenberg and Kalff 1993) and terrestrial inputs (Bloesch 2004, McCallister *et al.* 2004).

Soil type (Arheimer and Lidén 2000), lithology (Rosen and Jones 1998), vegetation cover (Galbraith and Burns 2007), and human activity can influence lake trophic status and broader ecosystem functions (Abell *et al.* 2011a). Even in large, deep lake systems where increased influx of external material from anthropogenic sources can initially be diluted by the lake (Abell *et al.* 2011a), the continual input of external energy can increase eutrophication and degrade water quality over time (Edmondson 1994, Jassby *et al.* 2003, Scavia *et al.* 2014). Well-known examples of rapid changes in lake trophic status include Lake Washington (Washington State, USA) and Lake Zurich (Switzerland). In both Lake Washington and Lake Zurich, treated sewage was discharged directly into the lake, causing the lake to become P-enriched and stimulating blooms of the blue-green cyanobacterium, *Oscillatoria rubescens* (Edmondson 1994, Schanz 1994). Diversion of treated sewage (Lake Washington) and construction of sewage treatment plants that included a phosphorus precipitation purification stage (Lake Zurich), resulted in a decrease in the density of *Oscillatoria rubescens* and an improvement in water clarity in both these lakes (Edmondson 1994, Schanz 1994).

Shifts in lake water quality have also been recorded in large lakes such as Lake Tahoe and Lake Erie in the USA. Lake Tahoe has experienced a significant loss of water clarity (a reduction of 6 m in 20 years) as a result of changes in algal species composition and abundance (Edgar 1999), with free-floating and benthic algal blooms occurring near highly developed areas along the shore. These changes in Lake Tahoe are linked to increasing P inputs as a result of urbanisation along the shores of the lake (Schuster and Grismer 2004), as well as atmospheric deposition of N (Hatch *et al.* 2001). In Lake Erie, P enrichment increased phytoplankton biomass, particularly the presence of nuisance and eutrophic diatom species in the first half of the twentieth century. As total phosphorus (TP) concentrations decreased between 1970 and 1983-87, water quality in the lake improved from mesoeutrophic/eutrophic conditions to oligotrophic/mesotrophic conditions (Makarewicz 1993).

1.3 New Zealand (NZ) lakes

1.3.1 Characteristics and challenges

New Zealand hosts a variety of lake types and sizes due to its active landscape, lithology, diversity of soil types and vegetation, and changeable maritime climate (Burns 1991). Many New Zealand lakes do not follow classic patterns found in

comparable lakes from the northern hemisphere (White and Payne 1978, Vincent 1983, Malthus and Mitchell 1989, Burns 1991). For example, NZ lakes are generally warmer in winter and cooler in summer than northern hemisphere lakes, and tend to be poly- or monomictic, as opposed to dimictic (Malthus and Mitchell 1989). New Zealand lakes also tend to exhibit deeper thermoclines in the summer than those in the northern hemisphere as high winds typical of New Zealand's maritime climate help increase the depth of the mixing zone (Davies-Colley 1988).

Some New Zealand lakes also differ from their northern hemisphere counterparts by exhibiting high algal biomass and phytoplankton productivity during winter mixing (White and Payne 1978, Vincent 1983, Bayer 2013, Bayer *et al.* 2015). Usually, decreasing irradiance and water temperature during the winter months inhibit phytoplankton biomass and growth. As irradiance increases in the spring, phytoplankton growth increases. However, in several large New Zealand lakes such as Lake Taupo (Vincent 1983), Lake Wakatipu, Lake Wanaka (Bayer *et al.* 2015), and Lake Coleridge (James *et al.* 2001), algal biomass and algal production rise during winter mixing when light levels and temperatures in the mixed layer are lowest. This increase in phytoplankton growth may stem from the replenishment of nutrients from deeper waters (Vincent 1983, Bayer 2013, Bayer *et al.* 2015), which may outweigh the inhibitory effects of cold temperatures and low light availability in these lakes.

In general, New Zealand lakes have low overall total nitrogen (TN) concentrations. In lakes in the Taupo volcanic zone on the North Island, low nitrogen concentrations are problematic as weathering of rhyolitic pumice beds (Timperley 1983) contributes significant quantities of phosphorus to these lakes, resulting in low N:P ratios. Low TN concentrations and N:P ratios can reduce the nutritional quality of phytoplankton (Checkley 1980) and promote cyanobacteria growth (Burns and Mitchell 1974, Malthus and Mitchell 1989, Edgar 1999). Poor nutritional quality of phytoplankton has been linked to reduced egg production potential of copepods (Checkley 1980), and may help explain low zooplankton to phytoplankton biomass ratios found in many New Zealand lakes (Malthus and Mitchell 1989). Low zooplankton:phytoplankton ratios mean more senescent phytoplankton cells are available to settle into the benthos, resulting in the increased sequestration of nutrients in the sediments. Thus, these New Zealand lake systems are highly susceptible to

"developing large internal loads of nutrients, which could result in increased sensitivity to accelerated eutrophication from any increase in external nutrient loads" (Malthus and Mitchell 1989).

1.3.2 Water quality

Compared with many other countries, New Zealand has good water quality, particularly in headwater areas adjacent to conservation land (Davies−Colley 2013). However, over the past 40 years, declines in water quality have been noted in New Zealand rivers and lakes as land development, particularly agricultural development, increases (Fish 1969, 1970, McColl 1972, White and Payne 1978, Vincent *et al.* 1984, Davies−Colley 2013). Agriculture contributes significantly to New Zealand's economy by generating jobs, creating export earnings and helping fuel rural and urban economies (Schilling *et al.* 2010). As farming intensification and diversification into new crops increases, so do stocking densities, fertilizer usage, feed production and water usage, which can increase sediment and nutrient influx into streams and lakes via surface runoff and subsurface flow (Foote *et al.* 2015). In 2010, 44% of monitored lakes in New Zealand were categorised as eutrophic to super-eutrophic, with trophic status increasing as pasture cover increased (Verburg *et al.* 2010).

Long-term studies on several North Island lakes have highlighted the connection between land use, nutrient inputs and lake water quality. A temporal comparison of changes in dissolved oxygen (DO) concentration in seven North Island lakes in New Zealand showed that DO decreased in at least six of the seven lakes from 1955 to 1970 (McColl 1972). The ratio of developed land to native forest or scrub in New Zealand lake catchments was closely related to the trophic status of these lakes, and the author predicted that if the percentage of exotic grassland, forest or residential development exceeded 45% of the total land area of a lake's catchment, the lake could be in danger of becoming eutrophic (McColl 1972). A later study by White et al. (1978) reported that one of the lakes in McColl's (1972) study, Lake Rotorua, experienced anoxic conditions in its bottom waters for a period of 5 to 12 days during the summer months when conditions were calm. As sediments in New Zealand lakes can support high internal nutrient loads, White et al. (1978) speculated that these anoxic bottom waters could result in significant nutrient releases (¼ to ½ of the total annual input from all other sources) from the sediments (White *et al.* 1978), although a separate study estimated lower release rates (3.6 mg m^{-2} d^{-1} DRP and 11.4 mg m^{-2} d^{-1}

instead of 250 mg m^{-2} d^{-1} dissolved inorganic nitrogen (DIN)) (Fish and Andrew 1980). Anoxic hypolimnetic conditions were also reported for Lake Rotoiti (Vincent *et al.* 1984), with the hypolimnion remaining anoxic for 3-4 months of the year. The authors also reported deteriorating water clarity and a shift in phytoplankton community structure since 1955, likely because of nutrient enrichment from upstream Lake Rotorua. Prior to 1991, sewage from the city of Rotorua was discharged directly into the lake, and made up roughly 50% of total phosphorus (TP) and 25% of N loading to the lake (Caruso 2000, Rutherford 2003).

Several large-scale studies have expanded on McColl's 1972 study linking lake trophic status to land use, reporting a link between trophic status, the loss of native grasslands and the proportionate increase in pastureland in lake catchments (Burns and Galbraith 2007, Galbraith and Burns 2007, Abell *et al.* 2011b). In a study of 101 lakes sampled on the North and South Islands, the proportion of high producing grassland in the surrounding catchment was the best predictor variable of in-lake TN and TP concentrations (38.6% and 41%, respectively). The proportion of exotic forest accounted for 18% of variance in TP concentrations in lakes, while urban development accounted for 3.7% of variance in TN (Abell *et al.* 2011b). In a wide-ranging study comparing 43 water bodies in Central Otago, New Zealand, Galbraith and Burns (2007) reported that lakes surrounded by pastureland had higher concentrations of N and P than those surrounded by native tussock, and that lake trophic status was associated with the proportion of the catchment modified by human activity (Burns and Galbraith 2007). In their study, 31% of variation in the microbial biomass of a given lake could be explained by the water quality data, with higher concentrations of nutrients correlating with the proportion of the catchment developed for pasture.

1.3.3 Lake Taupo: a case study

Few large, deep lakes in New Zealand have undergone intensive monitoring of water quality. However, Lake Taupo is one New Zealand Lake with well-documented changes in water quality. Situated on the North Island, Lake Taupo is New Zealand's largest oligotrophic lake (620 km^2, 186 m deep), with low in-lake nitrogen availability limiting algal productivity (Petch *et al.* 2003). The high water quality of the lake is an important amenity for regional tourism, which forms a substantial part of the local economy. A decline in water clarity throughout the winter months since 1976 (Edgar

1999) and the occurrence of potentially toxic blooms of cyanobacteria (*Anabaena spp.*) in 2001 and 2003 raised concerns over the state of Lake Taupo.

The decline in water quality in Lake Taupo is related to diffuse discharges of nutrients from agricultural run-off (Edgar 1999, Petch *et al.* 2003, Waikato 2007). Historically, Lake Taupo's catchment was covered in tussock grassland and native forest (Leathwick *et al.* 1995). With the advent of European settlement, this native vegetation was replaced with pastureland and pine forests. In the late 1990s, pastoral agriculture shifted from sheep and beef farming to dairying (Edgar 1999). Over the past century, nitrogen loads exported to the lake are estimated to have increased from 650 tonnes year^{-1} to 1200 tonnes year^{-1} (Waikato 2007), with inflowing stream N concentrations increasing between 50% and 300% since the 1970s (Petch *et al.* 2003). An increasing trend in chl *a* concentration (0.087 ± 0.029 mg chl *a* m^{-3} y^{-1}) within the lake was also reported from 1994 – 2003 (Gibbs 2011).

The difficulty in assessing land-water interactions in Lake Taupo stems from the long water residence time in the lake (11 years) and the lag time between increased nutrient inputs on the land and visible changes in the lake (Waikato 2007). Over time, N and P applied to soils as a result of intensive agricultural activities (e.g. application of agro-chemicals and animal manures) would move either laterally via overland or subsurface flow to streams and rivers, or vertically through the surface soil horizons to the groundwater (Arheimer and Lidén 2000). These nutrients can be stored within aquifers for decades before slowly moving into the lake. Thus, the changes currently being detected in Lake Taupo likely reflect past, as well as present, land use patterns. This means that nitrogen concentrations will likely increase in the lake, even if nitrogen loading from the catchment were to be held at current levels (Waikato 2007).

1.3.4 Central Otago lakes

Over the past 600 years, vegetation on the South Island of New Zealand has shifted from native forests to tussock-dominated grasslands to increasing areas of agricultural and urban development (McGlone 2001). Development in upland areas is relatively low intensity (Davies−Colley 2013), although studies on small lakes in the Central Otago region illustrate the impact even low-intensity development can have lake water quality (Burns and Mitchell 1974, Mitchell and Burns 1979, Bayer *et al.* 2008).

Lake Hayes and Lake Johnson are small (2.76 km^2 and 0.2 km^2, respectively) relatively shallow eutrophic lakes located in Central Otago. In the 1950s, catchment land use around Lake Hayes was primarily pastoral farming (Burns and Mitchell 1974). Between 1952-53 and 1969-72, changes were noted in transparency (a 1.3 m reduction in mean Secchi depth) and dissolved oxygen (DO) concentrations in Lake Hayes (Burns and Mitchell 1974). By the 1970s, bottom water was deoxygenated for more than 4 months of the year, which was a change from 1953-54 where these waters remained oxygenated throughout the summer (Burns and Mitchell 1974). Additionally, blooms of *Anabaena flos-aquae* were recorded in the lake between 1969 and 1971(Burns and Mitchell 1974), which had not been reported during early work on the lake (Jolly 1952). The changes suggested that Lake Hayes had become progressively more eutrophic since the 1950s. A more recent study of Lake Hayes recorded low N:P ratios in the lake, with pronounced enrichment of P and TN in hypoxic hypolimnetic waters during summer stratification (Bayer *et al.* 2008) that mixed with epilimnetic waters when the thermocline broke down. The authors speculated that P concentrations in Lake Hayes may reflect current and historical application of agro-chemicals, particularly superphosphate in the catchment, (Robertson 1988) as well as internal loading from anoxic sediments and the hypolimnion (Bayer *et al.* 2008).

Although management practices have been put into place to reduce nutrient inputs into the lake, Lake Hayes continues to be classified as eutrophic (Otago Regional Council 2009). This likely stems from continued inputs of nutrients via groundwater and the recycling of nutrients from the lake sediment as dissolved oxygen concentrations decline during summer stratification (Bayer *et al.* 2008). Inputs of these nutrients to the lake have been associated with algal blooms and degraded water quality (Bayer *et al.* 2008) creating a positive feedback mechanism to stimulate productivity (Burns and Mitchell 1974). A similar situation is apparent in Lake Johnson, where nutrients in the lake are recharged by groundwater seepage and temporary streams (Otago Regional Council 2009).

1.4 Lake Wanaka catchment

In the catchment surrounding Lake Wanaka (44° 42' S, 169° 09' E) (a large glacially-formed sub-alpine oligotrophic lake located near the main divide of the Southern Alps), vegetation cover is predominantly grassland and forest (Table 1), with

approximately 11% of the catchment used for urban or agricultural development (Rae *et al.* 2001). The catchment is mainly mountainous terrain, with more than 75% of the catchment considered moderately steep to steep (> 21° slope) (Livingston *et al.* 1986). Dominant soil types in the catchment include brown soils along steep hillsides, and pallic soils and recent fluvial deposits along valley floors (2016) (Figure 1). All three soil types in the region are formed from sedimentary greywacke schist, but drain differently, with brown soils and fluvial deposits draining reasonably well under moderate to high rainfall, and pallic soils draining poorly (Currie 2014). Nitrogen, P and OM concentrations in all of these soils are generally low (Leamy 1966). Brown soils have moderate P retention capabilities as the P binds with iron oxides in the soils, but these soils are likely to leach N as the soils drain freely (Leamy 1966, Currie 2014).

Table 1: Vegetation cover percentages for the catchment surrounding Lake Wanaka (based on 2008 Landcover Database version 3).

Vegetation Cover (%)			
Alpine grass/herb field	0.71	Exotic shrub land	0.16
Broadleaved Indigenous hardwoods	0.68	Gravel and rock/landslide	8.35
Urban	0.33	High producing exotic grassland	4.66
Deciduous hardwoods	0.14	Lake and pond	7.67
Exotic forest	0.09	Low producing exotic grassland	5.84
Indigenous forest	9.99	Scrub/shrub land	12.03
Fern land	5.18	Permanent snow/ice	2.02
Cropland/Vineyard	0.04	Tall tussock grassland	41.36
Depleted Grassland	0.01	Other	0.63

Agricultural land uses in the catchment mainly consist of dry-stock farming, with herds of sheep, beef cattle and deer. Farmers in the area use organic and conventional fertilizers to stimulate plant growth. The type of fertilizer used and the rate of application vary depending on landscape, soil type, pasture use and pasture age (Aspinall, personal communication). Conventional fertilizers used in the catchment tend to be nitrogen- and sulphur-rich. In the Matukituki Valley catchment, one large farm (10,500 stocking units) applies approximately 180 kg ha^{-1} of predominantly sulphur- (Sulphurgain 20S, 30S, Durasul, Pasturezeal high S) and nitrogen-rich (Urea, Cropzeal 16N, NRich 15K) fertilizers every other year (Aspinall, personal communication). Nitrogen- and sulphur-rich fertilizers are also applied in the Makarora River catchment, as well as fertilizers containing P (e.g. Sulphur Super 20, Sulphur Super 30, Cropmaster 15, Cropmaster DAP, 15% and 30% Potash S Super).

On one farm in the Makarora valley, 50 – 150 kg ha^{-1} of the N-rich fertilizers is applied annually, with application of urea and a 60:40 blend of ammonium sulphate and urea occurring at the end of winter to promote spring growth. On the same farm, application rates of S-rich fertilizers range from 100 – 650 kg ha^{-1} (Wanklyn, personal communication).
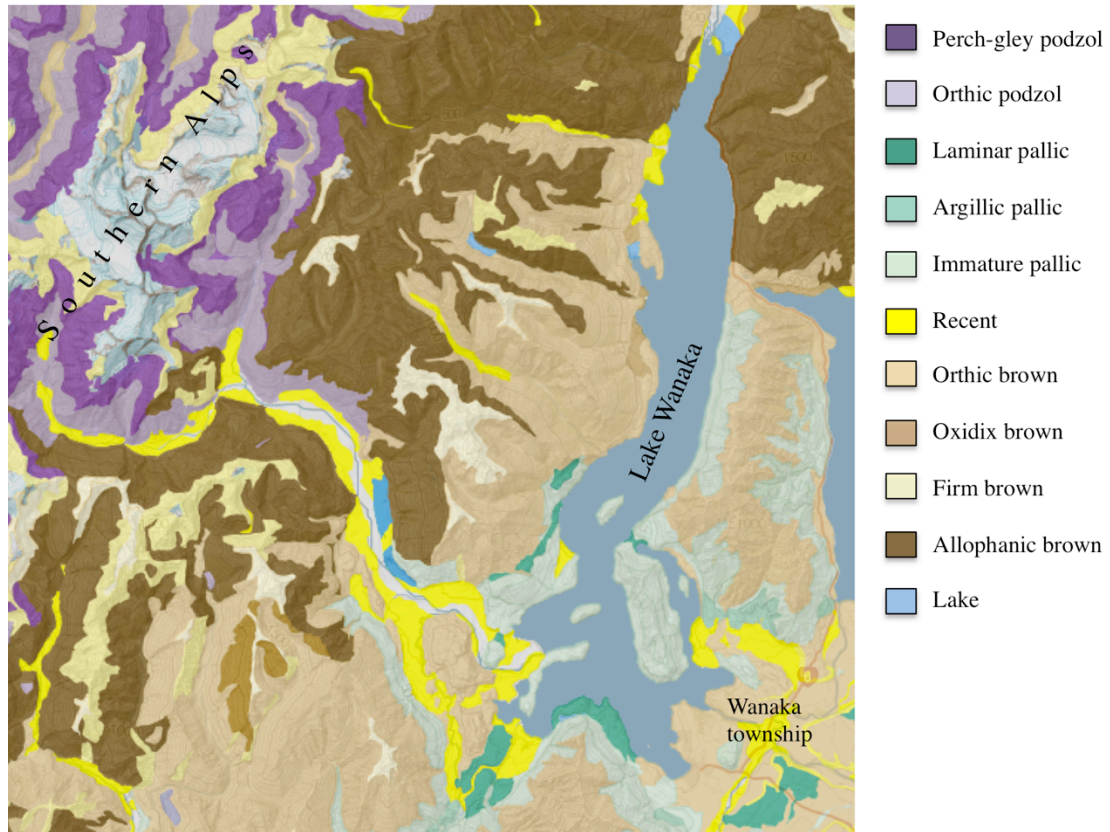
Figure 1: Soil map of the Lake Wanaka catchment. (Fundamental Soil Layers Map of the Wanaka catchment courtesy of Landcare Research)

In addition to high country farming, other developments around the Wanaka catchment include a golf course, vineyard, orchards (Rosen and Jones 1998) and the urbanised areas of Wanaka Township (located at the southern end of the lake) and the village of Makarora located upstream from the lake. In Wanaka Township, nitrogen- and sulphur-rich fertilizers are applied on sporting grounds and public spaces. Here, fertilizer application rates are approximately 40 - 90 kg ha^{-1} applied three times of the year.

1.4.1 The importance of determining land use influences on external energy inputs and microbial processes in Lake Wanaka

The quality of water in Lake Wanaka is an important amenity for the region, which supports a growing tourism, retirement and outdoor industry. Over ten years from 1991 to 2001, the population of Wanaka grew 41% (Lucas 2011) and it has increased another 51.3% between 2001 and 2013 (Statistics New Zealand 2013). While Lake Wanaka is currently classified as oligotrophic based on water clarity and nutrient and chl *a* concentrations (Otago Regional Council 2009), recent changes have been noted in the lake's phytoplankton. In 1975, surface water samples taken from the open water of Lake Wanaka indicated a diverse phytoplankton community dominated by Chlorophyte nanoplankton for much of the year (Clayton and Coleman 1976). Samples taken from the lake in 1994 (Naismith 1994) and 2002 (Galbraith, unpublished data) indicated algal biovolume was dominated by tiny picocyanobacteria. However, sampling from 2008-2012 has indicated a shift from a picoplankton-dominated algal community to the diatom *Lindavia intermedia* (formerly *Cyclotella bodanica*) (Nakov *et al.* 2015) making up roughly 40 – 85% of the algal biovolume in the water column (Bayer 2013).

Another recent change noted in the lake is the formation of organic aggregates, or 'lake snow'. Since 2003, fishermen have reported nuisance algal aggregates fouling fishing lines, and filamentous algae have occasionally clogged water intake valves for the Township of Lake Wanaka (Bodger *et al.* 2011). Organic aggregates in the water column are visible to the naked eye and are comprised of detrital material, phytoplankton, bacteria and other grazers (Bayer 2013). In samples taken from the lake in 2008 – 2010, organic aggregates were associated with cells of the phytoplankter *Lindavia intermedia* during the summer months (Bayer 2013, Nakov *et al.* 2015). While it is not clear whether the presence of lake snow is linked to the clogging water filters within the Wanaka Township water reticulation system, the shift in phytoplankton community appears to be related to the formation of organic aggregates.

Along with this change in phytoplankton community structure, lake sampling undertaken by the Otago Regional Council (ORC) from 2006 to 2009 indicated a slow increase in chl *a* concentration (0.56 to 0.74 mg m^{-3}) in Roy's Bay, a semi-enclosed arm of Lake Wanaka. Nitrogen concentrations also showed an increasing trend in this

bay (Otago Regional Council 2009). Urban, residential and agricultural development have been steadily increasing in the catchment surrounding Roy's Bay, and the increasing trends in chl *a* and TN may reflect changing land use patterns in the area. Because of the large volume of water in the lake, increased influx of external organic matter/energy to this system may be quickly diluted, resulting in no apparent change in microbial processing of organic material. However, the long residence time of the lake (> 6 years) may result in the steady build-up of organic material in the sediments, which could lead to in shifts in pelagic and benthic food web dynamics. While the increases noted to date are not enough to change the trophic status of the lake, the possibility of a decline in the water quality of the lake makes it important to understand underlying causes for these changes.

1.4.2 Objectives

The overarching goal of my thesis was to broaden our understanding of how land use practices affect aquatic ecosystems by examining how low-intensity development of grassland catchments affects nutrient dynamics and microbial activity in large high country lakes. Thus, research was conducted to trace variations in the movement of external material from minimally developed grassland catchments into a large, oligotrophic lake and to determine how this material affects biota in the lake.

Lake Wanaka was chosen as a study site because the catchment is dominated by native tussock grasslands, land use intensity in the catchment is relatively low, and the large, deep nature of the lake can dilute incoming nutrients, making it difficult to measure land use impacts simply by sampling open water sites. Furthermore, few studies have been conducted in Lake Wanaka and its surrounding catchment, and there is little information available concerning what impact (if any) low-intensity development in the catchment is having on the lake (Burns 1991, Otago Regional Council 2009). Research undertaken for this project included: intensive sampling of small streams and one of the main inflows to the lake during different seasons and hydrological conditions, following the path of two main tributaries out into the lake, and developing and carrying out laboratory-based experiments.

This thesis is made up of five main research chapters (Chapters 2-6), one of which (Chapter 2) has been provisionally accepted by the peer-reviewed scientific journal, *Marine and Freshwater Research*. The chapters are organised by decreasing spatial

scale, and move from the broader lake catchment to a more intensive analysis of how one main tributary influences the lake. Chapter 2 follows temporal variations in the movement of macronutrients and bulk DOC inputs into nine tributaries reflecting a gradient of increasing pasture cover, while Chapter 3 examines whether nutrients from a selection of these tributaries could be linked to transparent exopolymer particle formation in the lake. Chapter 4 follows the movement of inflowing water from two main tributaries to the lake (the Matukituki River and the Makarora River) and considers their effect on phytoplankton distribution and primary productivity in the lake. Chapter 5 looks at how farming in the Matukituki Valley affects the quality of organic matter being delivered to the lake and Chapter 6 examines what impact this terrestrially-derived DOM has on bacterial activity and productivity. A summary of each research chapter is provided below.

In Chapter 2, I hypothesised that i) DOC and N concentrations in tributary streams would increase with increasing land development in their catchments, but soils in the catchments would attenuate P input and ii) weather-related factors that increase hydrological connectivity in the landscape would enhance the influx of N and DOC to the tributaries. Sampling occurred over a four-year period and spanned eight streams and one river representing a gradient of increasing pasture cover. Physical and chemical variables measured in the stream water were compared with meteorological and landscape data. Agricultural development correlated positively with N and DOC concentrations in stream water, but was not significantly associated with P concentrations in streams. Weather-related variables were significant predictors of DOC, but not N. Temperature and soil moisture mitigated the influence of pasture cover on surface water DOC concentration under very dry or wet conditions. My results indicate that while concentrations of N and DOC entering Lake Wanaka increase as agricultural development in grassland catchments increases, weather and soil moisture conditions can mediate the amount of DOC transferred from soils into streams.

The aim of Chapter 3 was to determine whether external input of nutrients and dissolved organic carbon (DOC) could facilitate transparent exopolymer particle (TEP) formation in Lake Wanaka water; and whether TEP generation was related to the recent dominance by the diatom, *Lindavia*. I first wanted to determine whether

TEP concentration increased in response to inputs of catchment-derived nutrients and DOC. Next I wanted to determine whether algal growth in response to artificial nutrient enrichment promoted TEP formation. As *Lindavia intermedia* abundance increased in Lake Wanaka around the same time that organic aggregates began appearing in the lake, I hypothesised that TEP concentrations should increase in lake water treatments where this diatom is present. In four eight-day laboratory experiments, lake water was amended with water from streams representing a gradient of increasing pasture cover. In two subsequent 12-day experiments, a parallel set of treatments was amended with saturating concentrations of N and P. When lake water was enriched with N and P, algal growth, chl *a* concentration, diatom abundance and TEP increased substantially. TEP generation was not associated with abundance of *Lindavia intermedia*, although this may reflect constraints of the experimental set up.

While increased nutrient export from smaller streams will likely result in localised impacts on the lake, land use intensification in the catchments of the main tributaries can potentially extend into the open water. Thus, the aim of Chapter 4 was to understand how catchment-derived materials are delivered to Lake Wanaka under stratified and un-stratified conditions, and what effect this material has on phytoplankton biomass in the lake (as measured by chl *a*). The glacial origin of both the Matukituki and Makarora Rivers suggests the river water will likely plunge and either inflow along the bottom of the lake or interflow as a density current. The plunging plumes would contain higher concentrations of nitrogen, DOC and total suspended solids (TSS) than the lake, and could provide a wedge of nutrients to the metalimnion, thereby stimulating primary productivity. Water column profiles were taken along transects outside the Matukituki River mouth on ten occasions from 2009 to 2012, and on three occasions outside the Makarora River mouth between 2009 and 2010. While the Makarora River plume was not apparent on the dates sampled, cold river temperatures and high suspended solid loads produced a traceable plume outside the Matukituki River mouth. After initial turbulent mixing, the Matukituki River plume tended to plunge and interflow as a density current. A noticeable chl *a* underflow on three of the sampling dates indicated the river plume was either bringing in fluorescing material or supporting phytoplankton growth in the lake.

Dissolved organic matter (DOM) can also play an important role in regulating heterotrophic and autotrophic production in aquatic systems. Chapter 5 focused on how vegetation cover and land use can influence dissolved organic carbon (DOC) character by qualitatively comparing DOM in the Matukituki River and the lake. I hypothesised that deep lake water (100 m depth) and Matukituki River water would contain a greater proportion of aromatic DOM structures than shallow water (20 m depth) in Lake Wanaka, due to differences in source materials and exposure to bio- and photodegradation. I further hypothesised that the Matukituki River would contain a greater proportion of heteroelement formulae than the Lake as a result of land use activity in the Matukituki River Valley. Ultrahigh resolution mass spectra of DOM from the river and lake were analysed using van Krevelen diagrams to investigate molecular variations between sites. The Matukituki River water did not have more aromatic components, but did have significantly more CHO-S formulae than the lake. The river water also contained a high number of sulfonate-like formulae, which could reflect pesticide or fertilizer application in the catchment. The low number of sulphur-containing formulae in the lake compared with the river implies this material may be rapidly biodegraded, and indicates DOM from the Matukituki River could influence bacterial activity in the lake.

Chapter 6 examined how Matukituki River water influenced microbial physiological diversity and bacterioplankton activity, as compared to the lake. As nutrient limitation (particularly P) affects primary productivity in the lake, I hypothesised that seasonal changes in bacterial physiological diversity would be positively related to phytoplankton biomass in Lake Wanaka. As agricultural practices within the Matukituki River catchment influence the character of DOM being transported to the lake, I further hypothesised that bacterial activity would increase in response to increased availability of terrestrially-derived labile DOM, while bacterial productivity will increase with increasing P availability. Bacterial physiological diversity in the river and lake was monitored seasonally from 2012 to 2013 using Biolog ecoplates. Bacterial activity and productivity were determined via bioassays conducted in the austral winter, spring and summer of 2012- 2013. As N and P concentrations in Lake Wanaka are often low (i.e. growth-limiting), DOM lability was determined by adding saturating nutrient concentrations. In the Matukituki River, bacterial communities were consistently able to breakdown a diverse array of organic substances, reflecting

consistent exposure to a labile supply of DOM. In the lake, seasonal patterns in organic substrate use differed by depth, reflecting variations in thermal stratification affecting the movement of DOM into deeper waters. Additions of river water to lake water stimulated bacterial respiration and uptake of DOC and P, but did not affect bacterial productivity. This lack of apparent change in bacterial productivity likely reflects limitations of my experimental design. However, it may indicate that as scarce P supplies were depleted, lake bacteria mineralised DOC instead of incorporating it into the cell for growth and reproduction. If the latter explanation is the case, changes in nutrient and DOC export to the lake could lead to shifts in heterotrophic and autotrophic production, potentially facilitating a change in lake trophic status.

2 Land use, soil properties and weather conditions influence nutrient fluxes into a deep oligotrophic lake

2.1 Introduction

Land use intensification can increase nutrient and suspended solid loads leading to declining water quality in streams and lakes (Carpenter *et al.* 1998, Dauer *et al.* 2000, Meador and Goldstein 2003, Niyogi *et al.* 2003, Galbraith and Burns 2007, Abell *et al.* 2011b). Changing land use patterns can also alter stream hydrology (Allan 2004), affect UV penetration of surface waters (Findlay *et al.* 2001) and alter organic matter inputs (Wilson and Xenopoulos 2009). Such changes can intensify aquatic weed growth (Lewis *et al.* 2011), cause volatility in dissolved oxygen levels, reduce habitat availability, alter food webs (Williamson *et al.* 2015), attenuate light (Morris *et al.* 1995), alter trace metal bioavailability (Thurman 1985), and lower species diversity (Carpenter *et al.* 1998, Dauer *et al.* 2000).

Studies have shown that modification of native grassland systems can affect water yield (Mark and Dickinson 2008), erosion and runoff, leading to net declines in nutrient (McIntosh 1997) and organic carbon concentrations in soils (Farley *et al.* 2004). Yet studies comparing nutrient and dissolved organic carbon (DOC) fluxes in indigenous grassland and pasture streams are relatively infrequent in the literature. Studies that measure DOC input into streams often occur in forested systems (Bass, Bird *et al.*, 2011), or compare forested and pasture-dominated systems (Quinn and Stroud, 2002), while studies comparing streams draining native grassland and pasture-dominated catchments tend to focus on changes in macronutrient concentrations (Niyogi *et al.* 2003, Riley *et al.* 2003, Niyogi *et al.* 2007).

While many grassland zones around the world have been developed for farming, livestock grazing or other uses (Suttie *et al*. 2005), large areas of relatively unmodified indigenous grassland are still present on the South Island of New Zealand. The tall tussock grasses found in this region share many characteristics with forested systems; these plants are perennial and long-living (Moore 1955, Mark and Dickinson 2008) and root systems make up a significant proportion (23.6 – 44.7%) of their biomass (McIntosh 1997). Though several New Zealand studies compare macronutrient concentrations in tussock grassland and pasture catchments (e.g.

Niyogi *et al*. 2007), most of these studies do not include changes in dissolved organic carbon (DOC) concentration. Including DOC in studies of water quality is important since organic matter can control bacterial abundance and activity (Bernhardt and Likens 2002), alter trace metal bioavailability (Thurman 1985), attenuate light (Morris *et al.* 1995), increase surface water acidity (Hope *et al.* 1994) and affect the bioavailability of N (Bernhardt and Likens 2002).

One known New Zealand study (Riley *et al.* 2003) monitored changes in nitrogen (N), phosphorus (P) and DOC concentrations in streams draining modified and unmodified native grassland sub-catchments. However, this study did not take into account the mediating effects of weather-related variables on stream nutrient and DOC concentrations, which can mitigate or enhance input of exogenous material into surface waters (Wilson and Xenopoulos 2008). For example, high discharge events associated with rainfall can increase nutrient (Arheimer and Lidén 2000, Verheyen *et al.* 2015) and DOC export significantly (Bass *et al.* 2011), while prolonged dry periods can reduce the hydrological connectivity of the landscape, disrupting the transport of DOC to streams (Wilson and Xenopoulos 2008).

My study focused on eight tributaries draining into, or adjacent to, Lake Wanaka (South Island, New Zealand) from spring 2009 to autumn 2012. The Lake Wanaka catchment is predominantly covered in native tussock grasses, and the dominant soil types include brown soils along steep hillsides, and pallic soils and recent fluvial deposits along valley floors (Landcare Research 2016). Soils in the region are formed from sedimentary greywacke schist, but differ in drainage ability. Brown soils drain reasonably well under moderate to high rainfall, while pallic soils (formed from wind-blown silt) are poorly-drained (Currie 2014). Nitrogen, P and organic matter (OM) concentrations in all of these soils are generally low (Leamy 1966). Brown soils exhibit moderate P retention as the P binds with iron oxides in the soil, but may experience significant N losses as these soils drain freely (Currie 2014). Recent fluvial soils tend to have low nutrient retention capabilities (Leamy 1966, Currie 2014), with particularly high N-leaching rates occurring in coarse-grained sandy soils that allow rapid water movement (Vinten *et al.* 1994). While agricultural development in the Wanaka catchment is relatively low intensity, even low intensity pastoral development of hilly grassland catchments can increase in-stream nutrient concentrations and sedimentation (Niyogi *et al.* 2003, Riley *et al.* 2003) through

fertilizer application and feces deposition. Conversion from slow growing to highly productive vegetation can also increase soil organic matter concentrations, thereby increasing the amount of dissolved organic carbon available for export (Lambert *et al.* 2000, Hedley *et al.* 2009).

I studied how the conversion of native grasslands into pasture affects the concentrations and fluxes of macronutrients and DOC in headwater streams during periods of low to moderate flow. As soils in the Wanaka catchment are likely to leach N readily, and as increased soil organic matter (SOM) could increase the amount of DOC available for export, I hypothesised that:

Hypothesis (i): land use intensification will serve as a primary predictor of in-stream N and DOC concentrations by increasing the availability of organic matter and N in the soil. However, the relationship between land use and P concentration will be mitigated by the capability of the soil to retain P and low flow conditions.

As short-term variations in rainfall, soil moisture, and temperature can mediate the effect of agricultural development on stream DOC, N and P concentrations (Wilson and Xenopoulos 2008, Bass *et al.* 2011), I further hypothesised that:

Hypothesis (ii): factors that increased the hydrological connectivity of the landscape will significantly enhance stream nutrient and DOC concentrations through the subsurface movement/leaching of material into waterways.

While my study focused mainly on 2nd – 4th order streams in the Wanaka catchment, I also discuss the implications of anthropogenic development of larger catchments on lake nutrient status.

2.2 Material and Methods

2.2.1 Field sampling

All streams sampled in this study are tributaries draining into, or adjacent to, Lake Wanaka (44° 42'S, 169° 09'E), a large (192 km^2, 311 m deep), sub-alpine, oligotrophic (mean open water chl a = 0.49 mg m^{-3}, Secchi depth between 11 and 13.9 m) lake, located east of the Southern Alps in Central Otago, New Zealand (Figure 2).
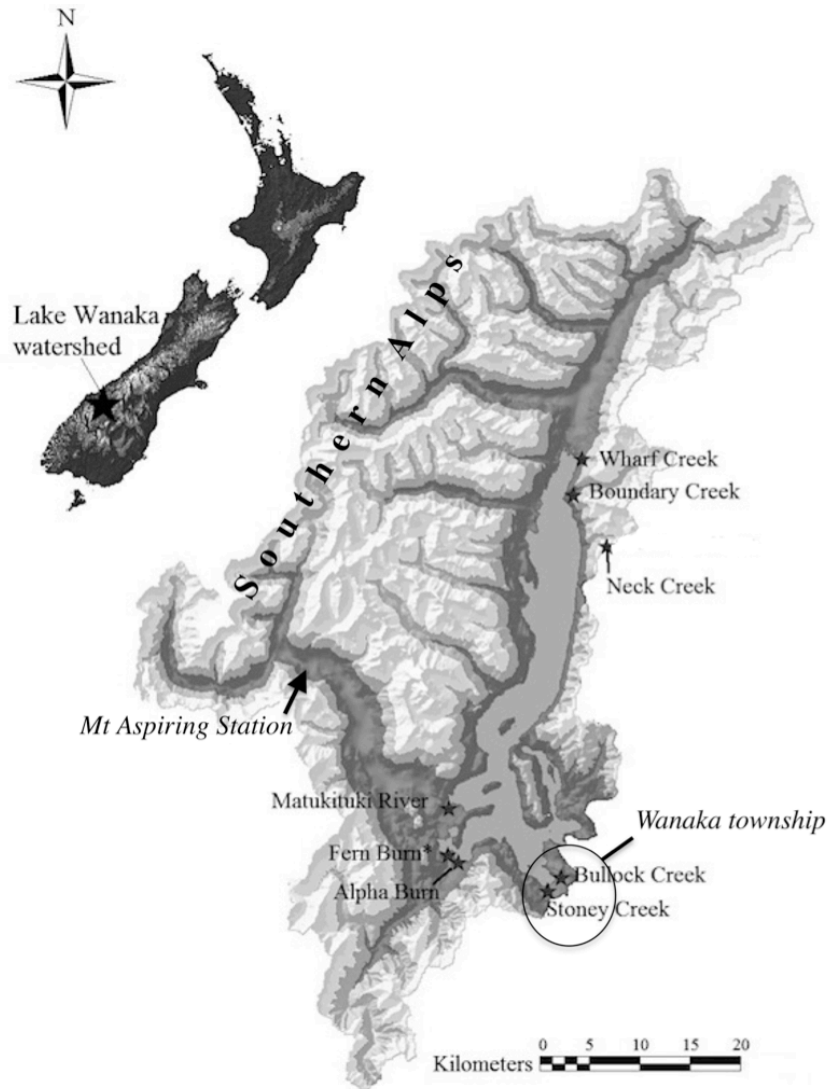
Figure 2: The Lake Wanaka catchment (44° 42'S, 169° 09'E) showing the approximate locations (★) of the streams sampled between 2009 and 2012 (Clockwise from top right: Wharf Creek; Boundary Creek; Neck Creek; Bullock Creek; Stoney Creek; Alpha Burn; Jack Hall Creek (a.k.a Fern Burn); the Matukituki River).

The mean annual temperature in the region is 16°C. Rainfall in the catchment is highly variable, with higher precipitation rates occurring closer to the Southern Alps, and drier conditions occurring further east. In general, Wanaka township, located near the southeastern corner of the lake, receives approximately 680 mm year^{-1} of rain (Rosen and Jones 1998), while westerly sites such as Mount Aspiring Station receive substantially more rainfall (2700 mm yr^{-1}) (LINZ 2005). The lake catchment consists of moderately well drained, mountainous terrain with basement lithologies of metavolcanic greenschist and quartzo-feldspathic grey schist (Rosen and Jones 1998). Soils in the catchment are well- to moderately-drained brown and podzolised soils

with small amounts of pallic and recent alluvial soils, and are considered to be of low and moderate fertility (Landcare Research 2016). Lake Wanaka is situated 300 m above sea level (a.s.l.) and the highest mountain in its catchment rises above 3000 m a.s.l. Water inputs to the lake are sourced from rainfall, snowmelt and glacier melt.

Table 2: Sub-catchment landscape characteristics and mean chemical concentrations for each stream listed in order of percentage pasture cover in the catchments. Abbreviated variable titles include: Ord indicates stream order, %P: pasture cover (%), %T: native tussock cover (%), %F: forest cover (%), S<15: terrain with a slope of 0 – 15° (%), S16–25: terrain with a slope of 16 – 25° (%), S≥26: terrain with a slope ≥ 26° (%), NO_3: nitrate-nitrogen (µg l^{-1}), DRP: dissolved reactive phosphorus (µg l^{-1}), DOC: dissolved organic carbon (mg l^{-1}), TN: total nitrogen and TP: total phosphorus (µg l^{-1}).

Tributary	Ord	%	%T	%F	S<15	S16–25	S≥26	DOC	NO_3	DRP	TN	TP
Wharf Creek	2	0	86	0	3.5	36.5	60	2.8	10.3	5.63	11.5	17.9
Boundary Creek	4	1	68	5	9	27	64	2.1	10.0	1.81	25.2	14.0
Neck Creek	3	3	74	11	8	31	61	2.0	1.7	1.25	13.6	3.2
Matukituki River	6	15	47	11	16	24	60	2.3	45.7	1.96	73	11.7
Fern Burn	4	42	48	2	27	23	50	3.4	76	1	126	5.3
Alpha Burn	3	48	37	3	25	19	56	3.9	387	1.78	419	8.2
Bullock Creek	2	55.5	0	6	82	10	8	3.0	478	0.96	652	3.5
Stoney Creek	2	68	8	2	44	23	33	4.9	288	3.03	437	20.4

During my study, eight tributaries (seven 2nd – 4th order streams and one 6th order river) with increasing proportions of pasture cover (Table 2) were sampled during the austral spring of 2011 through the autumn of 2012. In a pilot study, six streams were sampled during the spring and summer of 2009-2010 and autumn of 2011. All of the streams are spring-fed with additions of snowmelt in the spring except for Bullock Creek, which is mainly groundwater-fed from the Wanaka Basin, and the Matukituki River, which is partly fed by glacial melt water. Streams were sampled within 200 m of the stream outlet to Lake Wanaka at times of low to moderate flow. For safety and logistical reasons, flood events were not sampled. In each stream, measurements of temperature, oxygen concentration, conductivity and salinity were made using a YSI 6000 rapid-pulse environmental monitoring system (YSI Incorporated, Yellow Springs OH, USA), and pH was measured using a calibrated field IQ Scientific pH meter (IQ Scientific Instruments, Hach Company, Colorado, USA). Flow rate was measured using a Marsh-McBirney current meter (Marsh-McBirney Incorporated, Frederick, MD, USA) with readings taken at depths 40% above the stream bottom.

Discharge rates (Q) were calculated by measuring depth and flow velocities at equidistant points along a measured transect that extended across the stream channel.

A five-liter water sample was collected from each stream in an acid-washed polyethylene container and stored on ice, in the dark, until filtered. Water samples for determining nutrient concentrations were taken from the middle (or as far as safely possible) of each stream. Additional water samples were collected in duplicate acid-washed 50-ml polyethylene tubes for total nitrogen (TN) and total phosphorus (TP) analysis. Samples were filtered within eight hours of collection.

2.2.2 Sample processing and analysis

Water samples were kept at 4°C and in the dark until 250 – 500 ml were filtered through acid-washed pre-combusted Whatman GF/F (Pittsburgh, USA) filters (0.7 μm nominal pore size glass fiber) under low vacuum pressure (< 100 mmHg). Milli-Q (Millipore Corporation, Bedford, MA, USA) water held in a pre-sterilised polyethylene container was filtered alongside stream water for quality control purposes. Duplicate 50-ml subsamples for analysis of dissolved organic carbon (DOC), dissolved reactive phosphorus (DRP), nitrate-nitrogen (NO_3-N), and total dissolved nutrients (TDN) were collected in acid-washed 50-ml polyethylene tubes pre-rinsed with ultra-pure Milli-Q water.

Immediately after filtration, DOC samples were wrapped in aluminum foil to prevent photo-degradation, stored at 4°C and analysed within two-three days to one week of collection (Dafner and Wangersky 2002). Unfortunately, DOC samples were not acidified prior to storage, and bacteria capable of passing through the GF/F filters may have consumed or altered some of the DOC. Respiration could have led to a decrease in DOC, but it is unlikely DOC concentrations decreased substantially during the 7-day storage period. While bacterial respiration (BR) is stimulated at higher temperatures (20 – 25°C) (Berggren et al. 2010), respiration rates are low at colder temperatures (0 – 5°C). In studies using unfiltered water from temperate and boreal streams, carbon losses due to bacterial respiration ranged from <0.1 to 0.5 mg C l^{-1} wk^{-1} at 5°C (Roland and Cole 1999, Apple et al. 2006, Berggren et al. 2010). These changes in DOC concentration are similar to natural variations that occurred in laboratory blanks (0.10 - 0.35 mg C l^{-1}) and internal standards (0.17 – 0.47 mg C l^{-1}) when running the TOC analyser.

As samples taken during March and May 2011 were not processed within one week of collection, they were not used in the DOC analysis. DOC was measured on a Shimadzu Total Carbon Analyser TOC-V CSH (Shimadzu, Kyoto, Japan) using potassium hydrogen phthalate as a standard. Samples were purged with ultra-pure oxygen to remove DIC (dissolved inorganic carbon) and four injections were run for each sample, with the three closest concentrations averaged to give the DOC value. Chromophoric DOM (cDOM; determined by filtering samples through GF/F filters and measuring absorbance at 440 nm in a Shimadzu Spectrophotometer with a 10 cm path length), in the water samples rarely exceeded that of Milli-Q water (deionized and 0.2 μm-filtered water) and so this measure was discontinued.

Dissolved and total nutrient samples were analysed on a Skalar Auto-analyser (Skalar, Breda, the Netherlands) using standard colorimetric methods. TN and TP samples were digested using potassium peroxidisulphate, boric acid and sodium hydroxide and autoclaved 30 minutes prior to analysis. To minimise sample contamination in the field and lab, all filtration equipment and plastic ware were acid-washed and rinsed with Milli-Q water prior to sampling, and Milli-Q water was filtered alongside water samples to create field blanks. Field blanks and laboratory test tubes containing Milli-Q water were interspersed with field samples for quality control purposes and to ensure carryover between samples was negligible. Randomly chosen samples were re-run to account for drift in the instrument. Blank values were subtracted from field samples before analysis.

2.2.3 Data analysis

ArcGIS analysis was carried out in the Spatial Ecology Research Facility (SERF) in the School of Surveying, University of Otago. Stream catchments were delineated using River Environment Classification (REC) catchment database software provided by the Ministry for the Environment, New Zealand. Topographic slope was determined from raster images at a 1:25,000 scale (pixel size 2 m ground) using Topo vector data. Slope was quantified by counting the number of raster tiles with a slope of $1 - 77°$ in the stream catchment and buffer zone, and determining percentage area for each slope angle. Percentage gently ($< 15°$), moderately ($16 - 25°$) and steeply ($>$ $26°$) angled ground was then calculated.

Vegetation cover was determined using the New Zealand Land Cover Database version 3 (LCDB v.3) (Terralink and Landcare Research, Lincoln, New Zealand) (LCDB, 2012). Vegetation cover was determined for the stream catchment as well as within 100 m landward from each bank of the stream (Niyogi *et al.* 2007) by overlaying the catchment and the 100 m buffer boundaries onto the LCDB database. Localised rainfall and soil moisture data were based on virtual climate station (VCS) data provided by the National Institute of Water and Atmospheric Research (NIWA) CliFlo database, which are derived from the raw data recorded at 600 climate stations around New Zealand. Daily frequencies of rainfall were summed for the seven days prior to sampling, and analysed. Rainfall between sites varied by less than 15 mm on all sampling dates except in 29 October 2011 and 19 November 2011. On these dates, weekly rainfall at four northern sites (Wharf Creek, Boundary Creek, Sawyer Burn and Neck Creek) exceeded rainfall totals at two southern sites (Bullock Creek and Stoney Creek) by 20 to 30 mm.

Evaporation and soil moisture levels were averaged for the seven days prior to sampling. In the CliFlo database, soil moisture conditions are determined from the amount of rainfall entering the pasture root zone (defined as the top 150 mm of soil) and the amount lost by evapotranspiration (these variables are interpolated for the sub-catchments from rainfall, wind speed, temperature, solar radiation and relative humidity data obtained from 70 climate stations around the country over a 34-year period) (Tait and Woods 2007). Modeled soil moisture does not account for differences in soil type or vegetation cover. Air temperature data were obtained from VCS data and the "Aero AWS" climate station near Wanaka Airport.

2.2.4 Statistical analysis

Univariate and multivariate analyses were carried out using SPSS (v. 21.1, IBM) software. Data that were not normally distributed (i.e. some nutrient data) were $\log_{10}$ transformed before analysis. Between-group comparisons of median values of stream nutrients from different catchments were made using the Kruskal-Wallis non-parametric test when assumptions of normality or homoscedasticity were violated. As TP and DRP data were not normally distributed regardless of the transformation used, data were compared using non-parametric analyses. Simple and multiple linear regressions were performed on mean values and individual stream data that met the conditions for normality and homoscedasticity. Relationships between predictor

variables that did not change during the course of the study (i.e. landscape variables such as vegetation cover and catchment slope) and N, P and DOC were made using stream annual mean concentrations. As a focus of my study concerned how the nutrients transported in the tributaries could affect Lake Wanaka, flow-weighted mean nutrient and DOC concentrations (FWMC) were also calculated using the formula:

$$FWMC = \frac{\sum_1^n (c_i \; x \; Q_i)}{\sum_1^n Q}$$

where c_i = raw concentration, Q_i = stream flow rate at the time of sampling, and $\sum_1^n Q$ = sum of flow rate for the stream.

As sites were sampled multiple times, I used linear mixed-effects models to account for variability between sampling sites, and lack of independence among samples. The mixed-effects models related solute concentration to contemporaneous meteorological values (fixed effects) while accounting for cross-site differences (random effects). Adding parameters increases model complexity, and can increase likelihood due to overfitting. I used Bayesian Information Criterion (BIC) to select the best fitting model, because BIC penalizes the model based on the number of parameters used (Schwarz 1978).

As fixed effects are categorical independent factors, meteorological variables were analysed using dummy-coded grouping variables. Rainfall was grouped into 10 mm intervals, soil moisture was grouped by 10% intervals, and air temperature was grouped into 1 °C intervals. I used variance components to estimate the contribution of the random (i.e. pasture cover) effect to the variance of the dependent variable (i.e. DOC, N, P). Pearson's correlation was also used to compare univariate relationships among the variables. In all analyses, statistical significance was accepted if $p < 0.05$.

2.3 Results
2.3.1 Stream physico-chemistry
Generally, the catchments examined had low nutrient and DOC concentrations, representative of low intensity land use activities. Over the course of the entire sampling period, pH was circum-neutral, and dissolved oxygen (DO) concentrations indicated stream waters were usually well-aerated. DOC concentrations ranged from 1.14 to 5.69 mg l^{-1}, while TN and NO_3-N concentrations were quite variable, ranging

from 2.19 to 753 µg l^{-1} and from below detection to 624 µg l^{-1}, respectively (Appendix A, Table A1). Phosphorus concentrations were also quite variable, ranging from <1.0 to 8.91 µg l^{-1} for dissolved reactive phosphorus (DRP) and 1.38 to 86.99 µg l^{-1} for total phosphorus (TP). Comparatively, median trigger values of the Australia and New Zealand Environmental Conservation Council (2000) guidelines for the protection of upland rivers (> 150m a.s.l.) are: DRP = 9 µg l^{-1}, NO$_3$-N = 167 µg l^{-1}, TN = 295 µg l^{-1}, TP = 26 µg l^{-1}.

2.3.2 Relationships between nutrients and landcover

Total nitrogen, NO$_3$-N and DOC concentrations were consistently higher in farmed (Alpha Burn, Fern Burn) and urban catchments (Bullock Creek, Stoney Creek) than in catchments with minimal (< 5%) development (Boundary Creek, Wharf Creek, Neck Creek) (Table 2). Flow-weighting NO$_3$-N and TN concentrations did not increase the strength of the relationship between solutes and pasture cover, but did improve the fit of DOC regression models (Table 3).

Table 3: Linear regression models of dissolved organic carbon (DOC; mg l^{-1}), nitrate - nitrogen (NO$_3$-N) and total nitrogen (TN) (µg l^{-1}) to percent pasture cover (%Pas). f-w indicates flow-weighted mean concentration averaged over the sampling period.

Regression model	r	R^2	p	n
DOC = 1.98 + 0.032 %Pas	0.86	0.74	0.006	8
DOC$_{f-w}$ = 1.53 + 0.032 %Pas	0.87	0.76	0.005	8
LOG(TN) = 1.25 + 0.024 %Pas	0.95	0.90	<0.001	8
LOG(TN$_{f-w}$) = 1.28 + 0.021 %Pas	0.91	0.84	0.001	8
LOG(NO$_3$-N) = 0.88 + 0.028 %Pas	0.91	0.83	0.002	8
LOGNO$_3$-N $_{f-w}$ = 0.82 + 0.028 %Pas	0.88	0.79	0.003	8

Using raw data, N and DOC showed a strong, positive relationship with the proportion of the catchment covered in pasture (Table 3). The proportion of pasture cover in the catchment explained 90% of variability in mean stream TN concentrations and 83% of variability in mean NO$_3$-N concentrations (Table 3). Pasture cover also explained over 76% of variability in mean DOC concentrations in streams (Figure 3B).
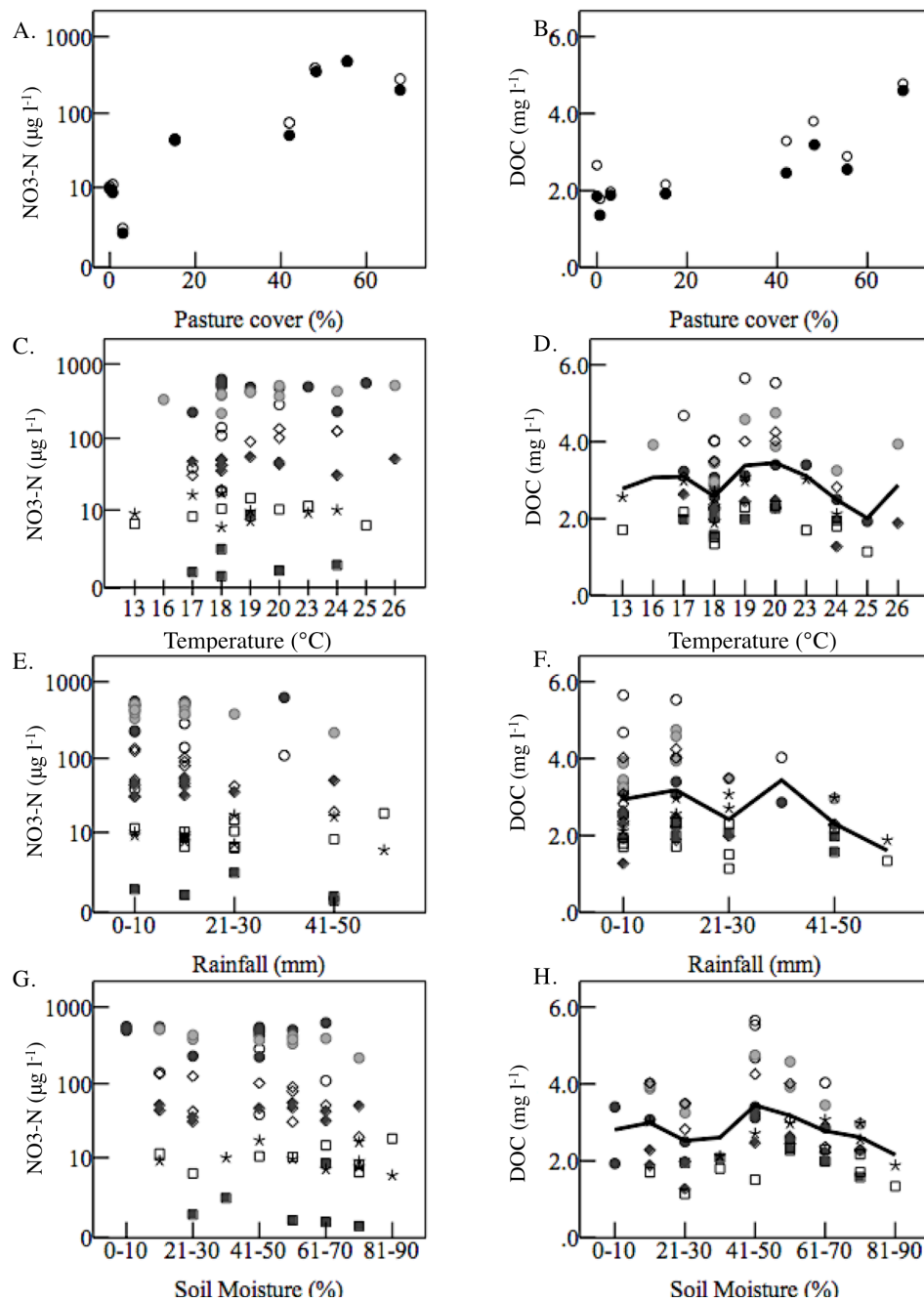
Figure 3: Nitrate-nitrogen (NO_3-N) ($\mu g\ l^{-1}$) A., C., E., F., and dissolved organic carbon (DOC) B., D., F., H., concentrations (mg l^{-1}) in relation to total rainfall (mm, summed over 7 preceding seven days), modeled soil moisture (% saturation, averaged over 7 preceding days) and percentage pasture cover in the catchment. Mean raw ($\circ$) and flow-weighted ($\bullet$) data are presented in Figures A and B. In Figures C through H., streams are denoted by: ($\bigcirc$) Stoney Creek (68% pasture cover); ($\bullet$) Bullock Creek (55.5% pasture cover); ($\bullet$) Alpha Burn (48% pasture cover); ($\diamond$) Fern Burn (42% pasture cover); ($\blacklozenge$) Matukituki River (15% pasture cover); ($\blacksquare$) Neck Creek (3% pasture cover); ($\square$) Boundary Creek (1% pasture cover); ($\star$) Wharf Creek (< 1% pasture cover). Black interpolation line in D. F. and H. represents mean fixed effects values.

In contrast, total (TP) and dissolved inorganic phosphorus (DRP) concentrations did not increase with increasing pasture cover (Figure 4 A and B). The highest DRP

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concentrations were recorded in Wharf Creek, which is predominantly covered in tussock and contains no pastureland (see Table 2; Figure 4C). Rainfall was moderate at the time of sampling (0.5 to 4 mm hr^{-1}). This high DRP concentration resulted in a weak positive correlation between DRP and percent tussock cover in the catchment (r = 0.319, p = 0.005). Very high TP concentrations (69.5 and 104.5 µg l^{-1}) were recorded in duplicate samples taken from Boundary Creek (BC) (< 1% pasture cover) in March 2011 under moderate rainfall conditions (Figure 4D). On the same date, DRP concentrations in Boundary Creek were 1.36 µg l^{-1} and 1.50 µg l^{-1}. As the sampling site for Boundary Creek was located downstream from State Highway 6, material flushed into the creek from the road may have contributed to the high TP concentration recorded on this date.
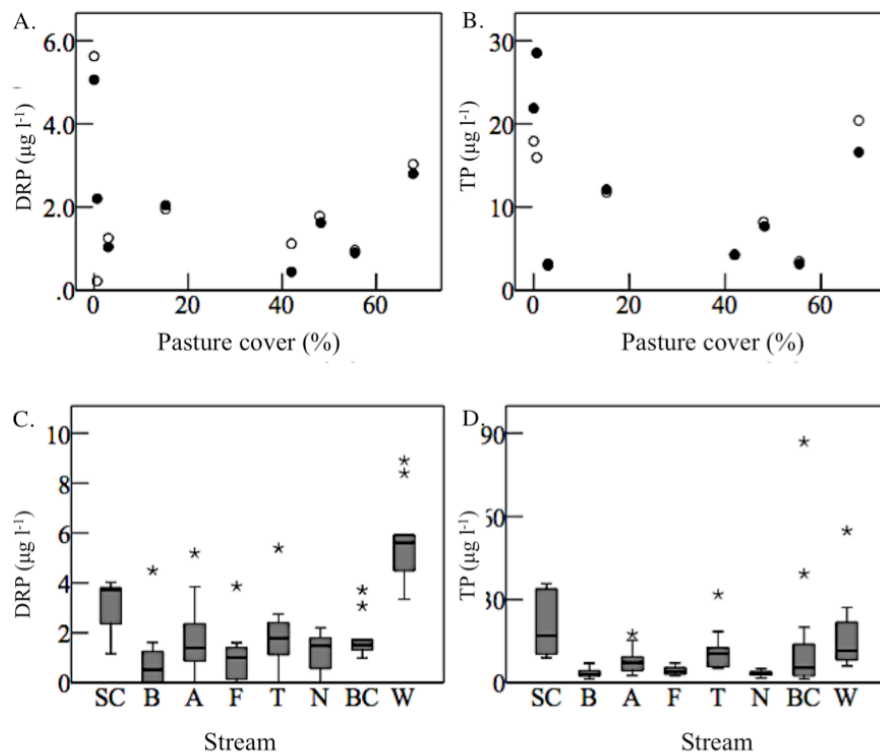
Figure 4: Dissolved reactive phosphorus (DRP) A., and total phosphorus (TP) B., concentrations in each stream by percent pasture cover in the catchment. Mean raw (O) and flow-weighted (●) data are provided. Boxplots of mean C., DRP and D., TP values for each stream are also given. (*) indicates an outlier. In Figures C., and D., SC = Stoney Creek, B = Bullock Creek, A = Alpha Burn, F = Fern Burn (Jack Hall Creek), T = Matukituki River, N = Neck Creek, BC = Boundary Creek, W = Wharf Creek.

Slope and pasture cover were positively related in my study (r = 0.79, p = 0.020), and it is likely some of the variation in stream solute concentration stems from differences in terrain between the catchments. However, the relationship between DOC and

gently sloping terrain ($r = 0.45$, $R^2 = 0.20$, $p = 0.265$, $n = 8$) was not as strong as between DOC and pasture cover (Table 3). Likewise, the relationship between in-stream N and gently sloping terrain was weaker (TN: $r = 0.84$, $R^2 = 0.70$, $p = 0.010$, $n = 8$; DIN $r = 0.70$, $R^2 = 0.50$, $p = 0.050$, $n = 8$) than the relationship between N and pasture cover (Table 3).

When the samples for each stream were analysed separately for each month, the extent of pasture cover accounted for at least 73% of the variability in TN ($r = 0.85 – 0.99$, $R^2 = 0.73 – 0.98$, $p < 0.05$, $n = 6 – 8$) and explained more than 47% of the variance in NO_3-N concentration ($r = 0.68 – 0.98$, $R^2 = 0.47 – 0.94$, $p < 0.061$, $n = 6 – 8$). Comparisons of monthly DOC measurements and pasture did not produce consistent results, with only five months (all sampled from October 2011 to April 2012) showing a significant ($r = 0.83 – 0.92$, $R^2 = 0.69 – 0.85$, $p < 0.05$, $n = 8$) relationship between DOC and pasture cover. The lack of a consistent association between DOC and pasture cover suggests other variables influenced the relationships between DOC concentration and land use in these tributaries.

2.3.3 Meteorological conditions and stream physico-chemistry

To determine whether meteorological conditions were influencing N, P and DOC in my study streams, we compared rainfall (summed over the preceding week), soil moisture (averaged over the preceding week) and air temperature (maximum on the day of sampling) with flow rate and concentrations of N, P and DOC at the time of sampling. Rainfall was consistently higher in steeper catchments located at the northern end of the lake (e.g. Wharf Creek, Boundary Creek, Neck Creek and Sawyer Burn) than in catchments located in the south (e.g. Bullock Creek and Stoney Creek). On two occasions (29 October 2011 and 19 November 2011), differences in weekly rainfall exceeded 10 mm. On all other sampling dates, differences in weekly rainfall between catchments did not exceed 10 mm, which was within the estimated error range of the virtual climate station data of 5 – 15 mm for catchments with elevations < 500 m (Tait *et al.* 2012).

Table 4: Linear mixed effects model of DOC concentration. Fixed effects: A. air temperature (°C); B: Soil moisture capacity (%); C: Rain: Rainfall (mm). Random effect Pasture = Pasture cover (%). Models are listed above each table. S.E. = standard error.

A.   DOC ~ (Fixed)Air temperature + (Random)Pasture + error

Fixed effect	Estimate	S.E.	Sig.	95% Confidence Interval (CI)	
				Low	High
Intercept	2.88	0.44	<0.001	1.95	3.78
13°C	-0.07	0.39	0.852	-0.86	0.71
16°C	0.19	0.45	0.681	-0.72	1.09
17°C	0.45	0.37	0.235	-0.30	1.20
18°C	-0.31	0.28	0.280	-0.87	0.26
19°C	0.49	0.30	0.103	-0.10	1.09
20°C	0.56	0.29	0.058	0.02	1.14
23°C	0.25	0.35	0.488	-0.47	0.96
24°C	-0.39	0.30	0.201	-0.99	0.22
25°C	-0.85	0.38	0.033	-1.63	-0.73
26°C	0[b]	0			

Random	Estimate	S.E.	Wald Z	Sig	95% CI	
					Low	High
Residual	0.13	0.03	4.52	<0.001	0.08	0.20
Pasture	0.96	0.53	1.82	0.069	0.33	2.83

B.   DOC ~ (Fixed)Soil moisture + (Random)Pasture + error

Fixed effect	Estimate	S.E.	Sig.	95% Confidence Interval (CI)	
Intercept	2.20	0.47	<0.001	1.21	3.15
0-10 %	0.62	0.52	0.233	-0.42	1.66
11-20 %	0.80	0.38	0.042	0.03	1.56
21-30 %	0.32	0.38	0.415	-0.46	1.09
31-40 %	0.43	0.43	0.315	-0.43	1.30
41-50 %	1.28	0.38	0.002	0.51	2.05
51-60 %	1.04	0.38	0.009	0.27	1.80
61-70 %	0.59	0.38	0.130	-0.18	1.36
71-80 %	0.35	0.38	0.361	-0.42	1.12
81-90 %	0b	0			

Random	Estimate	S.E.	Wald Z	Sig	95% CI	
					Low	High
Residual	0.21	0.05	4.57	<0.001	0.14	0.33
Pasture	0.79	0.45	1.77	0.077	0.26	2.40

C.   DOC ~ (Fixed)Rainfall + (Random)Pasture + error

Fixed effect	Estimate	S.E.	Sig.	95% Confidence Interval (CI)	
Intercept	2.23	0.52	<0.001	1.17	3.29
1-10 mm	0.59	0.40	0.152	-0.22	1.40
11-20 mm	0.98	0.40	0.019	0.17	1.79
21-30 mm	0.62	0.41	0.135	-0.20	1.44
31-40 mm	0.37	0.56	0.514	-0.76	1.50
41-50 mm	0.31	0.45	0.499	-0.60	1.22
51-60 mm	0b	0			

Random	Estimate	S.E.	Wald Z	Sig	95% CI	
					Low	High
Residual	0.26	0.05	4.73	<0.001	0.17	0.40
Pasture	0.95	0.54	1.76	0.078	0.31	2.89

In most streams, flow rate increased as conditions became wetter. In five of the tributaries, flow rate correlated positively with soil moisture or rainfall ($r = 0.64$ – 0.82, $p < 0.05$). Stream nutrient concentrations rarely correlated with flow rate. Exceptions include concentrations of TN ($r = -0.74$, $p = 0.034$) and NO_3-N ($r = -0.78$, $p = 0.013$) in Fern Burn, and NO_3-N in Alpha Burn ($r = -0.88$, $p < 0.001$), which tended to decrease with increasing flow rate. Dissolved reactive phosphorus concentrations increased with increasing flow in the Matukituki River ($r = 0.69$, $p = 0.040$).

No significant relationships were apparent between meteorological variables and in-stream N concentrations (Figure 3). However, rainfall ($F_{(5, 50.229)} = 2.686$, p = 0.032), soil moisture ($F_{(8,47.260)} = 3.947$, p = 0.001) and air temperature ($F_{(9, 46.054)} = 9.043$, p < 0.001) were all significant predictors of variation in DOC concentration, after accounting for cross-site differences. DOC ~ Air temperature + Soil moisture + Air temperature*Soil moisture + Pasture produced the best model based on BIC values (Appendix A Table A2).

The response of DOC to changes in air temperature varied across sites, even when allowing for differences between sites in the magnitude of the responses. DOC concentrations were generally highest when air temperatures were near 20°C, then decreased as temperature increased (Table 4; Figure 3D). Increasing temperature tended to correspond with drier soil conditions and lower DOC concentrations (Figure 5). DOC concentrations were highest when soil moisture content ranged from 41-50%. Past this point, DOC concentration decreased as soil moisture content continued to increase (Figure 3H). Similar to soil moisture, DOC concentration increased as rainfall increased from between 1-10 mm to between 11-20 mm (see Table 4; Figure 3F). When rainfall values exceeded 40 mm, DOC concentration tended to decrease in streams.
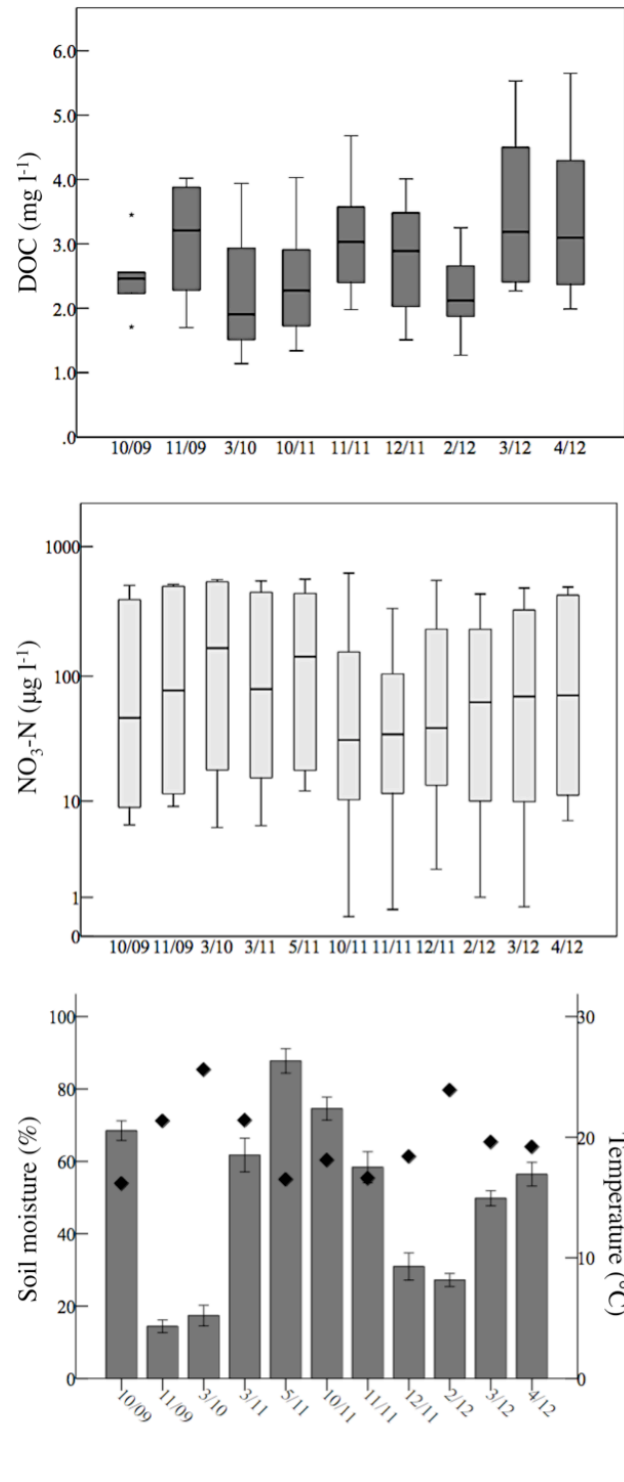
Figure 5: Boxplots of mean (top graph) dissolved organic carbon (DOC) and (middle graph) nitrate-nitrogen (NO$_3$-N) on each sampling date between October 2009 and April 2012. The bottom graph shows mean soil moisture content in the week prior to sampling (bars) compared with air temperature (◆). Dates on the x-axis are as follows: 10/09: October 14-16, 2009; 11/09: November 27-28, 2009; 3/10: March 7-8, 2010; 3/11: March 26-27, 2011; 5/11: May 20-21, 2011; 10/11: October 29-30, 2011, 11/11: November 19-20, 2011; 12/11: December 17-18, 2011, 2/12: February 4, 2012, 3/12: March 9-10, 2012, 4/12: April 1, 2012. DOC samples from March 26-27, 2011 and May 20-12, 2011 were not included in the study and are not present in Figure 5A.

The proportion of organic and inorganic N and P differed between streams draining modified (> 40% pasture cover) and unmodified (< 5% pasture cover) catchments, the Matukituki River and Lake Wanaka (Figure 6). Dissolved inorganic nitrogen (DIN = NO_3-N + NH_4) made up 50% of TN in the lake, similar to proportions in the Matukituki River (59.3%) and modified catchments (53.7%), while unmodified streams had smaller proportions of DIN (34%) than the lake. Modified streams had greater proportions of dissolved organic phosphorus (DOP) (27.3%%) and particulate phosphorus (PP) (40.8%) than open waters in Lake Wanaka, where DRP made up the majority of P (66.7%). In unmodified streams, the majority of phosphorus was in particulate (66.1%) and dissolved inorganic (24.3%) form. In the Matukituki River, 72% of P was in particulate form.
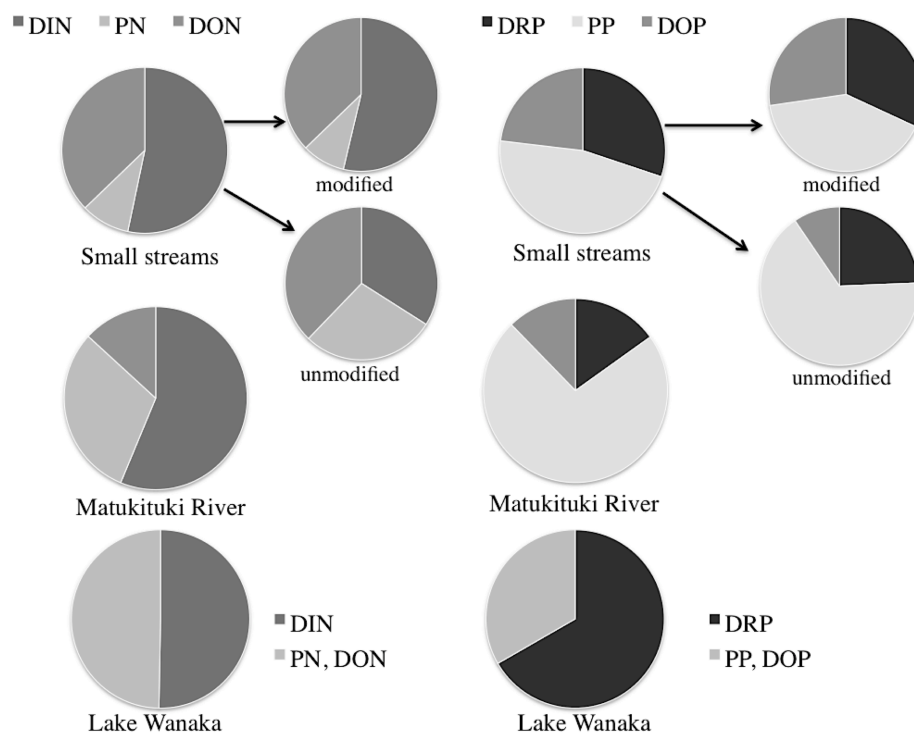
Figure 6: The proportions of nutrient fractions in waters of different origins. Particulate N (PN), dissolved inorganic N (DIN), dissolved organic N (DON), particulate P (PP), dissolved reactive P (DRP) and dissolved organic P (DOP). catchments are also grouped into modified (> 40% pasture) and unmodified (< 5% pasture).

2.4 Discussion

2.4.1 Landscape variables and stream physico-chemistry

In support of my first hypothesis that N and DOC concentrations will increase with increasing pasture development, TN, NO_3-N and DOC concentrations in the sampled streams were strongly positively correlated with pasture cover. The relationship

between N and percent pasture cover in my study is consistent with other studies of land use and nutrient enrichment in aquatic systems (Wang *et al.* 2001, Quinn and Stroud 2002, Niyogi *et al.* 2003, Niyogi *et al.* 2007, Wilson and Xenopoulos 2008). Previous research also supports the relationship I found between in-stream DOC concentrations and land use. The range of DOC concentrations in my study (0.8 to 5.7 mg l^{-1}) was similar to the range reported by Quinn and Stroud (2002) (0.10 – 8.80 mg l^{-1}), and is comparable to other studies conducted in hilly catchments (see Table 5). In general, agricultural development increases soil organic matter concentration, thereby increasing the amount of DOC available for export to nearby streams (Lambert *et al.* 2000, Hedley *et al.* 2009), although soil characteristics (Post and Kwon 2000), landscape (Dillon and Molot 1997) and weather conditions (Schiff *et al.* 1997), can affect the delivery of organic matter to waterways. Overall, DOC concentrations were low (< 10 mg l^{-1}) in my sampled streams, which probably stems from low soil organic matter content in the region (Leamy 1966, Brash and Beecroft 1987) and relatively good soil drainage in the catchment (Landcare Research 2016) as soil drainage capacity is inversely related to DOC concentration (Moore 1989, Wilson and Xenopoulos 2008).

Unlike N and DOC, P concentrations in my study streams were not significantly related to land use. While this finding supports my second hypothesis that P concentration will not increase as readily as N and DOC in stream water, it differs from several other New Zealand studies that report increasing TP and DRP concentrations with increasing pasture cover (see Table 5). Niyogi *et al.* (2003) reported DRP concentrations up to 12 times higher in streams with a high proportion of pasture cover than in undisturbed catchments. Similarly, Riley *et al.* (2003) found a strong correlation between DRP and TP concentrations and pasture development. Quinn and Stroud (2002), reported a 2.5- to 7.7-fold increase in TP concentrations from a catchment developed fully in pasture compared with one in native vegetation, with higher exports of TP occurring during wet winters. Verheyen *et al.* (2015) reported high P concentrations in forested streams during dry periods in the summer, which they attributed to warm temperatures stimulating microbial breakdown of leaf litter in the stream. In contrast, P concentrations were highest in streams draining pastureland during peak flows as overland flow supplemented P concentrations already in the stream (Verheyen *et al.* 2015).

Table 5: A comparison of terrain, vegetation, climate, and concentrations of N, P and DOC in 12 recent stream studies. Climate data listed as mean annual rainfall (Rain) and mean maximum and minimum air temperature (T). NO_3-N and DRP data presented in $\mu g \ l^{-1}$, while DOC data given as $mg \ l^{-1}$. Relevant findings from each study are described. Y-B = yellow-brown soil, W & X = Wilson and Xenopoulos, 2008.

Site	Terrain	Climate	Vegetation	Soil	Nutrients	Author	Findings
Sweden	Rolling hills	R: 620 T: -13.5 – 3.1	Forest (boreal)	Peat	DOC: 11.7 – 26.1	Agren et al. (2010)	High DOC concentrations during periods of high discharge in spring and after long winters, but lower if DOC export during the previous summer and autumn was high.
Daintree Nat'l Park, Australia	Steep	4900	Rainforest	Acidic brown	DOC: 0.9 – 5.3	Bass et al. (2011)	DOC concentrations increased with increasing stream flow rate/discharge. Changes in stream discharge affected the quality of DOC.
Westland NZ	Steep	R: 2400 T: 5 – 16	Forest and clear cut	Gravel & silty loam clay	DOC: 1.7 – 21	Moore (1989)	DOC concentrations were higher in streams draining logged catchments. During storm events, DOC concentrations increased with discharge prior to hysteresis.
Hamilton NZ	Hilly to steep	R: 1600 T: 13.7	Forest, pasture	Y-B	DOC: 1.5 – 2.28 NO_3: 99 – 858 DRP: 13.7 – 38.2	Quinn & Stroud (2002)	N species, TP and DOC concentrations were highest in streams draining pasture. Flow was strongly, positively related to NO_3-N concentration.
Hamilton NZ	Hilly to steep	R: 1600 T: 13.7	Forest, pasture	Y-B	DOC: 3.7 – 5.6 NO_3: < 1.0 – 536 DRP: <1.0 – 59	Findlay et al. (2001)	DOC concentrations were higher in surface and subsurface flow paths than in the groundwater. DOC quality was affected by these riparian flow paths.
Michigan USA	Gentle	*	Forest, cropland, urban	Gravel glacial till & sand	DOC: 0.81 – 7..98 NO_3: 14 – 17496 DRP: 2 – 111	Johnson et al. (2009)	DIN concentrations were significantly higher in agricultural streams, but DOC concentrations were not affected by land use. Higher concentrations of DIN did not stimulate uptake of DOC.
Ontario, Canada	*	*	Forest, cropland, wetland, urban	*	DOC: 1.7 – 24.1	W & X (2008)	DOC concentrations were not strongly influenced by the amount of agricultural development within the catchment. Soil drainage was the best predictor variable for DOC.
Scotland, UK	Hills	*	Forest, grassland/ moor	Peat	DOC: 7.6 – 10.8	Grieve (1984)	DOC concentrations were greater in forested than grassland catchments. Discharge was less able to explain variation in DOC concentrations in catchments with increasing extents of peaty soil.
Eastern Otago, NZ	*	*	Tussock and/or forest to farmland	*	NO_3: 5 – 1797 DRP: 2 – 101	Niyogi et al. (2007)	DIN, DRP and TP concentrations increased with increasing pasture cover. Tussock -dominated catchments had very low nutrient concentrations and little fine sediment.
Eastern Otago, NZ	*	*	Tussock, forest, pasture	*	NO_3: 6 – 2647 DRP: 2 – 35	Niyogi et al. (2003)	DIN and DRP concentrations were low in pristine streams and high in streams draining developed catchments.
Eastern Otago, NZ	Rolling hills	*	Tussock, pasture	schist	DOC: ≈ 2.7 – 7.5 NO_3: 3.5 – 33.6 DRP: ≈5 – 23	Riley et al. (2003)	Nutrient loads were higher in streams draining pasture than streams draining grazed or ungrazed tussock.
Central Otago, New Zealand	Hilly to steep	Rain: 680 T: 8 – 23	Tussock, pasture, forest, urban	Brown pallic & fluvial	DOC: 1.99 – 5.42 NO_3: 2.97 – 478 DRP: 0.96 – 5.63	Weaver et al.	NO_3-N, TN and DOC concentrations increased with increasing pasture cover. Weather conditions were weakly associated with NO_3-N and DOC concentrations but were not associated with TP or DRP.

It appears that the catchments in my study area were able to retain much of the current P added as fertilizers and stock faeces, possibly because pasture development was of low intensity and the minerogenic soils were able to effectively bind DRP. However, it should be noted that stream water samples were not collected during high flow events, and I have, therefore, underestimated particulate P and possibly DRP fluxes from these catchments. Although different flow regimes were captured during my study, the full range of hydrological flows (e.g., storm events) that could contribute to P input into streams was not sampled due both to logistical and safety issues related to 'flood chasing'. High flow events can significantly increase P influx into the stream water (House *et al.* 1998, Correll *et al.* 1999, Kolpin *et al.* 2000, Quinn and Stroud 2002), as rainfall and storm events increase P input to streams via surface runoff and subsurface flow (House *et al.* 1998, Kolpin *et al.* 2000). Furthermore, particulate P in riverbed sediments may be re-suspended as flow increases (House *et al.* 1998).

2.4.2 Meteorological conditions and stream physico-chemistry

While weather-related factors were significant predictors of DOC in stream water, the relationship between increasing hydrological connectivity and DOC concentration was not straightforward. The highest DOC concentrations were recorded in the late spring and autumn, when temperatures were warm and soil conditions were not extremely dry or wet. Low DOC concentrations were recorded in summer (February 2012) and early autumn (March 2010) under hot, dry conditions (Figure 5A). Dissolved organic carbon concentration also decreased as soil conditions approached saturation (Figure 3H). These results suggest the combined effect of temperature and soil moisture can mitigate the influence of pasture cover on surface water DOC concentration in the Lake Wanaka catchment.

Previous research has shown that the interplay of factors regulating DOC input in streams is complex. Warmer temperatures stimulate microbial decomposition of soil organic matter and DOC production (Moore and Dalva 2001), but precipitation or increased soil moisture content is necessary to leach that DOC from soils (Godde *et al.* 1996). When soil moisture conditions become very dry or very wet, relationships between DOC concentration and landscape variables can weaken (Wilson and Xenopoulos 2008). Under dry conditions, in-stream DOC concentrations may decrease, as stored water moves vertically to lower soil horizons and groundwater instead of to stream surface waters (Stieglitz *et al.* 2003). Increasing soil moisture

content as a result of rainfall or snowmelt can prolong contact between stored water and surface soils, increasing the connectivity of the upper and lower catchment. This increased connectivity allows for the lateral movement of water and solutes into streams (Stieglitz *et al.* 2003, McGuire and McDonnell 2010), and multiple studies report a positive relationship between stream discharge (related to rainfall or snowmelt) and DOC concentration (Meyer and Tate 1983, Moore 1989, Grieve 1990, Hinton *et al.* 1997, Clark *et al.* 2007, Wilson and Xenopoulos 2008, Bass *et al.* 2011). However, consistent or prolonged spells of high rainfall and runoff can ultimately result in hysteresis, where water from the upper catchment dilutes downstream DOC concentrations leading to a decrease in DOC (Meyer and Tate 1983).

In the Wanaka watershed, decreasing DOC concentrations with increasingly wet soil conditions may reflect frequent or repeated leaching of DOC from the soil, reducing the amount of DOC available for release (Godde *et al.* 1996). It may also reflect limitations of my sampling design. Steep terrain in the Wanaka watershed likely facilitates the rapid movement of water from the upper watershed during wet conditions, diluting solute concentrations in lower stream reaches. As sampling did not occur after significant rain events, I may have missed initial increases in DOC associated with rainfall or stream discharge.

Unlike DOC, TN and NO_3-N concentrations were not influenced by soil moisture content, rainfall, or changes in air temperature. These results contrast with the findings of Arheimer and Lidén (2000) who reported high in-stream nitrate concentrations during rainy periods and following snowmelt, and lower concentrations during dry periods. In their study, biological uptake and plant growth during summer months reduced the amount of N available for leaching, leading to lower concentrations of N in stream water (Arheimer and Lidén 2000). This is particularly true in small streams during low flow conditions, where algae can retain the bulk of nutrients in the stream (Marti *et al.* 1997). I did not observe a consistent pattern in N or P concentrations related to the growing season when comparing individual streams (data not shown). In general, the lowest NO_3-N concentrations occurred in my study streams in the late spring (Figure 5B) in conjunction with moderate flow conditions.

2.4.3 Implications for the lake

My findings indicate that even relatively low intensity land use clearly influences stream nutrient and DOC concentrations. In the largest tributary sampled, the Matukituki River, current nutrient and DOC concentrations are within the range of those in the lake (Figure 6). However, future anthropogenic development in this catchment could increase nutrient loading as urban and agricultural development appears to have done in my small, modified catchments. I caution that land use impacts on small streams should not be extrapolated directly to a large river catchment, because in-stream nutrient attenuation processes can vary substantially between small and large streams as river discharge and flow velocity affect nutrient spiraling length (Alexander *et al.* 2000, Hall *et al.* 2002). But in high discharge, fast-flowing rivers and smaller streams similar to those sampled in my study, land use has been shown to increase nutrient export, either through increased inorganic N loads or by altering stream metabolism (Alexander *et al.* 2000, Hall *et al.* 2002, Strayer *et al.* 2003, Hall *et al.* 2009). Thus, it is likely that increased agricultural or urban development in the Matukituki River catchment would promote increased concentrations of DIN, DOP (Figure 6) and DOC reaching the lake. Increased macronutrient and DOC loading to Lake Wanaka has the potential to stimulate changes in the pelagic microbial population, affect phytoplankton productivity (Bayer *et al.* 2015) and result in changes in food webs and community structure.

2.5 Conclusion

My findings show that N and DOC concentrations are higher in tributaries to Lake Wanaka that have greater pasture cover in their catchments. Although the intensity of agricultural activity and urbanisation in the region is relatively low, modification of catchments around Lake Wanaka appears to affect N and DOC loading to the lake. The relationship between land use and DOC export from these catchments is mediated by weather conditions, where DOC concentrations increase under warm temperatures and 'normal' soil conditions. In contrast, weather conditions did not significantly enhance the relationship between land use and N or P export in my study streams. My findings have potential relevance to other temperate, mountainous, catchments with low intensity agricultural development.

3 Factors related to production of transparent exopolysaccharide particles and the formation of lake 'snow' in Lake Wanaka

3.1 Introduction

Amorphous organic aggregates form through the coagulation of transparent exopolymer particles (TEP) (Alber and Valiela 1994, Kiørboe *et al.* 1994, Logan *et al.* 1995, Grossart *et al.* 1997, Farooq and Long 2001). TEP are optically clear polysaccharides that vary in adhesiveness by age and algal source (Alldredge *et al.* 1995, Passow and Alldredge 1995a), and are often considered to be exudate that is suspended in the water column (Alldredge and Gotschalk 1989). Initial particle formation occurs through the collision and clumping of small exudate particles (Logan *et al.* 1995), which then collide with other larger particles, including phytoplankton cells, as they grow in size and age (Alldredge and Gotschalk 1989, Logan *et al.* 1995). Large macroscopic aggregates found in lake and marine systems have been referred to as "snow" (e.g. Grossart et al. 1993; Logan et al. 1995).

Many species of diatoms and cyanobacteria are known to produce extracellular polysaccharides (Maurin *et al*. 1997, Simon *et al*. 2002), and extensive field evidence exists for aggregate formation at the termination of diatom blooms (Alldredge and Gotschalk 1989, Alldredge *et al*. 1993, Logan *et al*. 1995, Passow and Alldredge 1995a). Contrasting studies attribute TEP formation to either extracellular polysaccharide production during diatom blooms (Alldredge *et al*. 1995, Logan *et al*. 1995) or to nutrient limitation inhibiting biomass production but not limiting photosynthesis, resulting in algal cell exudation of polysaccharides into the water column (Smetacek 1985, Alldredge and Gotschalk 1989, Kiørboe *et al*. 1994, Engel *et al*. 2002). These two hypotheses are not mutually exclusive, as Penna et al. (1999) noted that several species of marine diatom were capable of increasing in biomass and producing extracellular polysaccharides under nutrient-limited conditions, and that extracellular polysaccharide material was present in the water during all phases of cell growth.
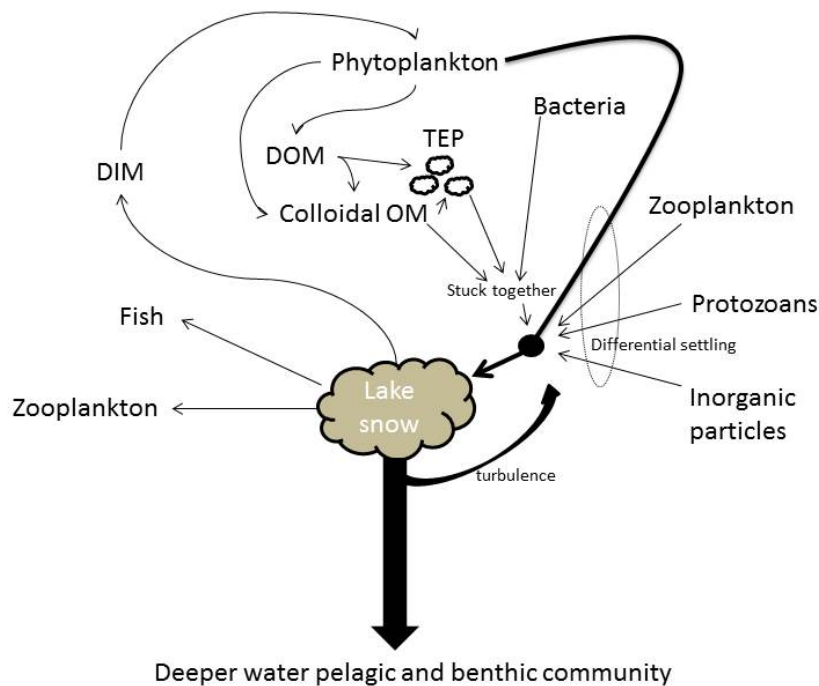
Figure 7: Schematic diagram of lake snow formation and uptake in pelagic waters. OM = organic matter; DOM = dissolved organic matter; DIM = dissolved inorganic matter (e.g. nutrients); TEP = transparent exopolymer particles.

In both low and high productivity systems, the presence of aggregated material in the water column provides nutrient-enriched micro-patches for colonisation by various microbial organisms (Berger *et al.* 1996), as well as a food source for larger components of the microbial food web (heterotrophic nanoflagellates, ciliates, rotifers, and crustacean grazers) (Figure 7) (Simon *et al.* 2002). Organic aggregate formation can also facilitate the vertical movement of organic matter to deeper waters, with rapid removal occurring in relation to particle size (Li and Logan 1995, Logan *et al.* 1995).   In non-bloom conditions, smaller particle sizes and more patchy distribution can lead to longer circulation of aggregates in the water column and gradual, as opposed to pulsed, sedimentation rates (Logan *et al.* 1995). As benthic primary production can substantially contribute to whole-lake primary production in low productivity systems (Vadeboncoeur *et al.* 2002), increases in pelagic primary production and the export of phytoplankton to the bottom of a lake can lead to shifts in benthic and pelagic community composition, food web dynamics (Chandra *et al.*

2005), oxygen concentrations and the release of bound nutrients into the water column (Genkai-Kato and Carpenter 2005).

While most work on organic aggregate production has occurred in marine settings, aggregate formation has also been reported in lake systems (Grossart and Simon 1993, Logan *et al.* 1995, Grossart *et al.* 1997, Grossart *et al.* 1998). In Lake Wanaka, lake "snow" has been recorded in monthly field samples from 2008 (Bodger *et al.* 2011), although anecdotal reports from local fishermen indicate it has been present in the lake in the summer since 2003. Microscopic observations of aggregates obtained from Lake Wanaka from 2008 to 2010 showed they were composed of algae, detrital material, bacteria and protozoans (Bayer 2013), and many of the aggregates contained the dominant diatom in the lake, *Lindavia intermedia* (P. Novis, Landcare Research, Lincoln). *Lindavia intermedia* has only recently become the dominant phytoplankter in Lake Wanaka (Naismith 1994, Galbraith and Burns 2007, Bayer *et al.* 2015). Preliminary analyses of diatom cases in sediment cores from Lake Wanaka indicate *Lindavia intermedia* (formerly identified as *Cyclotella stelligera*, *Cyclotella bodanica* and *Cyclotella* sp.) numbers increased substantially around 2003, which coincides with initial reports of organic aggregates in the lake (E. Saulnier-Talbot and M. Schallenberg, personal communication).

Factors driving organic aggregate formation in Lake Wanaka are currently unknown. My aim was to determine whether external input of nutrients and dissolved organic carbon (DOC) could facilitate TEP generation in Lake Wanaka water; and whether TEP generation was related to the recent dominance by the diatom, *Lindavia*. Previous studies have shown increased nutrient loads can stimulate algal growth in New Zealand lakes (Galbraith and Burns 2007, Abell *et al*. 2011b), and algal blooms have been positively linked to TEP generation in field and laboratory studies (Alldredge *et al*. 1995, Passow and Alldredge 1995a, Grossart *et al*. 1997). Transparent exopolymer particle precursor material has also been found to form from terrestrial leachates (Bozeman 2012), and increasing inputs of chromophoric DOC may facilitate TEP formation in low-productivity catchments (von Wachenfeldt and Tranvik 2008, Chateauvert *et al.* 2012).

In the Lake Wanaka catchment, tributaries with more pasture cover deliver more nitrogen (N) and DOC to the Lake (see Chapter 2). Phosphorus (P) concentrations in

inflowing tributaries are generally low (Appendix A, Table A1), and do not appear related to land use (see Chapter 2). However, P concentrations are often higher in streams (see Table A1) than in the open water of Lake Wanaka (DRP: < 1.0 - 1.7 μg l^{-1} between 10 – 150 m depth, 2008 – 2012). As catchment-derived material can stimulate phytoplankton growth (Galbraith and Burns 2007, Abell *et al*. 2011b), and facilitate organic aggregate formation in lake systems (von Wachenfeldt and Tranvik 2008, Chateauvert *et al.* 2012), I predicted that (i):

Prediction (i): TEP concentration will increase in response to inputs of catchment-derived nutrients and DOC.

During diatom blooms, high cell numbers can increase the total amount of polysaccharides produced, resulting in more rapid detection of organic aggregates in the water column (Alldredge *et al.* 1995; Alldredge and Passow 1995a). As algal blooms are linked to N and P availability, I predicted that (ii):

Prediction (i): Artificial nutrient enrichment will generate more TEP and result in more rapid organic aggregate formation than catchment-derived nutrients and DOC.

Lindavia intermedia is currently the dominant phytoplankter in Lake Wanaka (Bayer et al., 2015), and abundance of this species increased around the time organic aggregates were first reported in the lake (E. Saulnier-Talbot and M. Schallenberg, personal communication). If a change in phytoplankton community structure from picoplankton to diatoms, particularly *Lindavia*, is a driving factor for the production of TEP and formation of 'lake snow' in Lake Wanaka, I predicted that (ii):

Prediction (ii): TEP concentrations will increase with increasing presence/abundance of *Lindavia*.

3.2 Methods

In November 2011, December 2011, February 2012, March 2012, November 2012, February 2013 and May 2013, 10 litres of water was collected at 20 m depth from the open water of Lake Wanaka at Aspiring Basin (44º35.702 S 169º04.030 E). Lake water was pre-filtered through a 50-μm mesh screen to remove large grazers while retaining the dominant diatom, *Lindavia intermedia*. In May 2013, 10 l of water were also collected from the open water of Lake Wakatipu and Lake Hawea.

On the other six dates, five litres of water were collected from three tributaries to the lake that represent a gradient of increasing land use: Boundary Creek (1% pasture cover), Alpha Burn (48% pasture cover) and Bullock Creek (56% pasture cover + 21% urban). Water samples were kept in the dark and transported on ice back to the laboratory. Stream water was filtered through Milli-Q rinsed 0.22 µm pore size polycarbonate filters within 24 hours of collection to remove biota. Two litres of lake water were also filtered through 0.22 µm polycarbonate filters to act as exudate for the experimental control.

3.2.1   Stream and lake mixture experiments

During the austral spring through autumn, 2011-2012, experiments were run to determine whether catchment-derived inputs of N, P and DOC promoted TEP generation in Lake Wanaka water. In these experiments, lake water was amended with water from Alpha Burn, Bullock Creek and Boundary Creek (Table 6). Treatments were made by adding 150 ml of the 0.22 µm filtered stream water to each of 3 replicate 300-ml BOD bottles, and then adding 150 ml of 50 µm filtered lake water. The BOD bottles were loaded onto a plankton wheel to provide a constant rate of gentle turbulence throughout the study (Alldredge *et al*. 1995). Bottles were subjected to a 12 hour light (311 µmol photons $m^2 s^{-1}$): 12 hour dark cycle, and room temperature was maintained at 12°C ± 1.0°C. Each experiment lasted for 8 days, and samples were taken on day 0 and day 8 for TEP concentration, dissolved oxygen (DO), and nutrient concentrations. The eight-day endpoint was chosen as visible aggregates have been reported to form within this period in marine and lake systems (Alldredge and Jackson 1995), and to prevent excessive oxygen production in the BOD bottles.

Table 6: Initial 'N' = nitrate-nitrogen ($\mu g\ l^{-1}$), 'P' = dissolved reactive phosphorus ($\mu g\ l^{-1}$) and 'DOC' dissolved organic carbon ($mg\ l^{-1}$) concentration by treatment in the 8-day experiments. Treatments were mixtures of 50-μm-filtered Lake Wanaka water and 0.22-μm-filtered stream water exudate from stream draining draining pastoral land cover (Pasture), pastoral and urban cover (Pasture + Urban) and tussock land cover (Tussock). The control was a mixture of 50-μm-filtered Lake Wanaka water and 0.22-μm-filtered Lake Wanaka water.

	Control	Pasture	Pasture + Urban	Tussock
November 2011				
N	37.2	187.6	350.7	22.1
P	1.1	1.1	1.3	1.2
DOC	2.11	2.92, 3.01	2.68, 2.37	1.93, 2.14
December 2011				
N	39.8	158.1	123.7	18.2
P	b.d.	b.d	b.d.	b.d.
DOC	2.15	2.88	2.59	2.14
February 2012				
N	25.6	276.2	303.6	15.7
P	b.d.	1.4	b.d.	1.0
DOC	1.88	2.94, 2.52	2.57, 2.37	2.14, 2.02
March 2012				
N	16.2	*	*	14.7
P	b.d.	*	*	1.3
DOC	2.39 ± 0.18			2.33, 2.44

3.2.2 Nutrient enrichment experiments

Experiments were run in the austral spring (November) and summer (February) of 2012 – 2013 to determine how artificial nutrient enrichment affected TEP generation in the original stream mixtures. These experiments included the original mixture treatments, plus a parallel set of treatments that were amended with saturating concentrations of N and P. Initial concentrations of NO_3-N and DRP present in the mixture treatments and control are provided in Table 7. Nutrient-amended treatments were spiked with 1200 $\mu g N\ l^{-1}$ and 140 $\mu g P\ l^{-1}$ to promote high cell abundances and ensure detectable aggregation (Alldredge *et al.* 1995, Passow and Alldredge 1995a). These N and P concentrations reflect super-eutrophic conditions in New Zealand lakes (Bryers and Bowman 2000).

The November 2012 and February 2013 experiments were run for 12 days to allow more time for TEP generation (8-day experiment: TEP < 2.8 gum xanthan equivalents). TEP concentration was measured on day 0, 4, 8 and 12, while samples for chl *a*, phytoplankton enumeration, nutrients and DOC were taken at the start and end of the experiment. As the volume of water required for these additional samples was significantly larger than the 300 ml available in the BOD bottles, the experiment was run using 1-litre acid-washed polyethylene bottles. In November 2012, pH was

measured at the end of the experiment, while in February 2013, pH and alkalinity measurements were measured every second day (Appendix B, Table B1). Lids were removed from bottles for one hour every other day to allow for oxygen and carbon dioxide equilibration and to prevent pH reaching harmfully high levels.

Table 7: Initial nutrient concentration ± 1 standard error by treatment in the 12-day experiments. Treatments were mixtures of 50-μm-filtered Lake Wanaka water and 0.22-μm-filtered stream water exudate from streams draining pastoral land cover (Pasture), pastoral and urban cover (Pasture + Urban) and tussock land cover (Tussock). The control was a mixture of 50-μm-filtered Lake Wanaka water and 0.22-μm-filtered Lake Wanaka water. 'N' = nitrate-nitrogen (μg l^{-1}), 'P' = dissolved reactive phosphorus (μg l^{-1}), DOC = dissolved organic carbon (mg l^{-1}). b.d. = below the detection limit of the Skalar Autoanalyser.

	Control	Pasture	Pasture + Urban	Tussock
November 2012				
N	38.4 ± 0.3	162.9, 162.1	303.3, 303.9	24.7, 19.2
P	<1.0, 1.0	2.5, 2.7	1.4, 1.3	1.6, 1.3
DOC	2.4 ± 0.16	2.5 ± 0.03	3.2 ± 0.07	3.7 ± 0.06
February 2013				
N	22.7 ± 1.0	133.5	2.14.8, 253.5	12.1, 11.3
P	b.d.	0.9	b.d.	1.0, <1.0
DOC	2.9 ± 0.22	4.9, 7.7	16.6, 18.1	9.3, 9.4

In November 2012, chl *a* was extracted using standard fluorometric procedures (Parsons *et al.* 1984), but equipment breakdown meant only five treatments could be processed. Samples were not taken for phytoplankton enumeration during this experiment. In February, chl *a* was extracted from 5-ml replicate samples and quantified using a FLUOStar Omega plate reader (BNG Labtech, Ortenberg, Germany) following procedures described by Biggs and Kilroy (Biggs and Kilroy 2000).

3.2.3 TEP generation and *Lindavia* abundance

Dominant algae were identified and enumerated in the February 2013 experiment, and TEP concentration was compared to *Lindavia* counts. Transparent exopolymer particle concentration was also compared with *Lindavia* counts in an experiment run in May 2013. The May experiment differed from those in November and February because it compared TEP production in Lake Wanaka water with two other lakes (Lake Wakatipu and Lake Hawea), in which *Lindavia* does not make up a significant proportion of total phytoplankton composition. Using water from other lakes allowed me to determine whether TEP formation was indicative of processes occurring in Lake Wanaka, or if it was an artefact of the experimental design. Parallel treatments

were enriched with nutrients (1200 µgN l⁻¹ and 140 µgP l⁻¹) and run alongside the lake treatments.

3.2.4 TEP processing and analysis

Methods for the semi-quantitative determination of TEP abundance are described in Passow and Alldredge (1995b). Briefly, BOD bottles were gently inverted in ensure even mixing before an aliquot was removed and filtered through a 0.4 µm polycarbonate filter under very low (< 5 kPa) consistent vacuum pressure. To avoid clogging the filters, the volume of water filtered varied depending on the amount of TEP present (Passow and Alldredge 1995b). However, attempts were made to filter up to 50 ml of water, where possible. When the filter appeared dry, 0.5 ml of 0.22 µm-filtered Alcian blue dye (0.02%) (Sigma Aldrich, New Zealand) was added to the filtration tower and allowed to stain the filter for 2 to 5 seconds. Filters were rinsed with 2 ml of Milli-Q water, placed in 20 ml scintillation vials and acidified with 5 ml of 80% H_2SO_4 for two hours under gentle agitation on a shaker table (70 rpm minute⁻¹). Samples were inverted, then poured into a 1 cm glass cuvette to analyse the amount of light absorbed by each sample using a Shimadzu spectrophotometer (Shimadzu, Kyoto, Japan) (787 nm). For quality control purposes, triplicate 50-ml Milli-Q samples were filtered, stained, and analysed in the same manner as treatment water.

Xanthan gum was used as a calibration standard for each experimental run. Adding 15 mg xanthan gum powder to 200 ml of Milli-Q water and grinding the solution with a mortar and pestle created the xanthan gum solution. The solution was then poured into a beaker and stirred on a VELP Scientifica magnetic stirring hot plate (VELP Scientifica, Italy) for at least 30 minutes before grinding the solution again to break apart any gum xanthan clumps. To determine the dry weight of gum xanthan in the solution, 0.5 – 3.0 ml aliquots were filtered onto pre-weighed 25 mm, 0.4 µm-pore size polycarbonate filters. Additional 0.5 – 3.0 ml aliquots of gum xanthan solution were filtered and stained with Alcian blue. A calibration factor (f_x) was calculated by relating gum xanthan dry weight to absorbance reading from the stained filter, where:

$$f_x \ = \ W \times [(est_{787} - C_{787}) \times V_{st}^{-1}]^{-1}$$

where: W = dry weight of the standard (mg l⁻¹)

est_{787} = average absorption of the standard

C_{787} = blank absorption

V_{st} = volume filtered for staining (L) (Passow and Alldredge 1995b)

TEP concentration in natural water samples were converted to xanthan gum equivalents using the formula:

$$C_{TEP} = (E_{787} - C_{787}) \times V_f \times f_x$$

where: E_{787} = the absorption of the sample

C_{787} = absorption of the blank

V_f = volume filtered (L)

f_x = the calibration factor (mg) (Passow and Alldredge 1995b).

3.2.5 Nutrient and DOC processing and analysis

At the start and end of the experiment, 50-ml subsamples were taken from each treatment bottle for analysis of dissolved reactive phosphorus (DRP), nitrate nitrogen (NO_3-N), dissolved organic carbon (DOC) and total dissolved nitrogen and phosphorus. Samples were stored in acid-washed 50-ml polyethylene tubes pre-rinsed with ultra-pure Milli-Q water. Dissolved inorganic nutrient samples were analysed on a Skalar Auto-analyser (Skalar, Breda, the Netherlands) using standard colorimetric methods. To minimise sample contamination, all filtration equipment and plastic ware were acid-washed and rinsed with Milli-Q water prior to sampling, and Milli-Q water was filtered alongside water samples to create laboratory blanks. Test tubes containing Milli-Q water were interspersed with experimental samples and blanks for quality control purposes and to ensure carryover between samples was negligible. Randomly chosen samples were re-run to account for drift in the instrument. Blank values were subtracted from experimental samples before analysis.

Immediately after filtration, DOC samples were wrapped in aluminum foil to prevent photo-degradation, stored at 4°C and analysed within one week of collection (Dafner and Wangersky 2002). Unfortunately, DOC samples were not acidified prior to storage, and bacteria capable of passing through the GF/F filters may have consumed

or altered some of the DOC. Respiration could have led to a decrease in DOC, although it is unlikely DOC concentrations decreased substantially during the 7-day storage period. While bacterial respiration (BR) is stimulated at higher temperatures $(20 - 25°C)$ (Berggren *et al.* 2010), respiration rates are low at colder temperatures $(0 - 5°C)$. Studies of bacterial respiration rates at 5°C report carbon losses ranging from <0.1 to 0.5 mg C l^{-1} wk $^{-1}$ (Roland and Cole 1999, Apple *et al*. 2006, Berggren *et al*. 2010). These changes in DOC concentration fall within variations that occurred in laboratory blanks (0.10 - 0.35 mg C l^{-1}) and internal standards (0.17 – 0.47 mg C l^{-1}) when running the TOC analyser.

DOC was measured on a Shimadzu Total Carbon Analyser TOC-V CSH (Shimadzu, Kyoto, Japan) using potassium hydrogen phthalate as a standard. Samples were purged with ultra-pure oxygen to remove DIC (dissolved inorganic carbon) and four injections were run for each sample. The three closest concentrations averaged to give the DOC value.

3.2.6 Phytoplankton identification and enumeration
Phytoplankton enumeration and the identification of dominant algae were carried out using samples from the February 2013 and May 2013 experiments. Fifty- to 100-ml volumes of water were taken from each bottle at the start and end of the experiment, preserved with Lugol's iodine (5% v v^{-1}), and stored in clean, opaque polyethylene bottles until analysed. Samples were gently inverted to ensure adequate mixing before being poured into clean, dry settlement chambers. Depending on the productivity in the treatment, 25-ml or 50-ml settlement chambers were used. Chambers were covered on a stable, horizontal, level, platform, and algae were allowed to settle for 24 to 48 hours depending on the height of the settlement chamber.

Algal counts and phytoplankton identification to genus level were determined using an inverted microscope at either 400x to 1000x magnification using methods described by Wetzel and Likens (Wetzel and Likens 2001). A Whipple grid ocular was calibrated at both 400x and 1000x magnification using a stage micrometer. To enumerate phytoplankton within the sample, the Whipple grid was moved across the diameter of the chamber bottom in several passes. At each stop, all cells within the grid were counted before the grid was blindly moved to the next site. Enumeration

continued until at least 300 cells had been counted or more than 25 grids had been counted. Total cell counts in each sample were calculated as:

$$Total\ cells\ per\ ml = \frac{c * CA}{n * W * V}$$

Where c = the total number of cells counted

CA = the area of the settlement chamber (mm^{-2})

n = the number of Whipple grids counted

W = the size of the Whipple grid (mm^{-2})

V = the volume of the sample (ml).

For each sample, the total number of *Lindavia* cells were tallied by systematically counting cells along transects until the entire bottom of the sediment chamber had been covered.

3.2.7 Statistical analysis

All statistical analyses were carried out using SPSS (v. 21.1, IBM) software. TEP generation between treatment groups was compared using One-way ANOVA, or Kruskal-Wallis tests if assumptions of normality and homoscedasticity were not met. During each experimental run, initial and end nutrient concentrations were compared with the amount of TEP generated in each treatment bottle. NO_3-N concentrations were log_{10}-transformed prior to analysis. Pearson Correlations and linear regressions were used to compare TEP formation with concentrations of TEP, DOC, NO_3-N, DRP, chl *a* and pH in the 8-day and 12-day experiments. Significant differences in phytoplankton abundance and taxa were compared with TEP concentration using Pearson Correlations. In all analyses, statistical significance was accepted if $p \leq 0.05$.

3.3 Results

3.3.1 The influence of catchment-derived nutrients and DOC on TEP generation

The 8-day experiment was run four times from November 2011 to March 2012. Initial DOC ($F_{(3, 10)}$ = 16.676, p < 0.001) and N ($F_{(3, 10)}$ = 43.553, p < 0.001) concentrations were significantly higher in the Pasture and Pasture+Urban treatments than in the Lake Control, while initial P concentrations did not differ significantly

between treatments. Changes in dissolved oxygen (DO) never exceeded 1.1 mg l^{-1} during any run of the experiment.

While the amount of TEP generated differed between treatments, this difference was not statistically significant in November 2011, December 2011 and March 2012. The substantial within-treatment variability in TEP concentration on these dates may explain the lack of significant differences between the treatments. In February 2012, significantly more TEP was generated in the Tussock treatment than in the Pasture and Pasture+Urban treatments (χ^2 = 3.857, p = 0.050) (Table 8). However, the Tussock treatment did not generate significantly more TEP than the Lake Control.

Table 8: The amount of transparent exopolymer particles (TEP) (in xanthan gum equivalents ± 1 standard error) generated in each treatment group during four, 8-day experimental runs and 2, 12-day experimental runs. Treatments were mixtures of 50-μm-filtered Lake Wanaka water and 0.22-μm-filtered stream water exudate from streams draining pastoral land cover (Pasture), pastoral and urban cover (Pasture + Urban) and tussock land cover (Tussock). The control was a mixture of 50-μm-filtered Lake Wanaka water and 0.22-μm-filtered Lake Wanaka water. Significant difference between treatments are highlighted in bold.

	Control	Pasture	Pasture+Urban	Tussock
8-day experiments				
November 2011	1.19 ± 0.63	0.82 ± 0.17	0.95 ± 0.25	1.43 ± 0.49
December 2011	0.57 ± 0.12	0.47 ± 0.12	0.29 ± 0.11	1.16 ± 0.37
February 2012	0.19 ± 0.05	**0.16 ± 0.02**	**0.03 ± 0.03**	**0.95 ± 0.68**
March 2012	0.06 ± 0.03	*	*	0.15 ± 0.10
12-day experiments				
November 2012	0.14 ± 0.05	0.91 ± 0.21	0.25 ± 0.03	0.24 ± 0.10
February 2013	0.56 ± 0.13	-1.23 ± 0.26[†]	-1.58 ± 0.85[†]	3.14 ± 0.79

Only two treatments were run in March 2012. * indicates treatments that were not run in the March 2012 experiment.
[†] Initial concentrations of TEP were extremely high in Bullock Creek and Alpha Burn water, resulting in negative TEP values by day 8 in the Pasture+Urban and Pasture treatments.

TEP generation was not associated with initial DOC or nutrient concentrations during any runs of the 8-day experiment. TEP generation was also not significantly associated with ΔDO, or with nutrient or DOC uptake, during any of the 8-day experimental runs.

3.3.2 Nutrient enrichment and TEP
 The 12-day experiment was run in November 2012 and February 2013. In addition to the original mixture treatments, NO_3-N and PO_4-P were added at saturating concentrations (final concentration: 1200 μgN l^{-1} and 140 μgP l^{-1}) to parallel treatments, in order to stimulate an algal bloom. Transparent exopolymer particle

concentrations were significantly higher in nutrient enriched treatments compared with unamended mixture treatments in both November (χ^2 = 26.289, p < 0.001) and February (χ^2 = 14.17, p < 0.001). TEP generation increased steadily in both the mixture treatments and nutrient-enriched treatments in November (Figure 8 A and B), with aggregates visible in nutrient-enriched treatments by day 6. TEP concentrations were 2.8 to 9.3 times higher in nutrient-enriched treatments than the mixture treatments by day 12 (Figure 8).

In February, stream water used for two of the treatments (Pasture and Pasture+Urban) had very high initial TEP concentrations (2.71 and 5.64 gum xanthan equivalents, respectively). TEP concentrations decreased by day 4 in these treatments (1.31 and 3.13 gum xanthan equivalents, respectively), and then increased toward the end of the experiment to 8.30 and 11.63 gum xanthan equivalents (Figure 8D). TEP concentrations were 1.7 to almost 4 times higher, and chl *a* concentration was 7.5 to 34 times higher, in the nutrient enriched treatments than in the mixture treatments by day 12.

3.3.2.1. TEP generation in mixture treatments

Table 9: Between-treatment comparisons of nutrient uptake, or transparent exopolymer particle generation (TEP) during the 12-day experiments. K-W = Kruskal Wallis test, d.f. = degrees of freedom, Mixture = treatments amended with stream water only, Enriched = treatments amended with stream water + saturating concentrations of N and P (1200 μg N l^{-1} and 140 μg P l^{-1}), NO_3 = nitrate-nitrogen, DRP = dissolved reactive phosphorus.

Month	Experiment	Test	Variable	d.f.	F	χ^2	Sig
November		K-W	TEP			8.128	0.043
	Mixture	ANOVA	NO_3	3, 8	192.202		<0.001
		ANOVA	DRP	3, 7	29.043		<0.001
		K-W	TEP			10.068	0.018
	Enriched	K-W	NO_3			8.980	0.030
		ANOVA	DRP	3, 8	49.275		<0.001
February		K-W	TEP			8.803	0.032
	Mixture	ANOVA	NO_3	3, 7	104.292		<0.001
		K-W	chl *a*			0.411	0.938
		K-W	TEP			10.017	0.017
	Enriched	ANOVA	NO_3	3, 8	8.368		0.008
		ANOVA	DRP	3, 8	28.295		<0.001
		ANOVA	chl *a*	3, 8	20.796		<0.001

As in the 8-day experiments, there was no clear pattern between initial nutrient concentrations, nutrient uptake and TEP generation in the mixture treatments during the 12-day experiments. Transparent exopolymer particle generation was highest in the Pasture treatment in November and in the Tussock treatment in February (Table

9). In November, more NO_3-N was also taken up in the Pasture treatment than in the other treatments, and more DRP was taken up in all of the treatments than in the Lake Control (Table 9).

In February, TEP generation was not significantly associated with initial N or P concentrations or nutrient uptake, possibly because initial concentrations of TEP were extremely high in treatments containing stream water from the Pasture (Alpha Burn) and Pasture + Urban (Bullock Creek) catchments (Figure 8C). Algal biomass (measured using chl *a*) was also not significantly associated with nutrient uptake or TEP generation in the mixture treatments.

3.3.2.2. Nutrient-enriched treatments

TEP increased with decreasing DRP and NO_3-N concentrations in nutrient-enriched treatments in November (Table 10). In February, significantly more DRP was taken up in all of the treatment groups compared with the Lake Control (Table 9), but TEP generation was not significantly associated with initial nutrient concentrations or nutrient uptake (Table 10).

Table 10: Correlation matrix of transparent exopolymer particle (TEP) formation and chl *a* over 12 days compared with the change in nutrient concentrations in experiment bottles. Experiment bottles contained mixtures of 50 µm-filtered Lake Wanaka water and 0.22 µm-filtered stream exudate from streams draining pastoral land cover (Pasture), pastoral and urban cover (Pasture + Urban) and tussock land cover (Tussock). Nutrient-enriched experiment bottles, which were run in parallel to the original mixture treatments, also contained saturating concentrations of N and P (1200 µg N l^{-1} and 140 µg P l^{-1}). Italicised numbers indicate p < 0.05.

		Δ TEP	Δ NO_3	Δ DRP	Δ chl *a*
	Δ TEP	1	*-0.544*	-0.440	0.733
November: ambient	Δ NO_3		1	0.034	0.471
	Δ DRP			1	-0.467
	Δ TEP	1	*-0.533*	*-0.685*	0.578
November: enriched	Δ NO_3		1	*0.885*	-0.704
	Δ DRP			1	*-0.777*
	Δ TEP	1	-0.138	*	*0.458*
February: ambient	Δ NO_3		1	*	-0.103
	Δ DRP			1	*
	Δ TEP	1	-0.141	-0.242	0.200
February: enriched	Δ NO_3		1	*0.925*	*-0.830*
	Δ DRP			1	*-0.932*

Chlorophyll *a* was not significantly associated with TEP generation in nutrient-enriched treatments in either November or February (Table 10). However, chl *a* concentrations were significantly higher in all nutrient-enriched treatments than in the original treatments and nutrient-enriched control ($F_{(7,15)}$ = 52.494, $p < 0.001$).
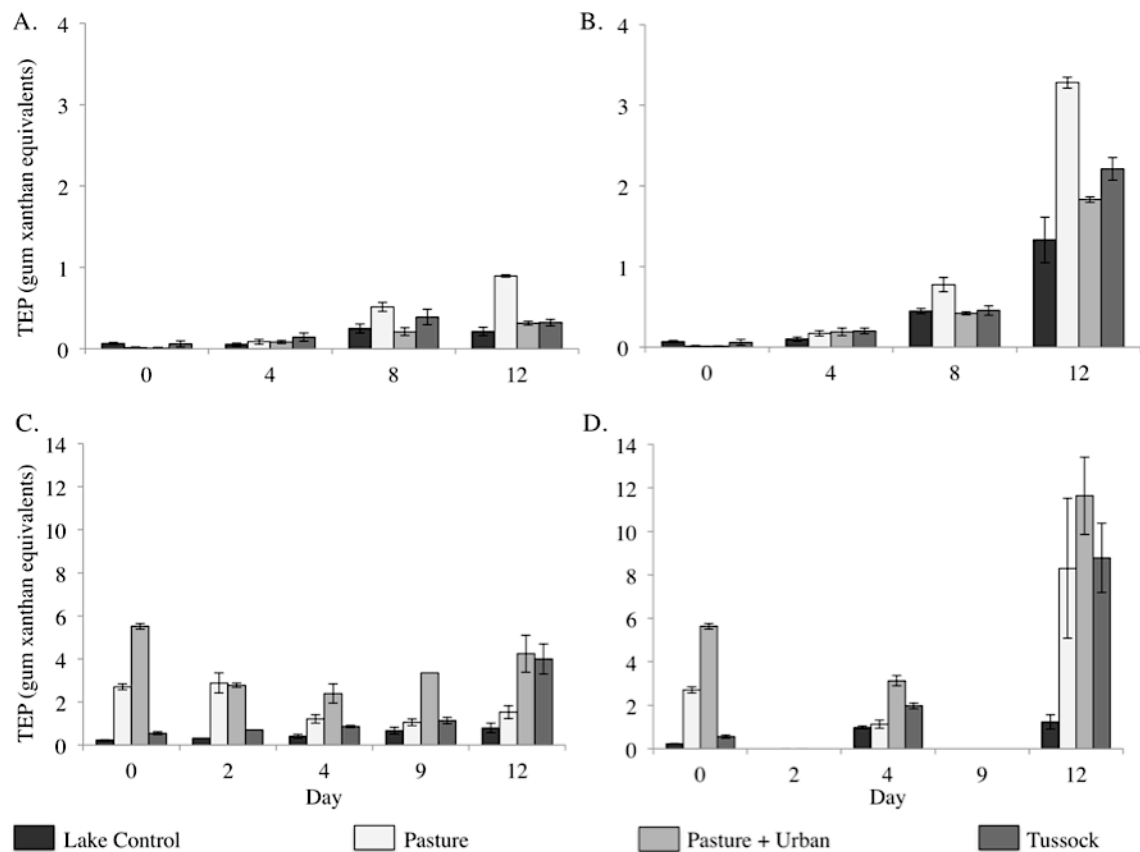
Figure 8: Concentration of transparent exopolymer particles (TEP) in A. mixture treatments and B. nutrient-enriched treatments from the November 2012 12-day experiment, C. mixture and D. nutrient-enriched treatments from the February 2013 12-day experiment. Thin vertical lines indicate ± 1 standard error.

In February 2013, nutrient enrichment significantly increased micro- and nano-phytoplankton abundance compared with original mixture treatments (Appendix B, Table B2). However, TEP concentration was not significantly associated with any algal class or genera in either the amended or original treatments, and phytoplankton community composition changed in all treatments during the experiment (Table 11). At the start of the experiment, diatoms made up approximately 11% of counted phytoplankton cells. By the end of the experiment, diatoms made up 1 to 67% of counted phytoplankton cells, while green algae, commonly *Ankistrodesmus,* (*Pseudo)sphaerocystis*, *Tetraspora*, *Botryococcus*, *Dictosphaerium*, and (possibly) *Stichococcus,* made up 8 to 88% of cells (Table 11; Figure 9).
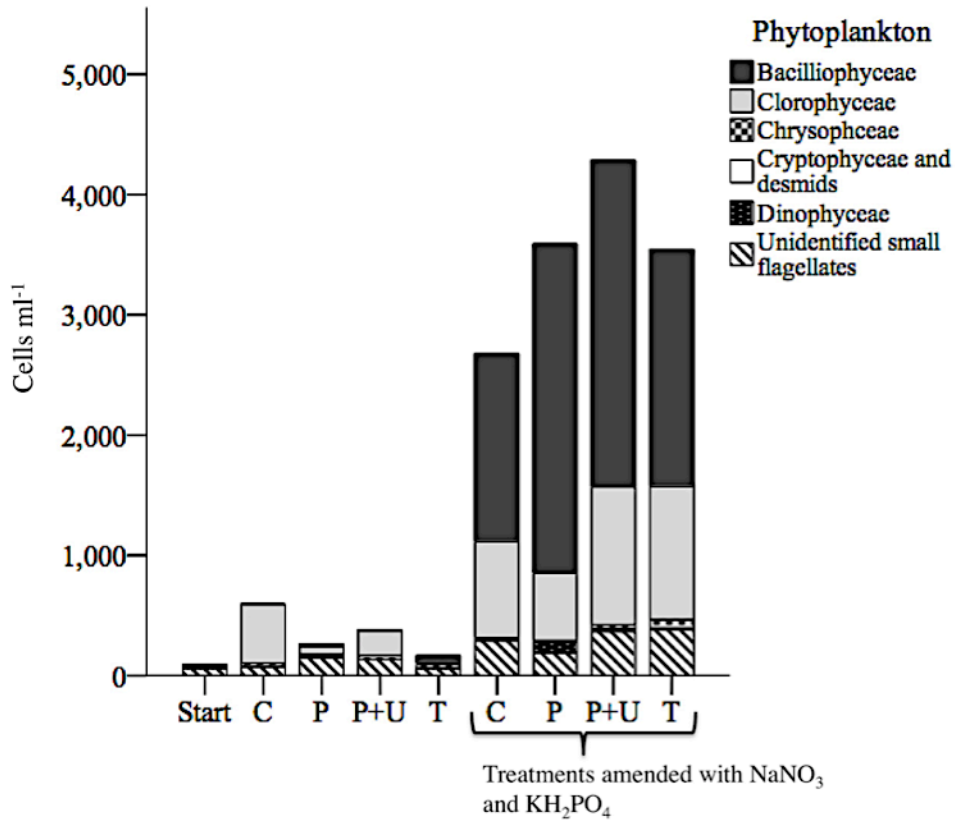
Figure 9: Composition and abundance of dominant phytoplankton taxa by treatment in the February 2013 experiment. C = lake water control; P = stream water from pasture-dominated catchment; P+U = stream water from Pasture + Urban-dominated catchment; T = stream water from Tussock-dominated catchment.

Table 11: Mean abundance of dominant micro- and nano-phytoplankton taxa (± 1 standard error) in the lake water at the start of the February 2013 experiment, and in each treatment at the end of experiment. ΔTEP = the amount of transparent exopolymer particles (TEP) generated over the course of the experiment. (P) Pasture, (P+U) Pasture+Urban, (T) Tussock. (*) highlights negative values, which were a result of very high initial TEP concentrations in these two treatments.

Treatment	Cells (ml^{-1})	Diatoms (ml^{-1})	Diatoms (%)	*Lindavia* cells (ml^{-1})	*Nitzschia* cells (ml^{-1})	ΔTEP
Start	89.3 ± 19.2	11 ± 0.6	13.6	5.7 ± 1.4	0.2 ± 0.2	0.2 ± 0.04
Control	120, 1071	4.9, 12.9	4, 1.2	1.6, 0	1.6, 6.5	0.6 ± 0.2
P	317, 208	36, 8.2	11.6, 3.9	3.3, 5.5	23.3, 2.7	-1.2 ± 0.3*
P+U	378.4 ± 101	12.2 ± 5.4	3.7	3.0 ± 0.6	3.0 ± 1.6	-1.4 ± 0.9*
T	165.4 ± 39.3	58.3 ± 51.7	25.8	5.4 ± 1.5	48.5 ± 47.5	3.5 ± 0.7
Control + nutrients	2677 ± 643	1562 ± 478	56.6	21.2 ± 5.4	1478 ± 499	1.3 ± 0.3
P + nutrients	3590 ± 218	2739 ± 143	76.6	22.7 ± 3.5	2607 ± 139	2.3 ± 0.4
P+U + nutrients	4296 ± 272	2719 ± 423	62.6	8.0 ± 4.8	2459 ± 426	6.0 ± 1.8
T + nutrients	3544 ± 175	1971 ± 250	55.2	27.1 ± 4.6	1777 ± 208	8.2 ± 1.6
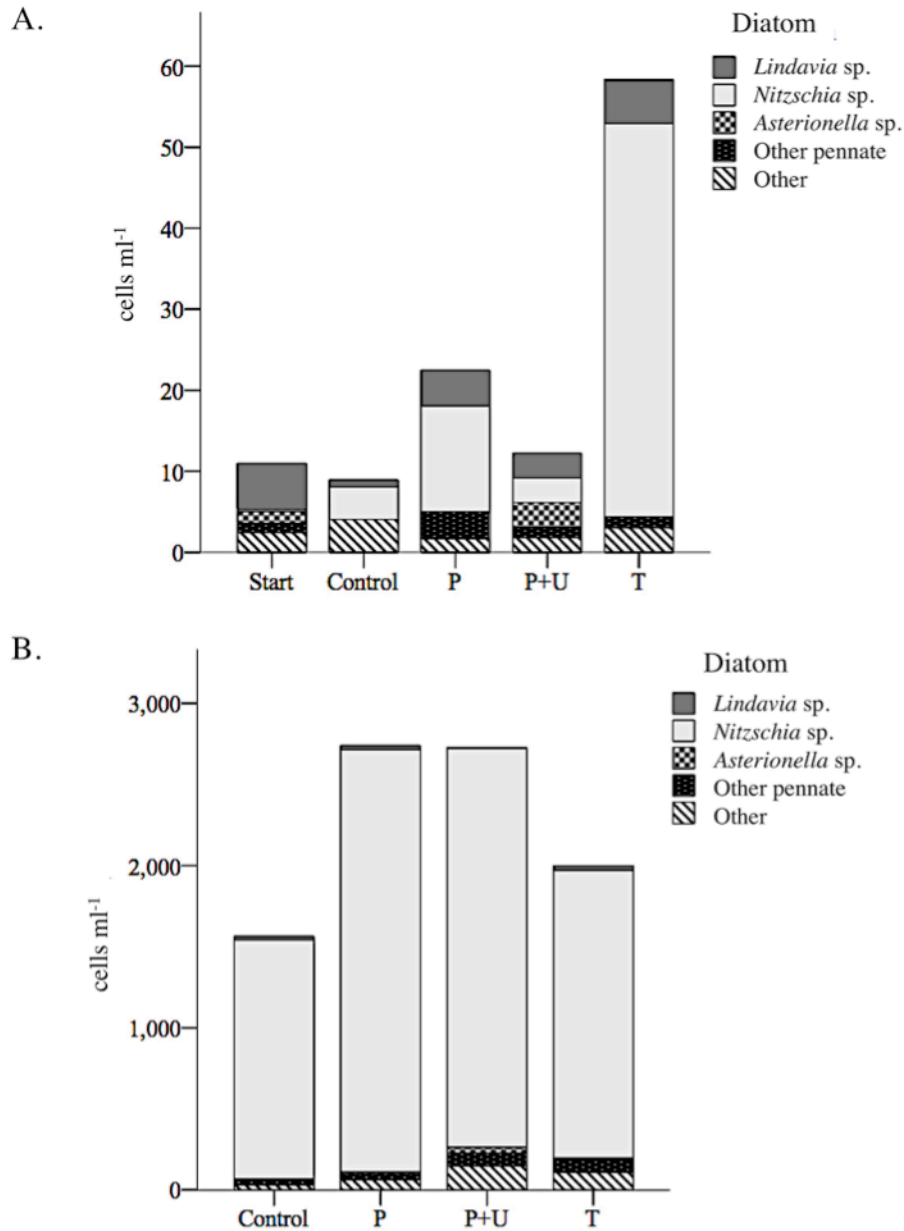
Figure 10: Diatom composition and abundance for each treatment by day 12 for A. original mixture treatments and B. nutrient-amended treatments in the February 2013 experiment. Control = lake water control; P = stream water from pasture-dominated catchment; P+U = stream water from Pasture + Urban-dominated catchment; T = stream water from Tussock-dominated catchment.

In nutrient-enriched treatments, diatoms made up a greater proportion of total counted cells (47 to almost 84%) (Table 11; Figure 9). *Nitzschia* sp. was the most prevalent diatom, making up 87-97% of diatoms (Figure 10) and 44-80% of total counted cells. Observational comparisons of live cells collected from treatments indicated that TEP

was associated with algal surface coatings on individual *Lindavia* cells and colonial green algae, as well as with aggregated material (Figure 11, Figure 12).
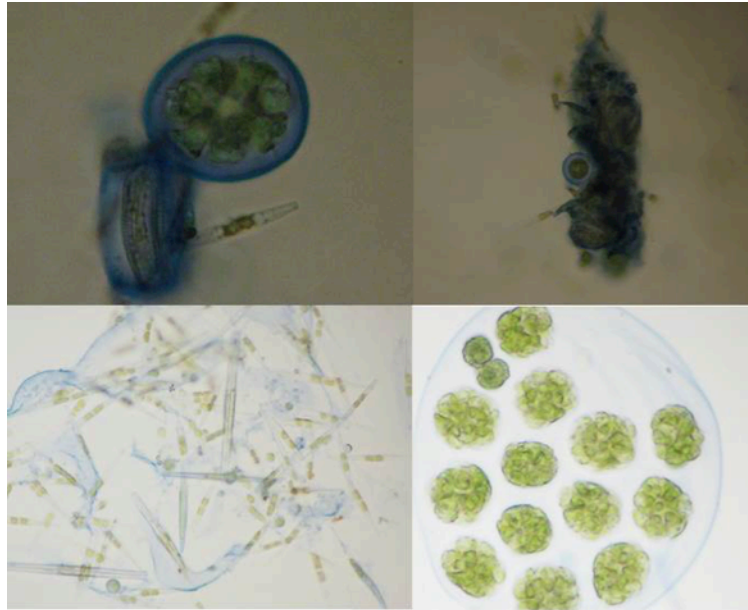
Figure 11: (clockwise from top left) Two *Lindavia* diatoms from different angles, plus *Nitzschia* (diatom) and colonial green algae associated with surface-coatings of TEP; aggregated material with *Lindavia* cell visible; colonial green alga (possibly, *Gonium*) with colourless envelope; loosely aggregated material dominated by *Nitzschia* cells.
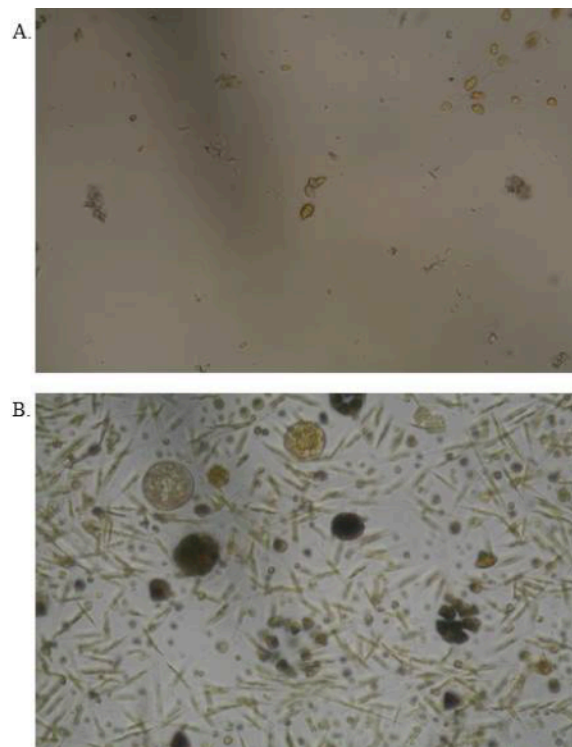
Figure 12: Phytoplankton biomass in A. initial concentrations in each mixture and B. end concentrations (after 12 days) in a nutrient-spiked mixture of Lake Wanaka and Alpha Burn water in February 2013.
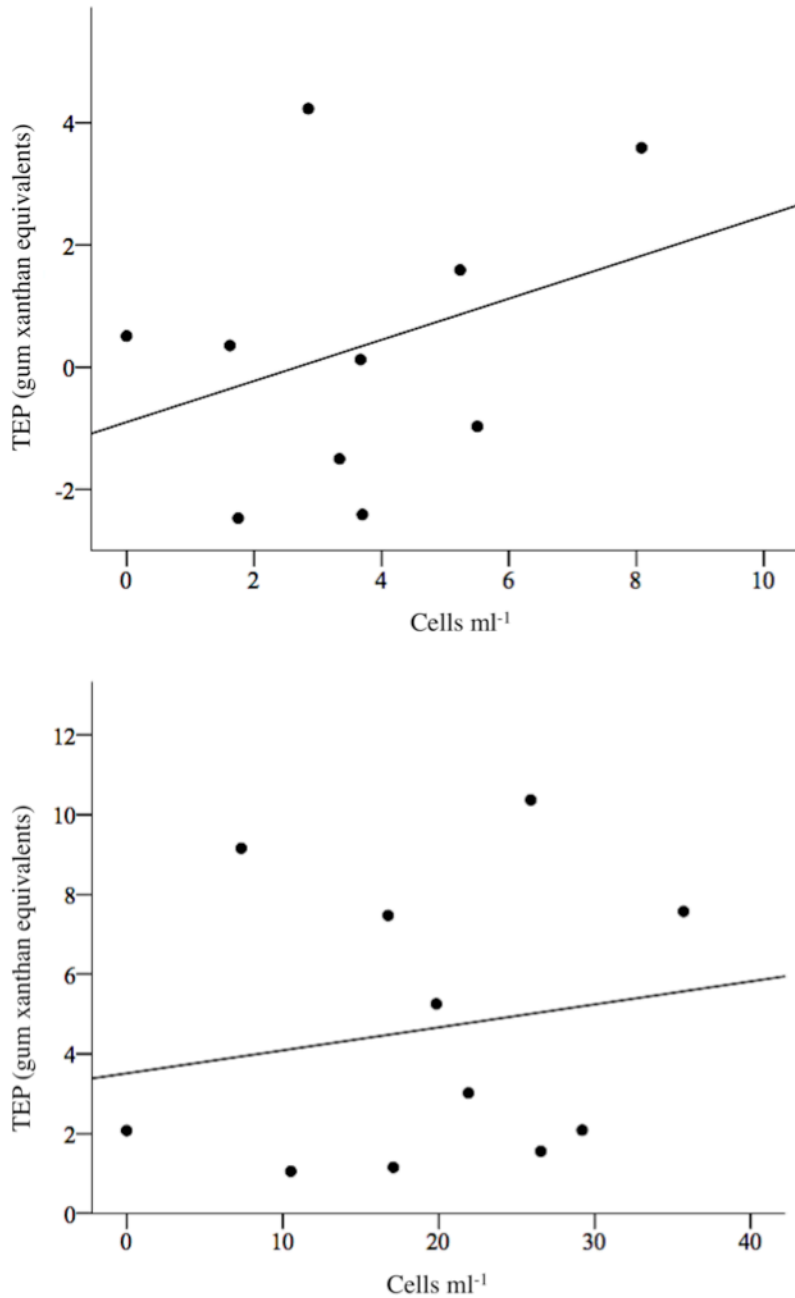
Figure 13: Scatterplot of TEP to *Lindavia* cell numbers (ml⁻¹) in (top graph) mixture and (bottom graph) nutrient-enriched treatments from the February 2013 12-day experiment.

3.3.3 TEP formation and *Lindavia* abundance

Transparent exopolymer particle concentrations were not positively associated with the presence or abundance of *Lindavia* sp (Figure 13). In February 2013, the number of *Lindavia* cells remained fairly constant in the mixture treatments. *Lindavia* numbers increased in nutrient-enriched treatments (Figure 10), but the proportion of *Lindavia* cells to total counted cells remained the same or decreased in almost all BOD bottles. At the start of the February 2013 experiment, *Lindavia* sp. made up 35.7 to 75% of total diatom cells in the different treatments (Figure 10). By the end

of the experiment, this genus made up 0.4-2.4% of total diatom cells in nutrient-amended treatments, and 5 to 86% of diatom cells in the original mixture bottles.

An inter-lake comparison of TEP generation was run in May 2013, to ensure TEP formation in the previous experiments was not simply a result of laboratory conditions. TEP formation was examined using water from Lake Wanaka and two lakes that do not experience 'lake snow' (Lake Hawea and Lake Wakatipu), and in which *Lindavia* is not present in significant numbers. Only lake water was used in this experiment; no stream exudates were added to any treatment. Parallel nutrient-amended treatments were run for each lake.

Over the course of the May 2013 experiment, phytoplankton composition changed in each treatment (Figure 14; Table 12). Significantly greater numbers of *Lindavia* and *Nitzschia* were present in the nutrient-enriched Lake Wanaka treatment than the Lake Wakatipu and Lake Hawea treatments (*Lindavia* $\chi^2 = 6.72$, $p = 0.035$, df = 2; *Nitzschia* $\chi^2 = 7.20$, $p = 0.027$, df =2) (Figure 14). The only significant increase in TEP ($\mu = 0.24$ gum xanthan equivalents) concentration occurred in the ambient Lake Wanaka treatment ($F_{(4, 10)} = 62.686$, $p < 0.001$). TEP concentrations in all other treatments were less than 0.05 xanthan gum equivalents.
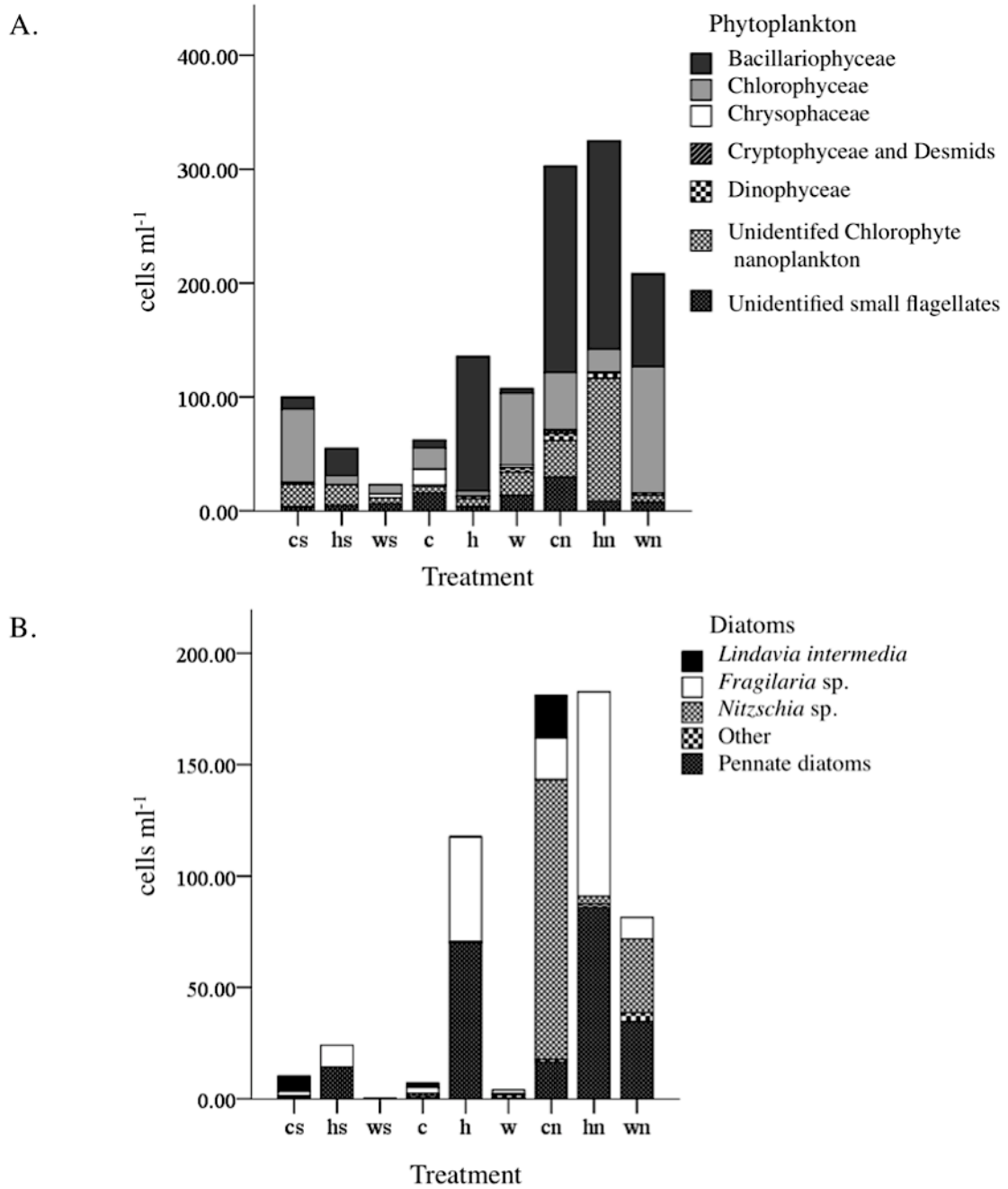
Figure 14: Composition and abundance by day 12 of (A) dominant phytoplankton taxa and (B) diatoms in c = Lake Wanaka (Control); h = Lake Hawea and w = Lake Wakatipu. cs = Lake Wanaka Start; hs = Lake Hawea Start, ws = Lake Wakatipu Start; cn = Lake Wanaka + nutrients (19 μM NO_3-N and 1.4 μM PO_4-P); hn = Lake Hawea + nutrients and wn = Lake Wakatipu + nutrients.

Table 12: Inter-lake comparison of start (S) and end values of phytoplankton abundance, including dominant taxa ± 1 standard error. Green (ml^{-1}) = the abundance of identified Chlorophyte algae. CS = Lake Wanaka Start; HS = Lake Hawea Start, WS = Lake Wakatipu Start C = Lake Wanaka (Control); H = Lake Hawea and W = Lake Wakatipu; CN = Lake Wanaka + nutrients (1200 µgN l^{-1} and 140 µgP l^{-1}); HN = Lake Hawea + nutrients and WN = Lake Wakatipu + nutrients.

	Total cells (ml^{-1})	Diatoms (ml^{-1})	diatom (%)	*Lindavia* (ml^{-1})	*Nitzschia* (ml^{-1})	Other diatom (ml^{-1})	Green (ml^{-1})
CS	100 ± 26	10.7 ± 1.2	13 ± 4.5	6.9 ± 2.4	0	3.4 ± 0.2	64.5 ± 26
HS	55 ± 4.3	24 ± 0.1	44 ± 4	0	0	24 ± 0.1	8.0 ± 1.3
WS	23.5 ± 7.4	0.5 ± 0.3	3.6 ± 2.5	0	0	0.5 ± 0.3	8.2 ± 5.8
C	62.0 ± 4.6	7.2 ± 1.8	11 ± 2	2.4 ± 0.5	1.2 ± 0.7	3.6 ± 1.2	18.5 ± 0.8
H	135 ± 16	118 ± 14.5	87 ± 1.6	0	0	118 ± 14.5	4.6 ± 1.2
W	107 ± 28.5	4.8 ± 0.3	5.4 ± 1.7	0.9 ± 0.6	0.4 ± 0.3	2.6 ± 1.2	62.7 ± 32.5
CN	303 ± 37	181 ± 35	57 ± 4	19.4 ± 4.7	126 ± 23	36.2 ± 7.2	50.5 ± 1.6
HN	325 ± 65	183 ± 81	40 ± 15	0	3.8 ± 0.6	179 ± 81.5	20.5 ± 3.9
WN	209 ± 23	81.9 ± 4.6	41 ± 10	0.3 ± 0.2	33 ± 2.1	47.9 ± 6.0	111 ± 17.9

3.4 Discussion

In all of the experiments, the algal community structure changed markedly from that observed in Lake Wanaka. Thus, the experimental results may not reflect in lake TEP drivers and dynamics.

3.4.1 The influence of catchment-derived nutrient and DOC inputs on TEP generation

The results from my study do not support my first hypothesis that catchment-derived nutrients and DOC would increase TEP generation in Lake Wanaka water. In the 8-day experiments, initial DOC and N concentrations were significantly higher in two of the treatment groups compared with the Lake Control (Table 6), but these two groups frequently had the lowest TEP concentrations (Table 8). None of the treatments had significantly higher concentrations of TEP than the Lake Control.

Other studies report a positive relationship between catchment-derived material and TEP production. In an estuarine system in Israel, anthropogenic effluents released into the water column promoted high phytoplankton biomass, resulting in 2 to 5-fold higher TEP concentrations than at oligotrophic sites further downstream (Bar-Zeev and Rahav 2015). In a study of 12 Swedish lakes, isotopic signatures and fluorescence index indicated settling organic aggregates were predominantly composed of allochthonous DOM (von Wachenfeldt and Tranvik 2008), and in a study of three arctic lakes, terrestrially-derived chromophoric dissolved organic carbon (cDOM) accounted for 53% of TEP-carbon (TEP-C) (Chateauvert *et al.* 2012).

In the latter study, both allochthonous DOM and TEP in lake water increased with increasing river discharge (Chateauvert *et al.* 2012).

The lack of a relationship between catchment-derived material and TEP in my study may reflect the study design (e.g. laboratory as opposed to field experiments; use bulk DOC concentrations instead of isotopes of cDOM). It may also reflect the hydrological condition of the streams at the time of sampling. Generally, water collection occurred at low to moderate flows, although samples were taken at high flows prior to the February 2013 experiment. On this date, DOC concentrations were elevated in the sampled streams (Appendix B, Table B3) compared with low flow conditions (Table 6), and initial TEP concentrations in the Pasture and Pasture+Urban treatments were very high. These high initial values may indicate TEP precursor material was present in the stream water (Figure 8C), but more work is required to substantiate a link between TEP, DOC and stream discharge in the Wanaka catchment.

The lack of a relationship between catchment-derived material and TEP may also reflect interactions between bacteria, DOC and TEP in the treatments. Bacteria have a complex, and occasionally contrary, relationship with TEP abundance. Not only can bacteria indirectly and directly contribute to TEP generation, but they also colonise and actively break down this substrate (Arnous *et al.* 2010). Under nutrient-limited conditions, bacteria can outcompete phytoplankton for nutrients (Thingstad *et al.* 1993, Mindl *et al.* 2005, Cunha and Almeida 2009) resulting in the release of TEP by phytoplankton. Bacteria can also actively produce TEP through the renewal of capsular material (Fazio *et al.* 1982, Ford *et al.* 1991, Passow 2002). This capsular material, composed of mucopolysaccharides (Ford *et al.* 1991), helps protect the cell from predators and toxins, regulates the transfer of ions at the cell surface, and helps concentrate nutrients (Fazio *et al.* 1982). Constant renewal of this capsular material results in release of older material into the water column, and natural bacterial assemblages are capable of generating between 195 to 377 µg xanthan gum equivalents a day (Passow 2002). In one study, bacterial carbon reportedly made up 3-10% of TEP-carbon (TEP-C) generated in lagoon water (Rochelle-Newell *et al.* 2010). As I did not measure bacterial abundance or activity, I cannot quantify the contribution of bacterially-produced carbon to total TEP-C in my treatments. Nor can

I determine whether the chemical composition of the DOC influenced bacterial activity and TEP production or uptake in the treatments. These parameters would be useful to include in future studies of organic aggregate formation in Lake Wanaka.

3.4.2 Nutrient enrichment and TEP formation

Algal blooms formed, and more TEP was generated, in nutrient-enriched treatments than in the mixture treatments. This supports my second hypothesis that nutrient enrichment would further increase TEP generation in lake and stream mixtures by stimulating algal growth. My data are consistent with previous studies linking nutrient availability, diatom blooms and TEP formation (Bodungen *et al.* 1986, Riebesell 1992, Kiørboe *et al.* 1994, Alldredge *et al.* 1995, Li and Logan 1995, Logan *et al.* 1995, Passow and Alldredge 1995a). For example, in a mesocosm study by Alldredge et al. (1995), high initial concentrations of NO_3-N (46 µM) silicate (SiO_4-Si) (45 µM) and phosphate (PO_4-P) (3 µM) stimulated a dense phytoplankton bloom dominated by diatoms, which was associated with an increase in TEP (Passow and Alldredge 1995a). In a separate study, Alldredge et al. (1995) reported that formation of large organic aggregates was related to increases in chl *a,* and that chain-forming diatoms such as *Chaetoceros*, *Thalassiosera*, *Leptocylindrus* and *Nitzschia* dominated phytoplankton biomass. Studies of TEP formation in Lake Constance have reported an association between pennate diatoms and surface films glued together by TEP (Grossart *et al.* 1997).

High initial TEP concentrations in the Pasture and Pasture+Urban treatments in the February experiment meant TEP generation was not significantly associated with initial nutrient concentration or nutrient uptake. However, a significant association between DRP uptake and TEP generation was apparent in the November 2012 nutrient-enriched treatments. While the results of my experiments cannot be extrapolated directly to Lake Wanaka, they indicate that increased nutrient availability (particularly P) in the lake could stimulate algal growth and promote production of 'lake snow' in Lake Wanaka.

3.4.3 TEP generation and *Lindavia*

My third hypothesis, TEP concentration will increase with increasing abundance of *Lindavia* sp., was not supported by the February 2013 or May 2013 experiments. In February, *Lindavia* abundance was not significantly associated with the amount of

TEP generated in any of the ambient or nutrient-enriched treatments. In May, a significant increase in TEP only occurred in the ambient Lake Wanaka treatment, while *Lindavia* abundance increased significantly in the nutrient-enriched Lake Wanaka treatment.

The lack of a significant relationship between TEP formation and *Lindavia* abundance may indicate that laboratory conditions were not optimal for *Lindavia intermedia* growth. *Lindavia intermedia* is a dominant phytoplankter in warm epilimnetic waters during the mid- to late summer (Interlandi and Kilham 1999). However, in Lake Wanaka, *L. intermedia* abundance is not positively associated with increased light intensity (Bayer 2013), and low light levels and low water temperature (4°C) have been used to successfully culture this phytoplankter in the lab (Theriot, personal communication to C. Burns). Although temperature was controlled to reflect the average temperature of Lake Wanaka in my experiments, the (relatively) warm water temperature (12°C) and saturating light levels during the experiment may have inhibited *L. intermedia* cell growth and extracellular polysaccharide production.

Other factors, including the removal of grazers during prefiltration, may also account for the lack of a relationship between *Lindavia* and TEP concentration. Grazers can influence phytoplankton composition by preferentially consuming certain phytoplankton (Sarnelle 2005). In Lake Wanaka, cladocerans like *Daphnia 'pulex'* are the dominant zooplankton throughout the year, and *Daphnia* preferentially consume small diatoms like *Nitzschia* and *Cyclotella* (Sarnelle 2005). *Nitzschia* is not a dominant phytoplankter in Lake Wanaka (Clayton and Coleman 1976, Naismith 1994, Bayer *et al.* 2015). As this diatom made up a significant proportion of total cell numbers in my treatments, the increase in *Nitzschia* cell numbers over *Lindavia* may indicate *Nitzschia* are better adapted to growth under laboratory conditions, or that grazer exclusion allowed the *Nitzschia* population to grow rapidly.

3.5 Conclusions

Under laboratory conditions, nutrient enrichment (particularly P enrichment) of Lake Wanaka water increased chl *a* concentrations, nano- and micro-phytoplankton abundance, and TEP generation. However, TEP generation was not associated with any particular diatom species, nor was it associated with catchment-derived N, P or DOC. As the algal community changed markedly over the course of these

experiments, the changes reported here may not reflect in-lake TEP drivers and dynamics. More intensive field and laboratory work needs to be carried out to determine what is causing the formation of organic aggregates in Lake Wanaka, and the possible effects (especially in the sediments) of the sedimentation of this material.

4 Intermixing depth and influence of two large river plumes on Lake Wanaka

4.1 Introduction

The path a river plume takes on entering a lake is a function of the density difference between the inflowing water and the lake surface water (Pickrill and Irwin 1982). In temperate climates, river and lake temperatures vary seasonally, and many lakes undergo seasonal stratification, where warmer, lower density water lies on top of colder, higher density water. When the lake is thermally stratified, a cold, sediment laden river plume may plunge along the bottom, or interflow where the density of the river water matches the surrounding lake water (Pickrill and Irwin 1982, Marti *et al.* 2011). However, a warm plume could flow over the surface of the lake if the river water is warmer than that of the lake (Pickrill and Irwin 1982).

Understanding changes in river inflow is important, as a river plume can influence the distribution of suspended material, nutrients and organic matter in a lake, which can play an important role in regulating both heterotrophic and autotrophic production (Hecky *et al.* 2003, MacKenzie and Adamson 2004, Emmerton *et al.* 2008, Johengen *et al.* 2008). Nutrient and organic carbon inputs can stimulate phytoplankton and bacterioplankton activity, particularly in nearshore areas (Scavia and Fahnenstiel 1987, Lohrenz *et al.* 1990, Lohrenz *et al.* 1992). A turbid plume interflowing into the epilimnion can cause initial light limitation, reducing phytoplankton productivity (Lohrenz *et al.* 1990, Lohrenz *et al.* 1992, McCullough *et al.* 2007), while a plunging plume can deliver exogenous sediment, nutrients and dissolved oxygen to the bottom waters near the lake bed, affecting benthic productivity (Johengen *et al.* 2008) and nutrient cycling.

Anthropogenic activity can alter river discharge patterns, affecting the depth and direction of an inflowing river (Loizeau and Dominik 2000). For example, hydroelectric dams built along the Rhone River helped prevent flooding and decreased suspended solid loads reaching Lake Geneva. However, the subsequent decrease in river water density and flow rate restricted the number and intensity of oxygen-rich underflows, potentially exacerbating oxygen-deficits in the deeper waters of the Lake (Loizeau and Dominik 2000). As terrestrially-derived material (e.g. total suspended solids (TSS), nutrients, dissolved organic carbon (DOC) and dissolved

oxygen (DO)) can help support biological activity in a lake system, and as anthropogenic activity frequently enhances productivity (Galbraith and Burns 2007, Abell *et al.* 2011b), it is important to understand the loads of terrestrially-derived materials to a lake system and where the loads are directed within the lake in order to better understand impacts of land use activities on lakes.

In Central Otago, New Zealand, Lake Wanaka is an important tourist and fishing destination known for its high water quality, ecology and scenic value. Recent changes to phytoplankton community structure (Bayer 2013) and the fouling of fishing lines and water intake filters by phytoplankton-derived organic aggregates have raised concerns about whether the lake is in a steady state. Urban, residential and agricultural development has been steadily increasing in the area, and increasing trends in chl *a* and TN concentration have been noted in bays close to developed areas (Otago Regional Council 2009). These trends are not statistically significant, although this may in part be due to the dilution potential of a lake as large as Lake Wanaka.

Lake Wanaka has two main inflowing tributaries: the Matukituki River and the Makarora River. The Matukituki River flows in from the west while the Makarora River flows in from the north. Both drain glaciated terrain, national parkland and low-intensity farmland. Currently, we do not know what impact (if any) the influx of terrestrially-derived material from these two tributaries may be having on Lake Wanaka.

The **aim of my study was to understand how catchment-derived materials are delivered to Lake Wanaka under stratified and un-stratified conditions, and what effect this material has on phytoplankton biomass in the lake (as measured by chl *a*)**. The glacial origin of both the Matukituki and Makarora Rivers suggests the river water will likely plunge and either inflow along the bottom of the lake or interflow as a density current. As plunging plume could provide nutrients to the metalimnion and hypolimnion, which in turn could stimulate phytoplankton growth in the mixed layer, I hypothesised that:

Hypothesis: Chlorophyll *a* concentration in Lake Wanaka will increase in the presence of the Matukituki and Makarora River plumes.

4.2 Materials and methods

Lake Wanaka is located in Central Otago along the eastern border of the Southern Alps in New Zealand. It is a large glacially formed lake covering approximately 180 km^2, whose deepest basin exceeds 300 m. The lake lies on a north-northeast axis and is long, narrow (maximum length of 45.5 km; width of 11.6 km) and steeply-sided (Irwin 1980). The lake drains to the southeast via the Clutha River.

The Matukituki River (catchment area: 799 km^2) is the major inflowing tributary to the lake from the west, originating at the Main Divide of the Southern Alps and flowing for roughly 50 km before discharging into the lake via a braided gravel-bed delta in West Wanaka Bay. The Makarora River (catchment area: 710 km^2) is the main inflowing tributary from the north, and flows for approximately 35 km before discharging via a braided gravel-bed delta into the northern end of the lake. Both river deltas are changeable. During the course of this study, the middle reaches of the Matukituki River mouth were occasionally blocked by shoals and/or sandbars, which disappear after high flow events. The mountainous terrain within these catchments restricts most agricultural development to the lower slopes and alluvial plains, with agricultural development consisting primarily of low-intensity farming of sheep, cattle and deer (Rosen and Jones 1998).

4.2.1 Sampling design

Plumes generated by the Matukituki and Makarora Rivers were monitored and sampled as they entered Lake Wanaka. The Matukituki River plume was sampled 10 times between 2009 and 2012. The Makarora River plume was sampled three times between 2009 and 2010. On each occasion, a series of stations extended from entrance mixing sites towards the open water of the lake. The entrance-mixing site was defined as the first sampling station on the transect leading from the river to the open water of the lake. The entrance mixing site was frequently 5-10 m from the river mouth. It is also referred to as the site of initial turbulent mixing. 'Open water' was defined as deep-water stations, far from the shore, where the influence of the river plume was not normally apparent. Two sites, the Aspiring Basin site for the Matukituki River plume (220 m deep, 5 km from river mouth) and S4 for the Makarora River plume (100 m deep, 2.3 km from river mouth) were sampled to represented 'open water' in the lake.
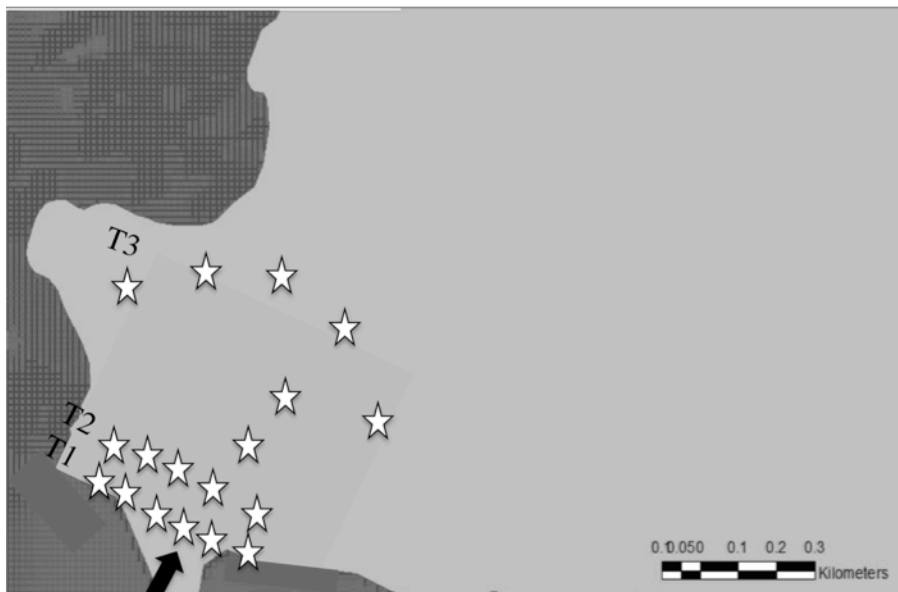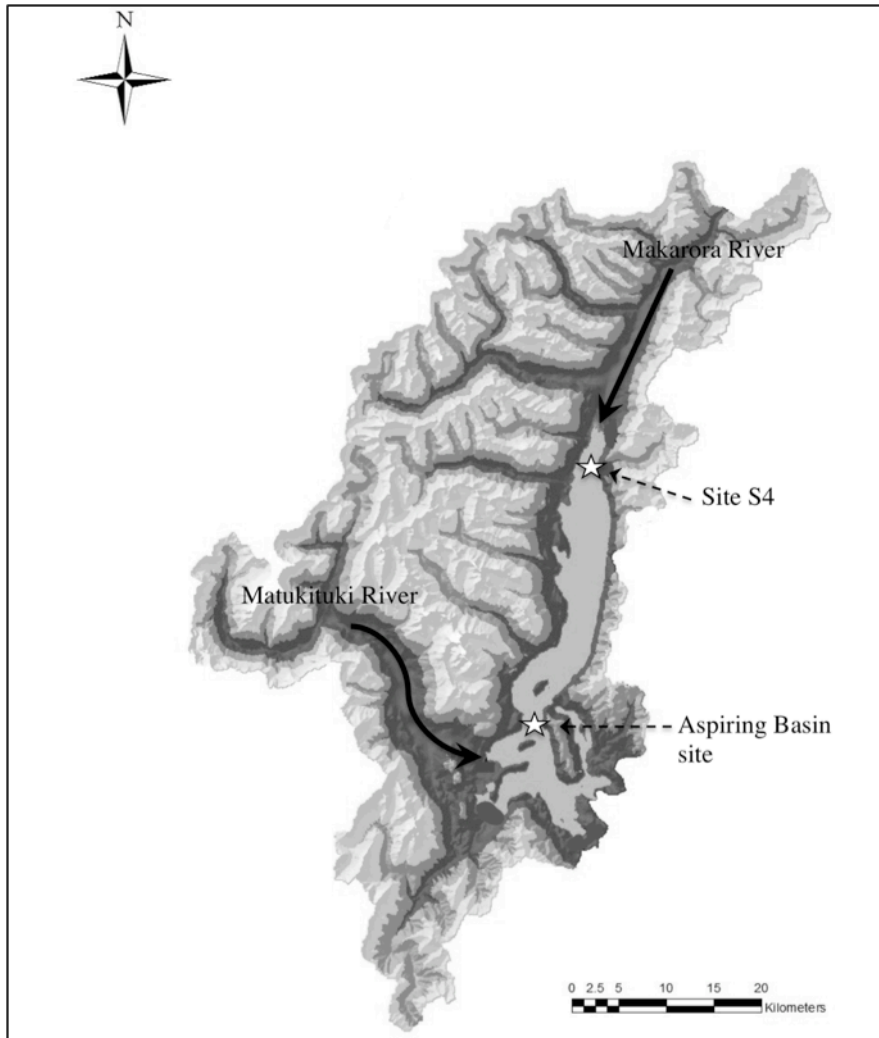
Figure 15: *Top map*: the Lake Wanaka catchment. Black solid arrows indicate the Matukituki and Makarora Rivers. The mid-basin sampling sites (Aspiring Basin and S4) are labelled on the map and the location is marked by (☆). *Bottom map*: CTD cast sites (☆) and transects (numbered T1, T2 and T3) outside the Matukituki River mouth.

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Both the Matukituki and Makarora River plumes were sampled in spring (September, November) 2009 and autumn (March) 2010. Sampling occurred in the morning between 8:00 and 11:30 h. At each station, water samples were taken for nutrients and dissolved organic carbon (DOC) at depths of 2 m and 10 m, and also 20 m, 40 m, 80m and 100 m, when possible. Water samples were collected in triplicate from the river and directly outside the river mouth, while duplicate samples were taken at all other lake sites. Samples were collected and stored in clean, 1-litre acid-washed polyethylene containers. CTD (Conductivity, Temperature, Depth) casts were made using a Seabird SeaCAT 19s profiler CTD (Seabird Electronics, Washington, USA), and measured temperature, conductivity, beam attenuation (optical backscatter), fluorescence, density and depth. Casting sites were determined using a global positioning (GPS) unit to an accuracy of $\pm$ 5 m. Temperature and suspended solid concentrations were used as tracers of the river plume. River measurements of temperature, oxygen concentration, conductivity and salinity were made using a YSI 6000 rapid-pulse environmental monitoring system (YSI Incorporated, Yellow Springs OH, USA). Hourly discharge rates for the Matukituki River were obtained from the National Institute of Water and Atmospheric Research (NIWA). Discharge rates were not available for the Makarora River.

Total nitrogen (TN) and total phosphorus (TP) samples were frozen prior to analysis. Upon thawing, these samples were digested using potassium peroxidisulphate, boric acid and sodium hydroxide and autoclaved 30 minutes prior to analysis. Samples were then processed on a Skalar Auto-analyser (Skalar, Breda, the Netherlands) using standard colorimetric methods. To minimise sample contamination, all filtration equipment and plastic ware were acid-washed and rinsed with Milli-Q water. Field blanks were interspersed with field samples for quality control purposes and to ensure carryover between samples was negligible. Randomly chosen samples were re-run to account for drift in the instrument. Blank values were subtracted from treatment samples before analysis.

Water samples analysed for DOC were kept at 4°C and in the dark until 250 – 500 ml were filtered through acid-washed pre-combusted Whatman GF/F filters (0.7 μm nominal pore size glass fiber) under low vacuum pressure (< 100 mmHg), within 8 hours of sample collection. For quality control purposes, field blanks were filtered alongside field samples using Milli-Q water held in a pre-sterilised polyethylene

container. Filtered DOC samples were wrapped in aluminium foil to prevent photodegradation and stored at 4°C until analysed within one week of collection. Dissolved organic carbon (DOC) samples were processed on a Shimadzu Total Carbon Analyser TOC-V CSH (Shimadzu, Kyoto, Japan) using potassium hydrogen phthalate as a standard. Samples were purged with ultra-pure oxygen to remove dissolved inorganic carbon (DIC) and four injections were run for each sample, with the three closest concentrations averaged to give the DOC value.

Unfortunately, DOC samples were not acidified prior to storage, and bacteria capable of passing through the GF/F filters may have consumed or altered some of the DOC. Respiration could have led to a decrease in DOC, but it is unlikely DOC concentrations decreased substantially during the 7-day storage period. While bacterial respiration (BR) is stimulated at higher temperatures (20 – 25°C) (Berggren et al. 2010), respiration rates are low at colder temperatures (0 – 5°C). Studies of bacterial respiration rates at 5°C report carbon losses ranging from < 0.1 to 0.5 mg C l^{-1} wk $^{-1}$ (Roland and Cole 1999, Apple et al. 2006, Berggren et al. 2010). These changes in DOC concentration fall within variations that occurred in laboratory blanks (0.10 - 0.35 mg C l^{-1}) and internal standards (0.17 – 0.47 mg C l^{-1}) when running the TOC analyser.

Table 13: Comparison of percent land cover in the Makarora and Matukituki catchments (based on LCDB v. 3 data).

Land cover (%)	Makarora	Matukituki
Built-up area/Urban	0.0	0.0
River/lake shoreline	1.1	2.3
Alpine gravel/rock/landslide	7.2	7.0
Snow/ice	1.3	3.8
Alpine grass	0.7	1.4
Exotic grassland	**2.5**	**15.8**
Tussock grassland	61.2	47.6
Fernland	1.6	4.6
Scrub/shrub	5.7	4.9
Exotic forest	0.1	0.3
Indigenous forest	18.6	11.3

The Matukituki River plume was sampled an additional seven times between 2011 and 2012. The Matukituki River plume was chosen for additional sampling as the plume was easier to identify from CTD beam attenuation data, and had a greater proportion of agriculturally developed land than the Makarora River catchment (Table

13). Sampling occurred in March, May and November 2011, and January, March, June and October 2012. These dates were chosen to reflect seasonal changes in density between the river and lake.

CTD casts were taken along three transects running parallel to the shoreline of Lake Wanaka outside the Matukituki River mouth (Figure 15). Transects followed the 10 m, 20 m and 50 m bathymetric contours of Lake Wanaka. Four to six casts were taken along each transect line, with a total of 15 to 18 casts taken per sampling event; 115 casts were taken in total. CTD casts along these transect lines included casting sites 1, 2 and 3 from pilot sampling in 2009-2010. A cast was also taken from the mid-lake Aspiring Basin site (max depth: 220 m). Contour plots of the CTD casts were used to pinpoint the main river plume channel and direction of the plume in the lake. Casts that overlaid the main river discharge site were used to develop profiles of the river plume entering the lake. Each of these profiles included at least three or five CTD casts of the water column. Profile plots were made with Golden Software SURFER v. 11 using a triangulation with linear interpolation gridding method.

Duplicate one-litre water samples were collected at 2 m depth from multiple sites on four sampling dates and analysed for suspended solid concentrations (SSC). Suspended solid concentrations were measured to calibrate beam attenuation (beam transmissometer) readings from the CTD. Suspended solids were calculated gravimetrically by filtering a known volume of water through a dry, pre-weighed 1.5 μm pore size filter. Filters were dried at 100 to 105°C for at least 3 hours and cooled over silica gel before weighing. Suspended solid concentration (SSC) was calibrated to CTD beam attenuation data ($r = 0.842$, $R^2 = 0.710$, $p < 0.001$, $n = 17$) using the formula (1):

$$(1)\ SSC = 10.023 + (-0.1 * (beam\ attenuation\ (\%)))$$

Temperature and SSC were then combined to calculate differences in density between the river plume and the lake water using the formula (Kaper and Engler 2013):

$$(2)\ \rho^1 = \rho^0 / (1 + \beta(t^1 - t^0)) + SSC$$

Where: ρ^1 = final density of the water (kg l^{-1})

ρ^0 = initial density of freshwater at 20°C (kg l^{-1})

β = volumetric expansion coefficient (1/°C)

t^1 = final temperature (°C)

t^0 = 20°C

SSC = suspended solid concentration (kg l^{-1}).

Chlorophyll *a* was calibrated to CTD fluorescence data (3) using a formula derived from 2008 to 2010 Lake Wanaka data (Bayer 2016) where:

$$(3) \; \text{chl } a = (2.2601 * \text{CTD fluorescence reading}) - 0.2578$$

4.2.2 Statistical analysis

All statistical analyses were carried out using SPSS (v. 21.1, IBM) software. Temperature, suspended solid concentration, chl *a* concentration and nutrient data were compared at each site and between sampling dates using Pearson's product-moment correlation coefficient to determine the distance and depth the river plume intermixed. Statistical significance was accepted if $p < 0.05$.

4.3 Results

Thermal stratification was well established in the lake during the summer (January 2012) and early autumn (March, 2010, 2011, 2012). The depth of the thermocline varied, but generally occurred between 20 and 90 m on these dates. During the winter (June 2012), temperatures at the Aspiring Basin site changed < 1.4°C between the surface and deep waters (> 100 m) of the lake. In the early spring (September 2009: and October 2012), water temperature at the Aspiring Basin site and S4 changed less than 0.6°C between the surface and deep water (> 100 m) in the lake.

On all 10 dates the Matukituki River plume was sampled, the river water was colder than surface waters of the lake (Figure 16), and contained more suspended material. The Makarora River was colder than lake surface waters in November 2009 and March 2010, and contained more suspended material than the lake in November 2009 (Table 14). As temperature data were not collected from the Makarora River in September 2009, comparisons between the river and the lake could not be made for this month.

Table 14: Physical and chemical data for the Matukituki, River Makarora River and Lake Wanaka by sampling date (m yr^{-1} = month year^{-1}). R = river and L = lake. For 9/09, 11/09 and 3/10, rivers are distinguished as T = Matukituki River and M = Makarora River. Makarora River only sampled in 2009 and 2010, all other dates represent Matukituki River data. (*) indicates missing data.

Date	T (°C)		DOC (mg l^{-1})		TN (μg l^{-1})		TP (μg l^{-1})		TSS (max)		DO (mg l^{-1})		Flow
m yr^{-1}	R	L	R	L	R	L	R	L	R‡	L	R	L	m^3 s^{-1}
9/09T	4	9.1	*	*	*	52.3 ± 16.6	*	0.6 ± 0.8	5.6	0.8	12.71	7.41	62.8
9/09M	*	9	*	*	*	*	*	*	1.05	0.9	*	7.43	*
11/09 T	8	11.5	2.7 ± 0.1	2.0 ± 0.6	109.5 ± 9.8	124 ± 37.9	< 1.0	< 1.0	1.5	0.7	*	7.34	43.8
11/09 M	9	9.9	2.4 ± 0.6	1.9 ± 0.4	42.4 ± 27.5	97.8 ± 34.5	< 1.0	< 1.0	0.6	0.8	*	7.43	*
3/10T	11.7	15.7	1.5 ± 0.1	1.8 ± 0.3	64.9 ± 14.1	54.6 ± 13.8	11.8 ± 2.9	1.7 ± 0.4	1.0	0.7	10.84	7.02	33.8
3/10M	10.2	14.1	1.6 ± 0.6	1.8 ± 0.2	168.6 ± 7.8	104.5 ± 18.4	1.3 ± 0.3	1.0 ± 0.6	0.6	0.9	10.84	7.13	*
3/11	11.5	13.6	*	*	56.2, 58.4	*	5.9, 5.6	*	6.43	1.7	10.54	6.83	66.1
5/11	*	13.6	4.1 ± 1.0	1.9	175.4, 157.4	*	5.42	*	4.4	0.8	*	7.05	30.7
11/11	8.5	10.8	2.6 ± 0.1	2.1	*	*	*	*	0.8	0.6	11.27	7.42	29.1
1/12	13.2	14.6	2.9	2.2	*	*	*	*	4.0	1.1	9.89	6.90	37.5
3/12	10.9	15.3	2.2 ± 0.2	2.3 ± 0.3	44.3	*	6.0	*	3.2	1.1	9.64	6.74	43.2
6/12	7.9	11.1	3.2 ± 0.4	2.8, 3.0	96.6	56.89	5.4	< 1.0	1.5	1.0	9.78	6.91	27.7
10/12	7.3	9.5	2.2 ± 0.2	1.4	286.8	47.27	3.8	3.1	4.9	1.2	*	7.97	50.7

R‡= parameter measured at the sites of initial turbulent mixing along transect T1
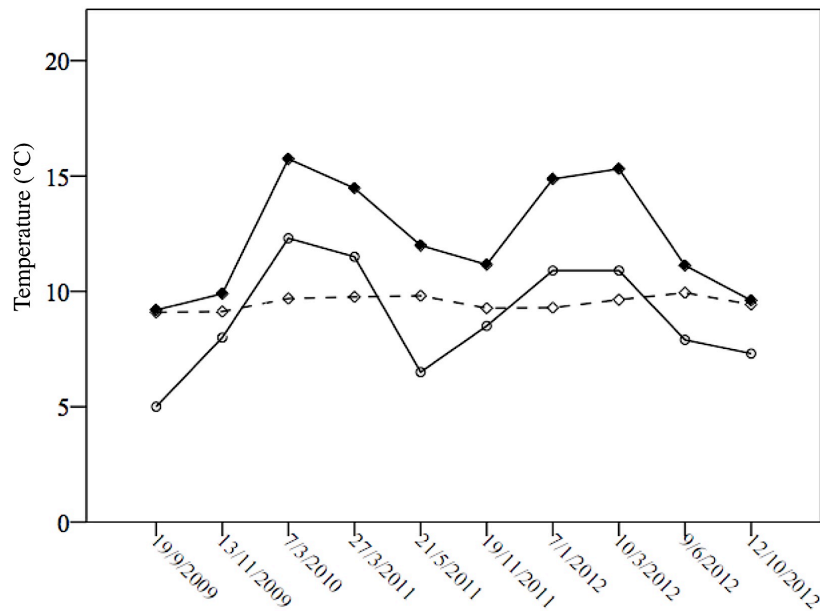
Figure 16: Comparison of water temperature in the Matukituki River (●) and the mean temperature of surface waters (> 20 m) (◆) and deep water (>150 m) (◇) at Aspiring Basin (44º35.702 S 169º04.030 E) at the time of sampling.

4.3.1 Direction of the river plume

Plume profiles at entrance mixing sites (located > 5 m outside the river mouth) were complex, with the river plume frequently interflowing as several distinct layers. These layers are apparent in density profiles from the entrance-mixing sites (Figure 17), as well as in temperature and suspended solid profiles (Appendix C Figure C1 and C2). Frequently, density profiles at the entrance-mixing site showed denser river water interflowing in surface to near-surface waters of the lake, with a small layer of colder water apparent at, or near, the lake bottom (Figure 17 E, G, H, I). This complex initial interaction between the river plume and the lake likely reflects turbulent mixing due to the steep drop-off of the river delta. Such turbulent mixing meant the density of the river plume did not always correlate with depth at entrance-mixing sites (Table 15).

Table 15: Correlations between density and depth at entrance-mixing sites (site '1') and at a second sampling site (site '2'), which continued to follow the trajectory of the river plume out into Lake Wanaka. Distance between site 1 and site 2 and average flow rate over the 24 hours prior to sampling are also given. ρ = density (kg l^{-1}). Bold font indicates when the lake was thermally stratified.

Date	ρ (kg l^{-1}) to depth (m) site '1'	Sig.	ρ (kg l^{-1}) to depth (m) site '2'	Sig.	Distance (m)	Flow rate (m^3 s^{-1})
19/09/2009	-0.908	<0.001	0.641	<0.001	107	62.8
19/09/2009	-0.619	0.001	0.157	0.435	499	*
13/11/2009	-0.872	<0.001	-0.511	<0.001	134	43.8
13/11/2009	0.816	<0.001	0.660	<0.001	800	*
07/03/2010	**-0.411**	**0.101**	**0.766**	**<0.001**	**520**	**33.8**
07/03/2010	**-0.681**	**0.021**	**0.631**	**0.037**	**349**	*****
26/03/2011	**-0.820**	**0.024**	**0.921**	**<0.001**	**137**	**66.1**
21/05/2011	-0.122	0.736	0.680	0.001	101	30.7
19/11/2011	0.889	<0.001	0.928	<0.001	20	29.1
07/01/2012	**0.542**	**0.014**	**-0.765**	**<0.001**	**60**	**37.5**
10/03/2012	**-0.310**	**0.303**	**0.850**	**<0.001**	**103**	**43.2**
09/06/2012	-0.528	0.078	0.352	0.099	25	27.7
12/10/2012	-0.877	<0.001	-0.730	<0.001	49	50.7

Occasions when density increased with increasing depth were November 2011 and January 2012 at entrance-mixing sites of the Matukituki River (Figure 17F and G), and November 2009 at Makarora River entrance-mixing sites (Figure 18). Although density increased with increasing depth at the Makarora inflow site in November 2009 (T: 9.71 – 9.79°C; SSC: 0.27 – 0.29 mg l^{-1}), there was minimal variation in temperature and suspended solid concentrations, which made it difficult to distinguish

the river plume. The same is true at the Matukituki River inflow site in November 2011 (T: 10.78 – 11.20°C; SSC: 56 – 80 mg l^{-1}). In January 2012, the Matukituki River plume mainly plunged, although a thin layer of dense water was also retained near the surface (Figure 17G; Appendix C Figure C1 and Figure C2).

Generally, once past the site of initial turbulent mixing, density increased with increasing depth, reflecting a plunging river plume (Table 15). Occasions where this did not occur include: October 2012, January 2012 (Figure 19) and November 2009 at sites outside of the Matukituki River. The lack of a positive correlation between density in depth in November 2009 and October 2012 possibly reflects weather conditions at the time of sampling, as high winds deposited noticeable quantities of dust on the lake surface, and wind-driven mixing may have stirred up shallower areas of the lake. In January 2012, the negative correlation between density and depth at site 2 could reflect the similarity between the inflowing river temperature and the surface temperature of the lake (see Table 14), or diel variations in the depth of the inflowing plume.
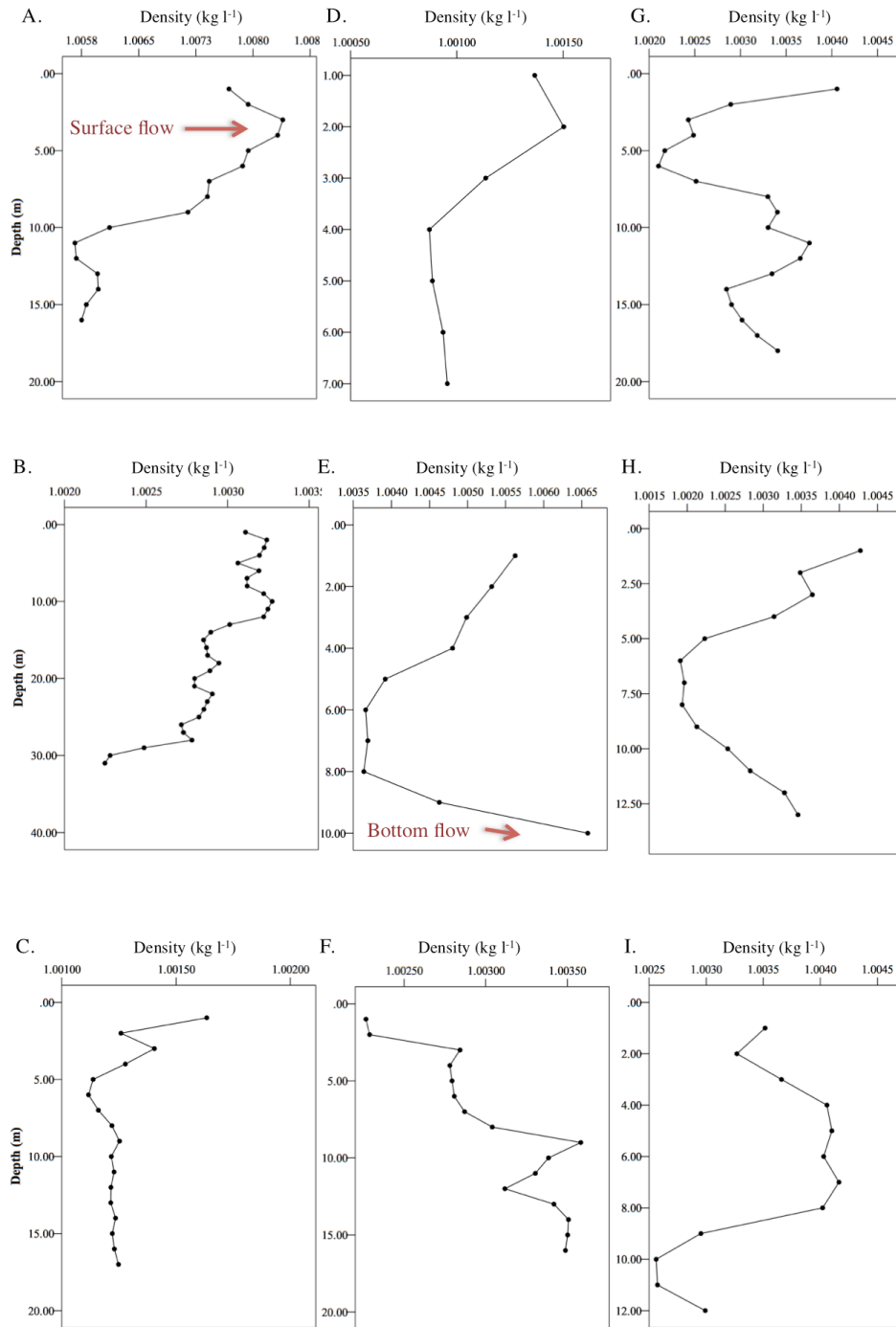
Figure 17: Density profiles at initial sampling sites outside the Matukituki River mouth (~10 m in September and November 2009; < 5 m on all other dates) in, A. September 2009, B. November 2009, C. March 2010, D. March 2011, E. May 2011, F. November 2011, G. January 2012, H. March 2012, I. June 2012. The red arrow(s) indicate the cooler water of the river plume.
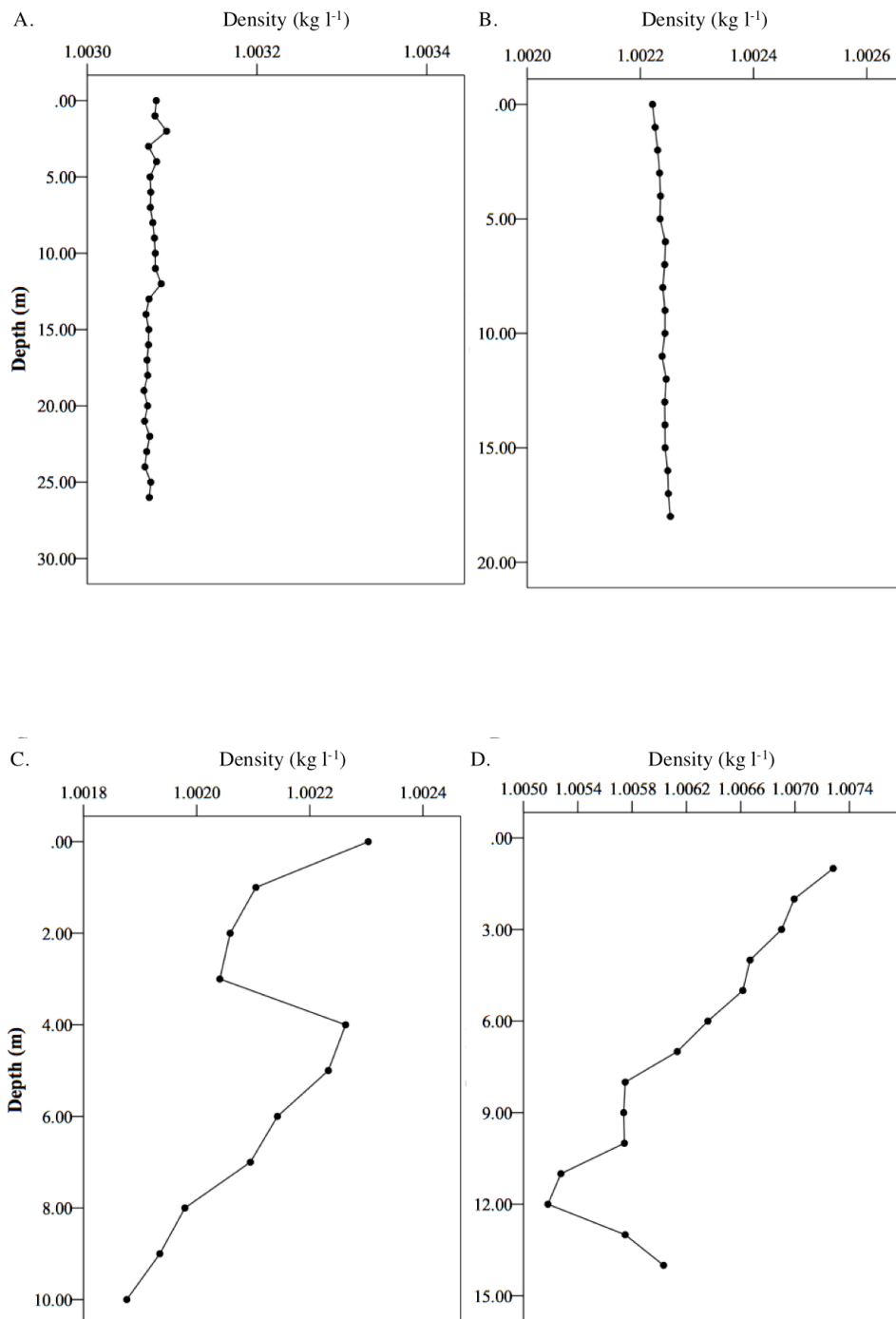
Figure 18: Density profiles at initial sampling sites in Lake Wanaka outside the Makarora River mouth (~ 10 m from the river mouth) in A. September 2009, B. November 2009, and C. March 2010, and directly outside the Matukituki River mouth in D. October 2012.

4.3.2 Material transported to the lake

Both the Matukituki River and Makarora River transported nutrients, dissolved organic carbon (DOC), dissolved oxygen (DO) and suspended solids to Lake Wanaka. Dissolved oxygen concentrations showed both the Matukituki River and mid-basin

sites in the lake were well oxygenated, and that Lake Wanaka waters remained well oxygenated with increasing depth (Appendix C, Table C1).

The Matukituki River had consistently higher total suspended solid concentrations (TSS) than Lake Wanaka (Table 14), and this parameter (Figure 20), along with temperature (Figure 21), proved useful for tracking the river plume out into the lake (also see Appendix C, Figures C3 through C9). Generally, bulk DOC and TN concentrations in the Matukituki and Makarora Rivers were not elevated compared to the lake, although TN concentrations were higher in the Makarora River than the mid-basin site in March 2010, and in the Matukituki River in June and October 2012 (Table 14). At entrance-mixing sites (located approximately 5 m from the river mouth), TN concentrations were either within range of, or less than, mid-basin TN concentrations. The exception occurred at the Makarora entrance-mixing site sampling station in March 2010, where TN concentrations averaged 192.63 ± 82.2 μg l^{-1} at 2 m depth and 101.53 ± 13.96 μg l^{-1} at 10 m depth, while mid-basin (S5) concentrations averaged 104.5 ± 18.4 μg l^{-1}. Dissolved organic carbon concentrations in the Matukituki River were within the range of DOC concentrations from the Aspiring Basin site on all sampling dates.

The Matukituki River frequently had higher TP concentrations than the lake (Table 14), but the differences between the river and lake were insufficient to allow tracking. The only sampling station in the lake where TP concentration exceeded background (mid-basin) concentrations was the entrance-mixing site for the Matukituki River. At this site, TP concentrations averaged 5.06 ± 3.27 μg l^{-1} in September 2009 and 5.96 ± 1.88 μg l^{-1} in March 2010. Total phosphorus concentrations were within the range of background concentrations by the next sampling station (100 m from river mouth in September 2009; 520 m from river mouth in March 2010). Total phosphorus concentrations at all sites were below detection in November 2009.
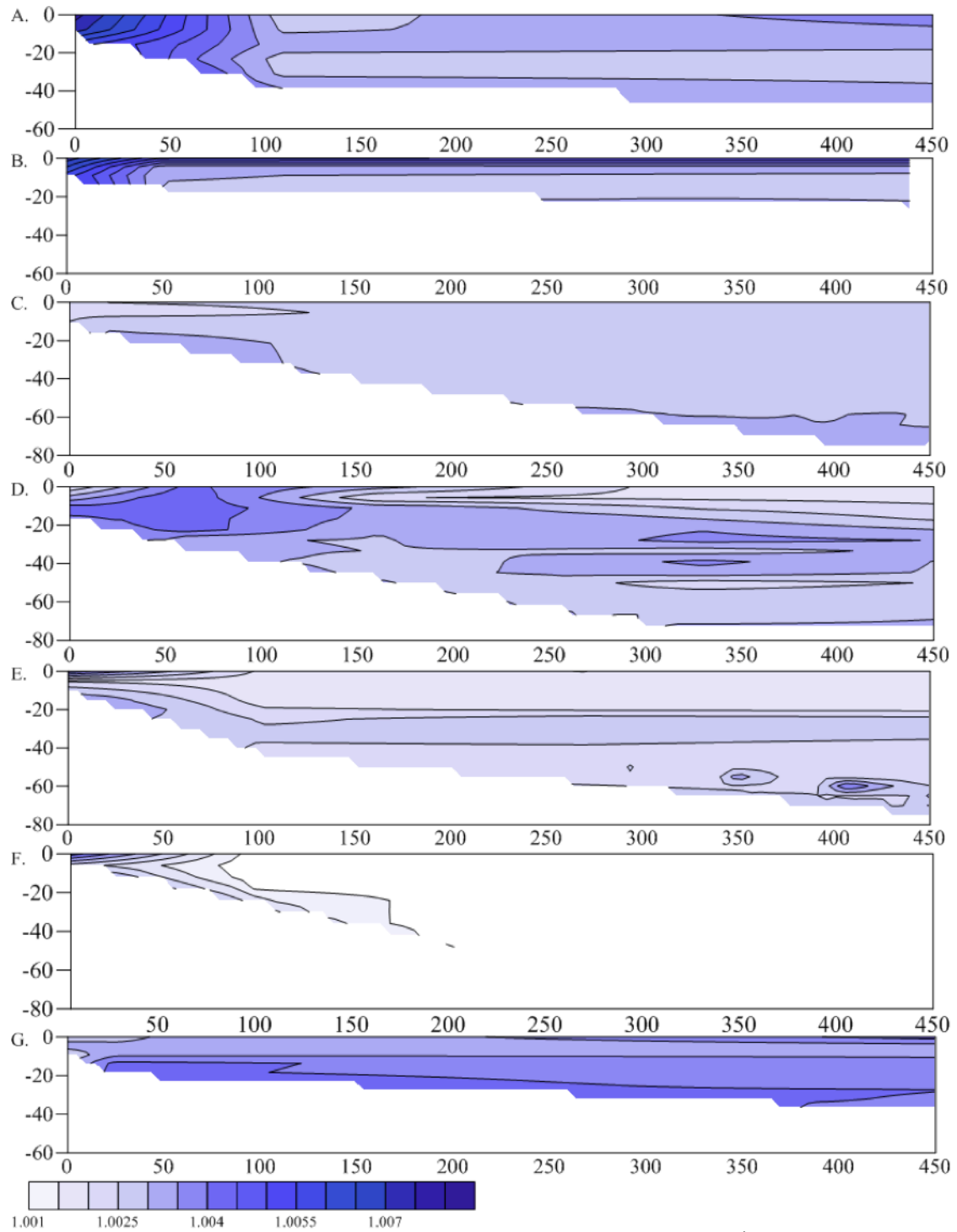
Figure 19: The Matukituki River plume as denoted by density (kg l^{-1}) in the spring (A. September 2009; B. October 2012; C. November 2011), summer (D. January 2012; E. March 2012), and late autumn/winter (F. May 2011; G. June 2012). Contour lines = 0.0005 kg l^{-1}. Depth (m) is shown on the Y-axis, while distance from the river mouth (m) is shown on the X-axis.
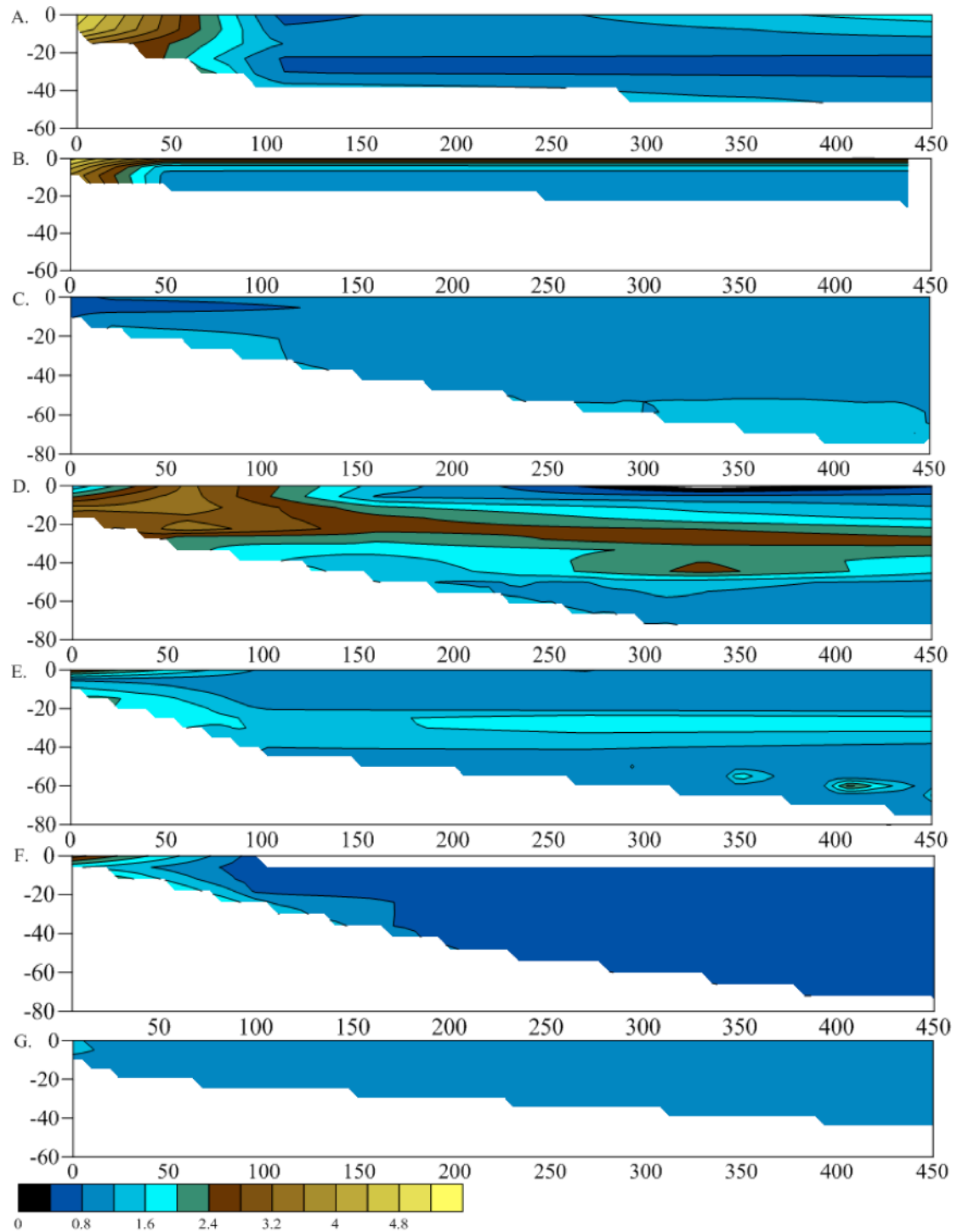
Figure 20: The Matukituki River plume as denoted by suspended solid concentration (mg l^{-1}) in the spring (A. September 2009; B. October 2012; C. November 2011), summer (D. January 2012; E. March 2012), and late autumn/winter (F. May 2011; G. June 2012). Contour lines = 0.4 (mg l^{-1}). Depth (m) is shown on the Y-axis, while distance from the river mouth (m) is shown on the X-axis.
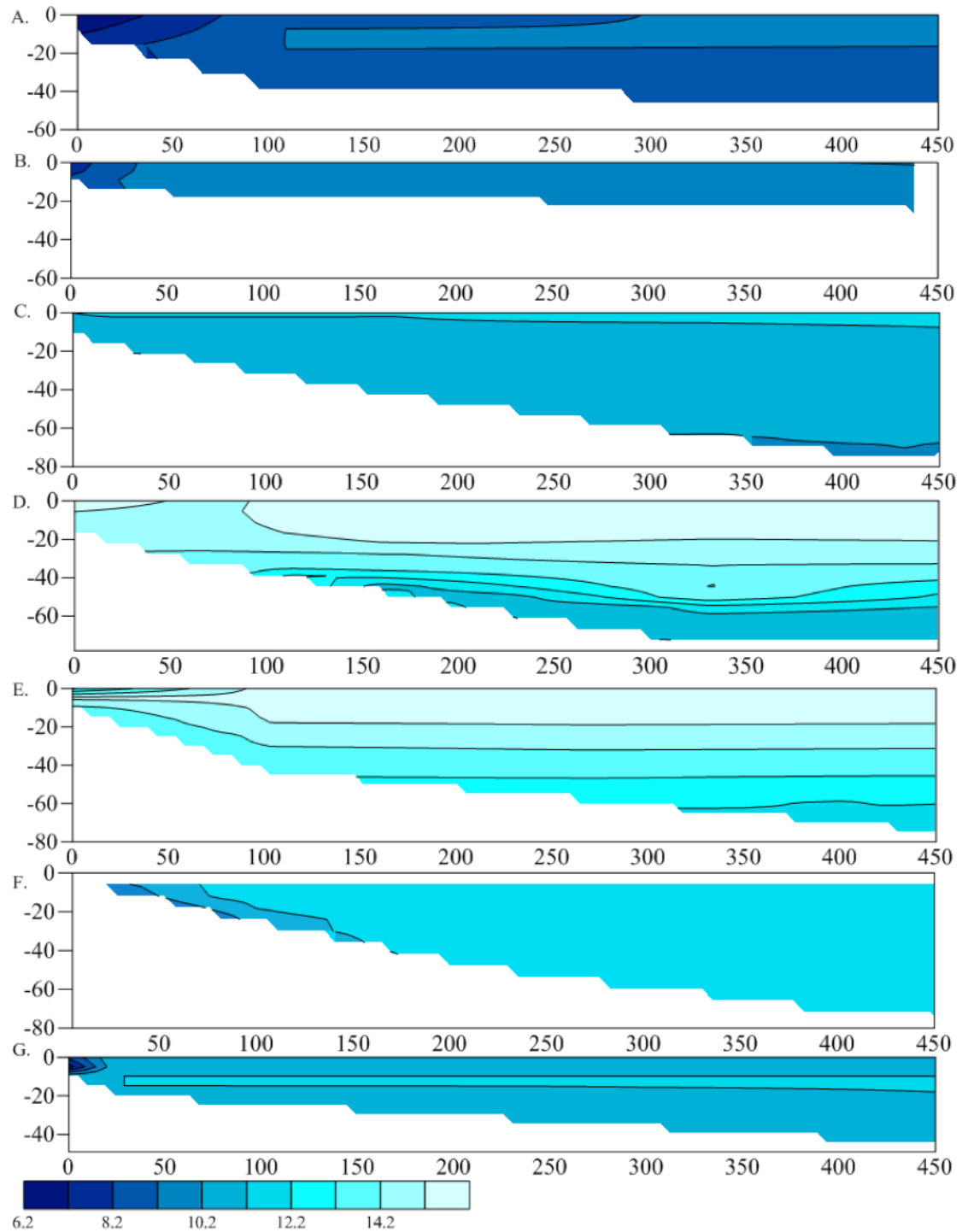
Figure 21: The Matukituki River plume as denoted by temperature (°C) in the spring (A. September 2009; B. October 2012; C. November 2011), summer (D. January 2012; E. March 2012), and late autumn/winter (F. May 2011; G. June 2012). Contour lines = 0.5°C. Depth (m) is shown on the Y-axis, while distance from the river mouth (m) is shown on the X-axis.

4.3.3 River plume dynamics and chlorophyll *a*

Once past the initial zone of turbulent mixing, chl *a* concentration was often higher between the layers of the Matukituki River plume (surface-flowing and interflowing) (see January 2012 in Appendix C, Figure C10; September 2009 Appendix C, Figure

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C11), and occasionally above the plume (see March 2012 in Appendix C, Figure C10). For example, in September 2009, density profiles at the Matukituki River entrance-mixing site showed the river plume interflowed near the surface, while a thin layer flowed along the bottom of the lake (Figure 19A). At this site, chl *a* concentrations were highest between the two plume layers (see Appendix C, Figure C11). Approximately 100 m out in the lake, these layers were apparent as an interflow at 15 m and an underflow at 40 m. Again, chl *a* concentrations were highest in the waters between these two layers, with a chl *a* maximum occurring at approximately 30 m (see Appendix C, Figure C11).

On three dates, an underflowing or interflowing chl *a* layer extended from the river mouth out into the lake (Figure 22). In May 2011, a low chl *a* underflow extended 150 m from the river entrance out into the lake (Figure 22 F). A similar underflow extended approximately 10 m out into the lake in June (Figure 22G). In March 2012, an interflowing chl *a* layer was apparent out to 150 m (Figure 22E). Evidence of a chl *a* underflow (May 2011, June 2012) and interflow (March 2012) suggests the river plume was either bringing in fluorescing material or supporting phytoplankton growth in the lake.
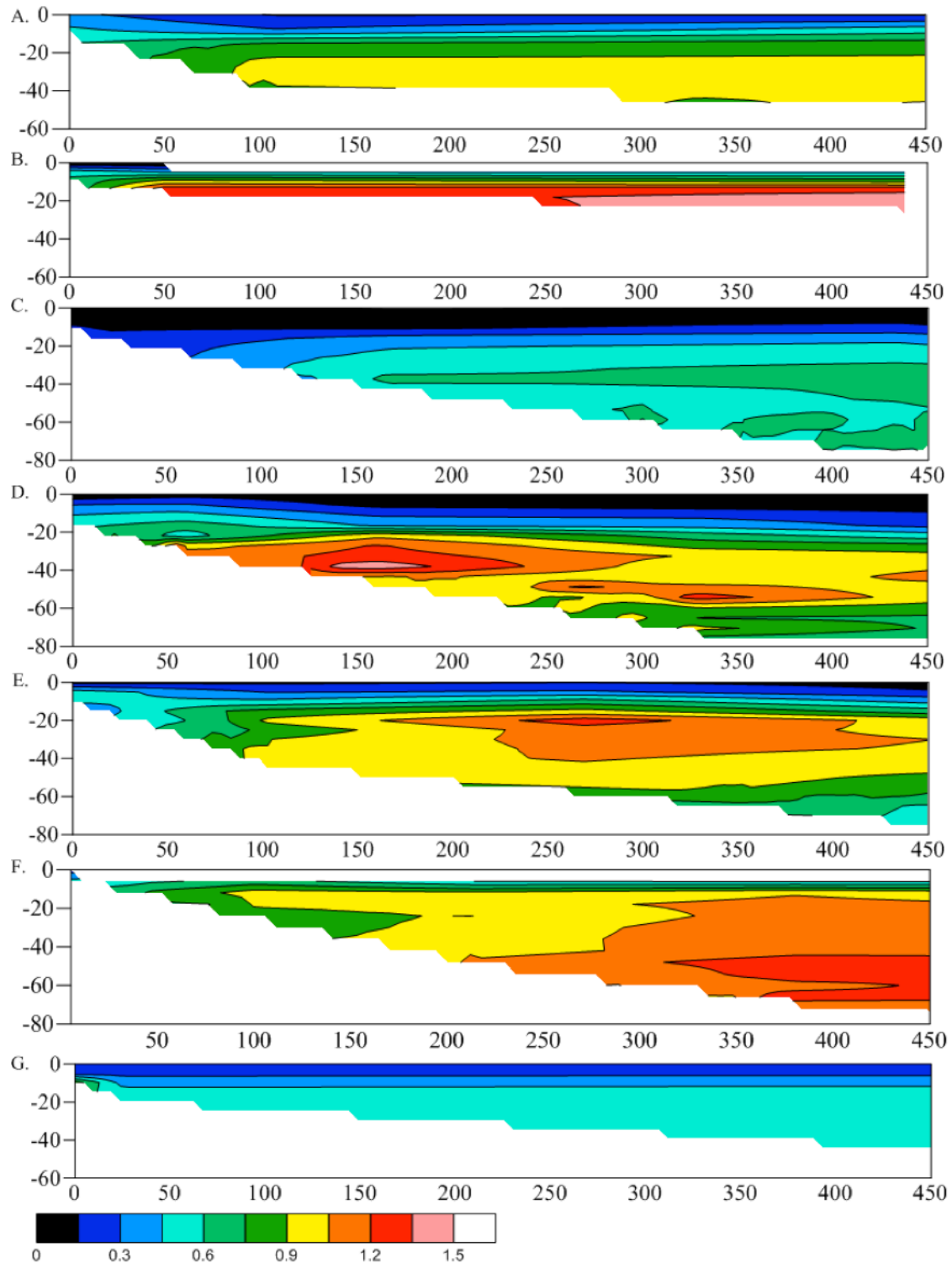
Figure 22: Water column profiles of chlorophyll a concentration (mg m^{-3}) from the mouth of the Matukituki River moving towards the open water of Lake Wanaka. Spring (A. September 2009; B. October 2012, C. November), summer (D. January 2012; E. March 2012), and late autumn/winter (F. May 2011; G. June 2012) profiles are included. Contour lines = 0.15 mg m^{-3} for all profiles. Depth (m) is shown on the Y-axis, while distance from the river mouth (m) is shown on the X-axis.
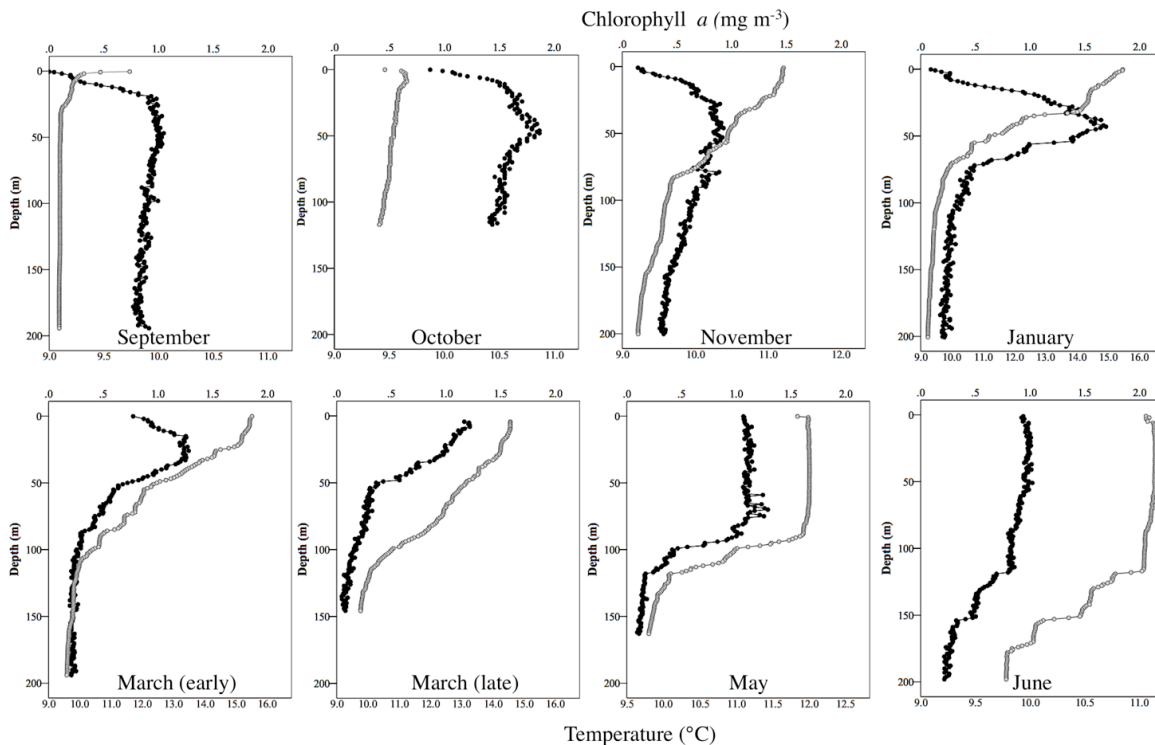
Figure 23: Chlorophyll *a* (black line) and temperature (grey line) profiles at the Aspiring Basin open water site (44º35.702 S 169º04.030 E) in Lake Wanaka at different times of the year. The scale for chl *a* (mg m^{-3}) is given on the top axis of each graph. The scale for temperature (°C) is given on the bottom axis of each graph. Sampling dates are as follows: September: 19 September 2009; October: 12 October 2012; November: 19 November 2011; January: 7 January 2012; March (early): 10 March 2012; March (late): 26 March 2011; May: 21 May 2011; June: 9 June 2012.

The highest chl *a* concentrations (0.87 - 1.87 mg m^{-3} below 20 m depth) were recorded at the Aspiring Basin site in October 2012, despite cool water temperatures associated with the early spring (Figure 23). The Aspiring Basin site was always sampled in the morning (between 8:00 and 9:00 h) to avoid high sunlight intensity associated with solar noon. However, non-photochemical quenching of the surface water fluorescence profile may still have occurred.

At mid-lake sites (e.g. Aspiring Basin or S4, approximately 2 km south of the Makarora inflow), chl *a* tended to decrease as the density of the surrounding water increased in the summer and autumn/early winter when the lake was either thermally stratified or when thermal stratification was breaking down. On these occasions, algal growth was restricted to the mixed layer above the thermocline. Instances where chl *a* was not significantly correlated with density at the mid-lake site occurred in the spring when the lake was isothermal (Table 16).

Table 16: Pearson correlations between density and chl *a* concentration at mid-lake sites (AB = Aspiring Basin for the Matukituki outflow, S4 = arbitrary site north of Minaret Basin for the Makarora outflow. Bold font indicates $p < 0.05$.

site	19/09 2009	13/11 2009	07/03 2010	26/03 2011	21/05 2011	19/11 2011	07/01 2012	10/03 2012	09/06 2012	12/10 2012
AB	**0.77**	0.004	**-0.63**	**-0.98**	**-0.95**	0.13	**-0.51**	**-0.89**	**-0.98**	-0.28
S4		**-0.36**	-0.16							

4.4 Discussion

4.4.1 Direction/depth of the river plume

Matukituki River water was consistently colder and more turbid than Lake Wanaka. Thus, one would expect the river plume to plunge and flow along the bottom of the lake, or interflow as a density current. However, at entrance-mixing sites, the plume frequently intermixed as several distinct layers, with a thin layer plunging along the bottom. A similar pattern was not obtained for the Makarora River plume as suspended solid concentrations and water temperatures were frequently similar between the river and the lake.

The initial surface suspension of the Matukituki River plume is likely related to the bathymetry at the river-lake interface, as a steeply sloping bed at the mouth of the delta could cause a fast flowing plume to initially lift away from the channel bottom even if the plume was more dense than the lake water (Spigel *et al.* 2005, Mackay *et al.* 2011). Turbulent mixing produces anomalies in T, conductivity and SSC profiles, reflecting complex layers of river and lake water in underflow and interflow (McCullough *et al.* 2007). As distance from the river mouth increases, these anomalies can become less pronounced (McCullough *et al.* 2007).

The Matukituki River water tended to plunge as it moved out in the lake, although the thermal structure of the water column affected whether the plume interflowed or underflowed. When thermal stratification was well established in the lake, the river plume tended to plunge and interflow as a density current above the thermocline (Figure 19D and E). When the lake was isothermal or weakly stratified, the depth of intermixing was more variable, with the plume either interflowing (Figure 19A) or plunging along the bottom (Figure 19A, C, F). The one occasion where the plume appeared to flow along the surface (Figure 19B), likely reflected deposition of dust on the lake surface as a result of windy conditions at the time of sampling.

The propensity of the Matukituki River plume to sink and flow as a bottom, or density, current is supported by the likely presence of sublacustrine channels in the lake bed. The CTD casts taken from March 2011 through October 2012 included sites that possibly follow one of these channels out into the lake. Bathymetric maps showing the possible sublacustrine channel in relation to the Matukituki River plume are presented in Appendix C (Figures C12 and C13). The channel is similar to ones described by Irwin (1980), which originated near the mouth of the Matukituki River and extended out past Rabbit Island (approximately 5 km from the river mouth). These channels were likely scoured out by underflows and density currents related to the river plume (Irwin 1980). Irwin (1980) described similar channels outside the Makarora River mouth as well as outside the Rees and Dart River Mouth in nearby Lake Wakatipu (Central Otago, New Zealand).

While I did not record any instances of continued surface flow beyond the initial mixing zone in Lake Wanaka, theoretically the Matukituki and Makarora River plumes could overflow along the surface given the right conditions. In Lake Wakatipu, a similar large (289 km^2), deep (380 m) monomictic oligotrophic lake located on the South Island of New Zealand, underflows generally predominate from the headwater delta, which drains two large, glacially-fed rivers. During warmer months, large diurnal variation in river density occasionally produced an interflowing layer near the base of the thermocline (Pickrill and Irwin 1982). Based on density data extrapolated from the Rees River, the authors speculated that diurnal variations in river temperature could result in the plume occasionally flowing along the surface in summer, as river temperatures increased during the late afternoon and water density decreased. On one occasion, turbid waters from the Makarora River were reported to extend up to 100 m from the river mouth during calm conditions (Irwin 1980). Thus, these plumes can surface flow for a considerable distance into Lake Wanaka when conditions allow.

4.4.2 Export of terrestrially-derived material to the lake

At the Matukituki River entrance-mixing sites, the river plume contained higher concentrations of TSS and marginally higher concentrations of total phosphorus (TP) than the mid-basin (Aspiring Basin) site, while total nitrogen (TN), dissolved organic carbon (DOC), and dissolved oxygen (DO) concentrations were usually within range of background concentrations in the lake. The similarity between riverine and lake

TN, DOC and DO concentrations made the river plume signal too weak to track for a great distance using these parameters. Although the Matukituki River (TP: 5.79 to 12.57 µg l^{-1}) generally had higher TP concentrations than background concentrations in Lake Wanaka at the time of sampling (Lake Wanaka TP: < 1.0 to 1.7 µg l^{-1}, with 8.3 µg l^{-1} = maximum recorded TP on 22/03/2009), this variable was also not a good indicator of the river plume as concentrations were at, or near, the range found in lakes classified as oligotrophic (TP: 4.1 to 9.0 µg l^{-1}).

The marginally elevated TP concentrations in the river compared with the lake could reflect land use in the catchment, as application of conventional fertilizers and/or manure can increase soil P concentrations above what is necessary for plant growth (Gburek *et al.* 2000). Currently, the majority of P transported by Matukituki River is in particulate form (see Figure 6 in Chapter 2), which potentially reflects the export of surplus P bound to clay particles and metal hydroxides to the lake via surface runoff (Gustafsson *et al.* 2012). As TP concentrations were not elevated in the Makarora River compared with the lake, this may reflect the smaller proportion of land in the catchment devoted to pasture cover, or that far fewer samples were taken from the Makarora River, and sampling did not occur during or following high flow events which can increase P export to downstream water bodies (Correll *et al.* 1999, Line *et al.* 2000).

Unlike TN, TP, DOC and DO, suspended solid concentrations (SSC) proved a useful parameter for tracking the Matukituki River plume (Figure 20, Appendix B). While suspended solids transported by the Matukituki River are probably glacial in origin, agricultural activity also likely contributes some of the suspended solid load via soil compaction and stock trampling of river banks (Gburek *et al.* 2000). As the Matukituki River plume tends to plunge as it moves out into the lake, the turbid waters would be unlikely to attenuate light and restrict phytoplankton growth in relatively shallow waters in the lake (McCullough *et al.* 2007).

4.4.3 Chlorophyll *a* and the Matukituki River plume

Attempts were made to avoid sampling at solar noon, but chl *a* profiles were not corrected for fluorescence quenching. Exposure of phytoplankton to high light stress can lead to non-photochemical quenching, as phytoplankton in the upper euphotic zone inhibit fluorescence in order to protect their photosystems from excess light

energy (Hamilton *et al.* 2010). The result is fluorescence readings that are under-representative of phytoplankton abundance at the surface (Fennel and Boss 2003, Sackmann *et al.* 2008). Low chl *a* concentrations are apparent in surface water in September, October, November, January and March, which may be indicative of non-photochemical quenching of the fluorescence profile, although the March 2012 beam attenuation profiles does not indicate the presence of high concentrations of particulate matter in surface waters (Appendix C Figure C14).

The apparent chl *a* underflow/interflow in March, May and June supports my hypothesis that chl *a* concentration will increase in the presence of the river plume. While chl *a* concentrations were often low at the lentic-lotic boundary, this may reflect decreased light availability (Smith and Demaster 1996), as the initial turbulence of the plume entrained suspended solids near the surface of the lake. Low chl *a* concentrations also likely reflect initial dilution by the river plume (Mackay *et al.* 2011). Once past the initial zone of turbulent mixing, chl *a* concentration was often higher between the layers of the plume (if surface flowing and interflowing) (Appendix C, Figure C10 and Figure C11), and occasionally above or below the plume. Other studies of river plume influences on phytoplankton biomass also report an increase in chl *a* as hydrological conditions change from river-dominated zones to the lake-transition zones (Kimmel *et al.* 1990, Mackay *et al.* 2011). Along this gradient, algae are increasingly able to utilise enhanced inflowing nutrient concentrations as light penetration improves and initial riverine dilution decreases (Mackay *et al.* 2011).

In Lake Wanaka, the highest chl *a* concentrations were recorded at the mid-basin site not directly impacted by the river plume. While the Matukituki River plume likely extends out as far as the Aspiring Basin site during flood events, the river plume (as determined by density) was not apparent in any of the CTD profiles taken at this site. At the mid-basin site, chl *a* maxima were not related to thermal stratification or a particular season, as maxima occurred both when the lake was mixed (October 2012) and when thermal stratification was well established (January 2012). However, thermal stratification did influence the distribution of chl *a* in the water column. When the Lake was stratified, chl *a* concentrations were highest in the epilimnion. When the Lake was mixed, chl *a* concentrations were more uniformly distributed throughout the water column (Figure 23). Chlorophyll *a* concentrations remained

relatively high during the winter and early spring (0.63 − 1.87 mg m^3), which could reflect good water transparency combined with nutrient replenishment from deeper waters in the lake (Otago Regional Council 2009, Bayer 2013, Bayer *et al.* 2015).

4.5 Conclusions

Plumes from the main inflowing tributaries to Lake Wanaka can potentially extend far into the lake. Temperature, suspended solid concentrations and density were useful parameters for tracing the Matukituki River plume as it flowed into the Lake. Turbulent mixing at the lentic-lotic boundary resulted in complex layers of light and dense waters. As the river plume extended into the lake, it tended to plunge and either interflow or underflow depending on the thermal structure of the water column. Currently, nitrogen and DOC concentrations in the Matukituki River are within the range of those in the lake (see Chapter 2), while phosphorus concentrations exceed background concentrations in the lake. Catchment-derived materials exported to the lake by the Matukituki River plume appear capable of stimulating phytoplankton growth, and controlling the depth of phytoplankton growth, in nearshore waters.

5 Potential Influence of Low-intensity Land Use on Dissolved Organic Carbon Character in the Matukituki River and Lake Wanaka

5.1 Introduction

Dissolved organic matter (DOM) plays an important role in regulating heterotrophic and autotrophic production in aquatic systems (Hecky *et al.* 2003, MacKenzie and Adamson 2004, Emmerton *et al.* 2008, Johengen *et al.* 2008). The quality and quantity of DOM can control bacterial biomass and activity (Scavia and Fahnenstiel 1987, Cotner *et al.* 2000, Findlay *et al.* 2001, Bernhardt and Likens 2002), alter bacterial community structure (Crump *et al.* 2003), affect trace metal bioavailability (Thurman 1985), attenuate light (Morris *et al.* 1995), increase surface water acidity (Hope *et al.* 1994), and affect the bioavailability of N (Bernhardt and Likens 2002, Johnson *et al.* 2009). While determining the quantity of DOM within a system is important, the quality of DOM (determined by its chemical composition and molecular structure) determines how it reacts with its environment (Davis and Benner 2005, Johnson 2008, Wagner *et al.* 2015). Generally, DOM that is rapidly bioavailable (i.e., high quality DOM) is enriched with N and P and of low molecular weight to facilitate enzymatic transformation and transport across membranes (Freese *et al.* 2007).

The source of the DOM influences its chemical composition and how it is processed in the environment. Algal exudate, comprised of high concentrations of freshly-produced carbohydrates (Biddanda and Benner 1997) or other low molecular weight (LMW) aliphatic material such as proteins or organic acids, is highly labile and tends to be rapidly biodegraded (Hansen *et al.* 2016). In comparison, terrestrially derived DOM (tDOM) spans a continuum from relatively unaltered, easily identifiable plant residues to plant and animal material that has been strongly altered by microbial and chemical processes (Biddanda and Benner 1997). While tDOM from agriculturally-modified catchments tends to be less structurally complex and can contain a greater proportion of heteroatoms than forested or wetland-dominated catchments (Wilson and Xenopoulos 2009, Wagner *et al.* 2015), DOM sourced from woody vegetation tends to contain a higher proportion of humic substances such as lignins and tannins (Benner 2004), or their soluble microbial degradation products. As these humic

substances have been strongly altered through decomposition and humification processes, they tend to be enriched in aromatic structures that can be relatively resistant to further microbial processing (Johnson *et al.* 2009, Cerdán *et al.* 2016). However, these substances can be highly photoreactive, and can photochemically break down into more bioavailable photoproducts (Mead *et al.* 2013).

Biodegradation and photodegradation can alter DOM composition. Biodegradation of DOM can lead to the rapid incorporation or mineralisation of high quality (i.e., low molecular weight aliphatic) DOM (Hansen *et al.* 2016), while photodegradation can break large DOM molecules into smaller, more bioavailable photoproducts that can then be taken up by microorganisms (Moran and Zepp 1997). Biodegradation occurs in both photic and aphotic zones, but the influence of photodegradation decreases with increasing depth, which can result in chemically distinct DOM in surface waters and deep waters (Moran and Zepp 1997). For example, in the open ocean, surface DOM is predominantly composed of recently produced labile biological substances, while deep (> 200 m) DOM consists primarily of old (3000 to 4300 year old) biologically refractory humic material (30–80%) (Mopper and Schultz 1993). In coastal areas, lakes and rivers, pronounced light attenuation as a result of coloured DOC, turbidity or productivity can restrict photolysis and the production of more biologically labile photoproducts to surface waters (Moran and Zepp 1997).

Determining DOM composition in aquatic systems is difficult due to the complexity and diversity of the naturally occurring DOM pool (Kim *et al.* 2003, Gonsior *et al.* 2011, Mead *et al.* 2013). Recent advances in ultra-high resolution spectrometric and spectroscopic techniques offer detailed information on the molecular composition of naturally sourced DOM. One such method, Electrospray Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR-MS), can characterise thousands of individual molecular formulae typically found in a given DOM sample (Stenson *et al.* 2002, Koch *et al.* 2007, Tremblay *et al.* 2007, Hertkorn *et al.* 2008, Gonsior *et al.* 2011). A qualitative technique, FT-ICR-MS has been used to differentiate between marine and freshwater sources of DOM (Tremblay *et al.* 2007, Gonsior *et al.* 2011) and between the molecular composition of DOM collected in rainfall from coastal compared to continental storms (Cottrell *et al.* 2013, Mead *et al.* 2013). However, few studies applied ESI FT-ICR-MS to look at differences in

DOM composition within a large lake and between a lake and its inflowing tributaries (Minor *et al.* 2012), particularly in catchments with minor agricultural development.

Lake Wanaka is a large (192 km^2), deep (311 m), oligotrophic lake located in Central Otago, New Zealand (44° 42'S, 169° 09'E). Waters in the lake are clear (Secchi 11-13.9 m) and productivity is low (mean chl *a* = 0.49 mg m^{-3}). The clarity and long hydraulic residence time in Lake Wanaka (> 6 years) would allow DOM in surface waters to become severely photobleached, potentially producing smaller, more bioavailable photoproducts. Biological activity in surface waters would also produce labile, low molecular weight (LMW) aliphatic DOM. In deep waters (> 100 m) of the lake, photolysis will not affect DOM bioavailability, as photosynthetically active radiation drops to below 1% of its surface value by 40 m depth in Lake Wanaka (Bayer 2013). As biodegradation of sinking, senescent autochthonous and terrestrially-derived material would remove labile DOM from the water column leaving behind DOM that is more resistant to microbial degradation, I hypothesised that:

Hypothesis (i): Deep lake water will contain a greater proportion of aromatic structures than near-surface waters in Lake Wanaka

Flowing into the western side of Lake Wanaka, the Matukituki River drains national parkland and low-intensity farmland. Land cover in the Matukituki River catchment is similar to the overall Wanaka catchment (see Table 1 in General Introduction) with the majority of land cover comprised of tussock grassland (47.6%), and woody vegetation (forest (11.6%) + scrub/shrub (9.6%) = 21.1%), pastureland (15.8%) and snow and ice (10.8%). Woody vegetation is not the dominant land cover in the Matukituki Valley, but makes up a significant proportion (1/5) of vegetation in the catchment. As aromatic DOM concentrations are higher in soils beneath patches of woody vegetation than in between patches of woody vegetation (Cerdán *et al.* 2016), DOM in the river may contain recalcitrant (but photochemically active) humic substances. As the short residence time and high turbidity of the Matukituki River will likely limit photodegradation of aromatic riverine DOM, I hypothesised that:

Hypothesis (ii): The Matukituki River will contain a greater proportion of aromatic structures than near-surface water in Lake Wanaka

In addition to woody vegetation, a small proportion (15.8%) of the Matukituki Valley is covered in pastureland. The growth of highly productive vegetation and presence of animal manure can not only increase inputs of less humified and more labile OM to streams, but can also increase the molecular diversity of DOM (Ohno *et al.* 2007, Wilson and Xenopoulos 2009, Bida 2013), particularly the presence and abundance of heteroatoms (i.e., atoms other than carbon and hydrogen) in the DOM signature (Wagner *et al.* 2015). As the volume of Lake Wanaka would dilute inflowing material from the Matukituki River, I hypothesised that:

Hypothesis (iii): The Matukituki River water will have proportionally more heteroelement DOM formulae than Lake Wanaka.

5.2 Methods
5.2.1 Study site and field sampling
Lake Wanaka is located in the high country of Central Otago, New Zealand. Land development is the area is relatively low intensity (Davies-Colley 2013), although this region is experiencing a slow shift in vegetation cover from tussock-dominated grasslands to increasing areas of agricultural and urban development (McGlone 2001). The mountainous terrain surrounding Lake Wanaka restricts most urban and agricultural development to alluvial plains and the glacially-formed basin at the southern end of the lake. Agricultural development in the area (including the Matukituki River Valley) consists primarily of sheep, beef cattle and deer farming (Rosen and Jones 1998).

In June 2012, duplicate 5-L water samples were collected in Lake Wanaka from 20 m and 100 m at the Aspiring Basin site (44º35.702 S 169º04.030 E) to determine the character of DOM in the lake. Samples were collected using a 5-L Niskin water sampler, and stored in acid-washed polyethylene containers that had been thoroughly rinsed with Milli-Q water. On the same morning, two duplicate 5-L water samples were collected from the Matukituki River for the same purpose. Matukituki River water samples were taken from the middle of the river in the main flow approximately 20 cm below the surface. All water samples were stored on ice in the dark and transported back to the laboratory.

5.2.2 Bulk DOC concentration

Triplicate 50 ml water samples from each container were filtered through a Durapore® PVDF membrane 0.22 μm filter. The filter was wrapped in aluminium foil to prevent photo-degradation and stored at 4°C to determine bulk DOC concentration. Bulk DOC samples were analysed on a Shimadzu TOC-V CSH Total Carbon Analyser (Shimadzu, Kyoto, Japan) using potassium hydrogen phthalate as a standard. Samples were acidified to pH 2.5 using analytical grade HCl, then purged with ultra-pure oxygen to remove dissolved inorganic carbon (DIC). Four injections were run for each sample, with the three closest concentrations averaged to give the DOC value.

5.2.3 Characterisation of DOM

The remaining large-volume water samples were immediately filtered through a Millipore GV 0.22 μm filter, acidified to pH 2.2 ($\pm$ 2) and stored for one month at 4°C in acid-washed and Milli-Q rinsed polyethylene containers until solid phase extraction (SPE). Optimal water volumes filtered for SPE were determined using maximum recorded DOC concentrations in the river and lake in order to achieve a target eluate concentration of > 10 μg C ml^{-1}. The Agilent Bond Elut PPL SPE cartridges (1 g PPL resin) were activated with 5 ml methanol (HPLC-grade) and the methanol was rinsed off with acidified Milli-Q water. Each water sample was then gravity-fed (< 20 ml min^{-1}) through the activated cartridge. After the extraction, cartridges were rinsed with 5 ml of acidified Milli-Q water, and dried under a vacuum. After drying, each cartridge was flushed twice with 5 ml of HPLC-grade methanol, eluting the solid-phase extracted DOM (SPE-DOM) into amber 20 ml vials that had a lid with a Teflon-coated silicon seal. Samples were analysed using a 12 Tesla Bruker Solarix Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) instrument at the Helmholtz Center for Environmental Health, Research Unit Analytical BioGeochemistry, Germany, to obtain ultra-high resolution mass spectra of the SPE-DOM. Ions were produced via electrospray ionization (ESI) in negative ion mode, and molecular formula assignments were based on the following elements: $^{1}H_{0-\infty}$, $^{12}C0_{0-\infty}$, $^{16}O_{0-\infty}$, $^{32}S_{0-\infty}$, $^{34}S_{0-3}$, $^{14}N_{0-10}$ (Gonsior *et al.* 2011). Samples were injected at 3 μL min^{-1} and 500 scans were averaged at a mass-to-charge ratio (*m/z*) between 147 and 1000. The FT-ICR-MS was calibrated internally using arginine

clusters and again externally using known exact DOM molecular formulae (Timko *et al.* 2015).

Duplicate samples were averaged before the formula assignments (intensities of each m/z ion). This meant that lower abundance m/z ions were averaged with slightly higher abundance ions in the replicate sample, resulting in more peaks over the relative abundance threshold, and an overall increase in the number of assigned formulae for the averaged spectra. Summary data from the duplicate samples are available in Appendix D, Table D1.

Van Krevelen diagrams (van Krevelen 1950, Kim *et al.* 2003) were used to investigate molecular variation between sites. Van Krevelen diagrams are constructed from elemental ratios of hydrogen to carbon (H:C) on the y-axis and oxygen to carbon (O:C) on the x-axis. While molecular formulae that have the same elemental ratios overlap on these diagrams, major chemical classes in the DOM can produce characteristic H:C and O:C ratios that cluster within a particular region of the diagram. These regions can help indicate the source DOM material, although formulae falling within these characteristic ranges do not definitively identify the compounds (Kim *et al.* 2003). This is because no structural information is available from mass spectrometric results alone, and a high number of isomers may be present. The van Krevelen diagrams were also useful for visualising the presence and abundance of aromatic compounds after applying a general aromaticity index.

To distinguish aliphatic compounds from aromatic compounds, the general aromaticity index (AI) developed by Koch and Dittmar (2006; updated in 2016) was used. Koch and Dittmar (2016) calculated AI by first calculating the double bond equivalents (DBE) of the molecular core of the original molecular formula. The DBE of the molecular core (DBE_{AI}) is calculated by subtracting all functional groups that potentially contribute to Double Bond Equivalents (DBE) from the original molecular formula ($DBE_{AI} = 1 + (C - O - S - \frac{1}{2}(N + P + H))$). Next, C_{AI} is determined by the formula $C_{AI} = C - O - S - N - P$, to get the respective number of carbon atoms in the molecular formula (Koch and Dittmar 2006, Koch and Dittmar 2016). The aromaticity index was then calculated as:

$$AI = \frac{DBE_{AI}}{C_{AI}}$$

In addition to *AI,* a modified aromaticity index (AI_{mod}) was also calculated, where:

$$AI_{mod} = \frac{1 + C - \frac{1}{2}O - S - \frac{1}{2}(N + P + H)}{C - \frac{1}{2}O - N - S - P}$$

The AI_{mod} index accounts for the fact that carboxyl groups are made up of both carboxyl oxygen that is bound with sigma (σ) bonds (the first bond formed between carbon and another atom), as well as carbonyl groups that are bound with π-bonds (second or third bonds between atoms) (Koch and Dittmar 2006, Koch and Dittmar 2016). The modified aromaticity index is less conservative than the original aromaticity index, and increases the number of compounds identified as aromatic.

Double bond equivalents and DBEs normalised to the number of carbons (DBE/C) were also used to analyse the different DOM signatures from the three sites. Double bond equivalents represent the degree of unsaturation of a compound (or the number of double or triple bonds potentially present in a compound), which affects the number of hydrogen atoms a compound can bind (Minor *et al.* 2012). Unsaturation increases with an increasing number of double bonds, and unsaturation combined with low H:C and O:C values, can be indicative of aromatic structures (Minor *et al.* 2012).

5.2.4 Analysis of the data

Statistical analyses were carried out using SPSS (v. 21.1, IBM) software. Chi-square (χ^2) statistical tests were used to determine whether the proportion of aliphatic to aromatic compounds was the same at the three sites. The tests were also used to determine whether the proportion of heteroelement formulae were the same for each site. In a few instances, quantitative comparisons were made using One-way ANOVA, or Kruskal-Wallis tests if assumptions of normality and homoscedasticity were not met. In all analyses, statistical significance was accepted if $p \leq 0.05$.

5.3 Results

In June 2012, bulk DOC concentration was 3.32 ± 0.49 mg l^{-1} in the Matukituki River, which was similar to bulk DOC concentrations at the two sites in Lake Wanaka (3.05 ± 0.24 mg l^{-1} at 20 m and 3.25 ± 0.004 mg l^{-1} at 100 m). In general, bulk DOC concentrations in the Matukituki River (1.27 to 3.81 mg l^{-1}, mean DOC $= 2.46 \pm 0.57$ mg l^{-1}, n = 16) were similar to the lake (mean lake DOC $= 2.04 \pm 0.47$, range $= 1.43$ to 2.91 mg l^{-1}, n = 10) when sampled during low to moderate flows (flow rate < 65 m^3 s$^-$

^{1}). The Matukituki River flow rate can be as high as 1000 m^3 s^{-1} in flood, and flow rates greater than 100 m^3 s^{-1} are not uncommon. Hence this 'average' DOC concentration does not reflect the amount of material brought in by the river during high flows.

5.3.1 Aromaticity of DOM

Aromaticity index data are presented as both *AI* and *AI$_{mod}$* in Table 17. Van Krevelen diagrams using calculations of *AI$_{mod}$* are presented in Figure 24, while diagrams using calculations of the more conservative *AI* formula are available in Appendix D (Figure D1).

Table 17: Total number of carbon, hydrogen and oxygen (CHO), carbon, hydrogen, oxygen and sulphur (CHO-S) and carbon, hydrogen, oxygen and nitrogen (CHO-N) containing formulae in each sample. Total number of aliphatic (≤ 0.5), aromatic (> 0.5) and conservatively defined aromatic (≥ 0.67) CHO, CHO-S and CHO-N are also given. Aliphatic and aromatic groups were determined using both the Aromaticity Index (*AI*) and the modified Aromaticity Index (*AI$_{mod}$*).

	Total number of formulae			≤ 0.5			> 0.5			≥ 0.67		
Site	CHO	CHO-N	CHO-S	CHO	CHO-N	CHO-S	CHO	CHO-N	CHO-S	CHO	CHO-N	CHO-S
							AI					
R	2305	716	**1215**	2206	706	**1215**	99	10	0	6	0	0
20	2274	810	616	2175	798	614	99	12	0	7	0	0
100	2607	1071	544	2431	1046	543	**176**	25	0	**39**	3	0
							AI$_{mod}$					
R	2306	718	**1215**	1867	529	**1212**	438	188	**3**	94	37	0
20	2275	811	616	1823	587	610	451	223	4	103	57	0
100	2608	1072	544	2023	770	538	**584**	301	5	**173**	67	0

Analysis of DOM composition using FT-ICR-MS revealed a lot of overlap in DOM signatures between the two lake sites, with the majority of the compounds at each site being aliphatic (Figure 24, Appendix D Figure D1). However, the two lake sites did not have similar proportions of aliphatic to aromatic compounds (χ^2 = 13.13, *p* = 0.0003) (see Appendix D, Table D2). Fewer than expected aromatic compounds were present in water from the 20 m site, while more than expected aromatic compounds were present in water from the deep (100 m) site. Deep-sourced (100 m) lake water had a significantly higher number of averaged double bond equivalents (DBEs) (χ^2 = 6.292, *p* = 0.012) and DBEs normalised to the number of carbons (DBE/C) than the

shallow water (20 m) site (Table 18; Appendix D, Table D3). Number-averaged H:C values (Appendix D, table D3) were significantly lower at the 100 m site than at the 20 m site ($\chi^2 = 8.949$, $p = 0.011$) (Table 19).

Similarly, FT-ICR-MS revealed a lot of overlap between the Matukituki River and the shallow water of the lake (Table 17). Like both lake sites, the Matukituki River DOM was predominantly composed of aliphatic compounds. The river and 20 m lake site had similar proportions of aliphatic to aromatic compounds, but the river and 100 m lake site did not ($\chi^2 = 13.96$, $p = 0.0002$). Fewer than expected aromatic compounds were present in the river water, while more than expected aromatic compounds were present in 100 m lake water (Appendix D, Table D4). Matukituki River water contained the lowest number of DBEs and DBE/Cs of all three sites (Table 18; Appendix D, Table D3).

Table 18: Average number (± 1 standard deviation) of double bond equivalents (DBE) and DBE normalised to the number of carbons (DBE/C) in CHO, CHO-N and CHO-S formulae found in the Matukituki River (River), at the shallow water site in Lake Wanaka (20 m), and at the deep water site in the lake (100 m). Lake water from 100 m depth with the highest number of DBE and DBE/C, are highlighted in bold.

Site	CHO		CHO-S		CHO-N	
	DBE	DBE/C	DBE	DBE/C	DBE	DBE/C
River	9.94 ± 3.89	0.47 ± 0.16	5.64 ± 2.64	0.29 ± 0.40	9.01 ± 2.56	0.53 ± 0.13
20 m	10.37 ± 3.95	0.49 ± 0.15	5.98 ± 2.92	0.32 ± 0.15	9.28 ± 2.61	0.55 ± 0.12
100 m	**10.69 ± 4.13**	**0.50 ± 0.16**	**6.47 ± 2.92**	**0.35 ± 0.15**	**9.84 ± 2.77**	0.55 ± 0.12

5.3.2 Heteroelement DOM signature

More than half of the assigned compounds from the three sites (54.4 – 61.7%) contained only carbon, hydrogen and oxygen (CHO) (Table 17). Carbon, hydrogen oxygen and nitrogen (CHO-N) formulae made up 16.9 to 25.4% of all formulae from the three sites, with the highest diversity of CHO-N compounds occurring in the deep waters of Lake Wanaka (1071 unique formulae out of 4224 total formulae, or 25.4%) and the lowest abundance of CHO-N formulae occurring in the Matukituki River (716 unique formulae out of 4240 total formulae, or 16.9%). In contrast, the greatest diversity of carbon, hydrogen, oxygen and sulphur (CHO-S) formulae was found in the Matukituki River (1215 unique formulae) (see bold font, Table 17), while the least diversity of CHO-S formulae were found in the deep water of the lake. No CHO-P formulae were detected by mass spectrometry.

River and lake DOC signatures did not differ significantly in mean molecular weight or O:C (Table 19). However, H:C was significantly higher in Matukituki River water than the other two sites ($F_{(2, 2369)}$ = 24.639, p < 0.001). Chi-square tests showed the proportion of CHO, CHO-N and CHO-S formulae differed significantly between the River and Lake (χ^2 = 388.139, p < 0.001) (Table 20).

Table 19: Average mass (kDa), O:C and H:O ratio (± 1 standard deviation) for carbon, hydrogen and oxygen (CHO) compounds; carbon, hydrogen, oxygen and nitrogen (CHO-N) compounds, and carbon, hydrogen, oxygen and sulphur (CHO-S) compounds in the Matukituki River (R) and Lake Wanaka at 20 m (20) and 100 m (100) depth in June 2012. Mean H:C values differed significantly between sites and are highlighted in bold.

Site	CHO			CHO-N			CHO-S		
	Mass	O:C	H:C	Mass	O:C	H:C	Mass	O:C	**H:C**
R	442.5 ± 128	0.48 ± 0.15	1.15 ± 0.32	376 ± 81.4	0.49 ± 0.1	1.13 ± 0.24	431 ± 0.55	0.44 ± 0.14	**1.52 ± 0.28**
20	449.6 ± 129	0.49 ± 0.15	1.12 ± 0.31	377 ± 82.9	0.50 ± 0.1	1.10 ± 0.23	417 ± 123	0.44 ± 0.14	**1.47 ± 0.30**
100	449.1 ± 133	0.48 ± 0.17	1.10 ± 0.32	392 ± 92.3	0.48 ± 0.11	1.09 ± 0.22	419 ± 121.5	0.44 ± 0.15	**1.42 ± 0.30**

Table 20: Chi-square (χ^2) contingency table comparing the proportion of carbon, hydrogen and oxygen (CHO-), carbon, hydrogen, oxygen and sulphur (CHO-S) and carbon, hydrogen, oxygen and nitrogen (CHO-N)-containing formulae at each site. Observed (Obs), expected (Exp) and cell-specific chi-square values are given. River: Matukituki River; 20 m: 20 m depth in Lake Wanaka; 100 m: 100 m depth in Lake Wanaka.

		River	20 m	100 m	Total
CHO	Obs	2305	2274	2607	
	Exp	2503.69	2186.89	2495.42	7186
	χ^2	15.77	3.47	4.99	
CHOS	Obs	1215	616	544	
	Exp	827.48	722.78	824.75	2375
	χ^2	181.48	15.77	95.57	
CHON	Obs	716	810	1071	
	Exp	904.83	790.34	901.84	2597
	χ^2	39.41	0.49	31.73	
Total		4236	3700	4222	12158

A comparison of CHO-S compounds using van Krevelen diagrams showed molecular ions with high H:C ($\approx$ 2) and O:C (< 0.6) ratios were present in the river and shallow waters of the lake (sampled at 20 m), but were missing from deeper (100 m) lake waters. All molecules falling within this range were present in the river water. These compounds were mainly $C_8 - C_{13}$ (see bold font, Table 21) with neutral mass ranging from 225 to 330. The relative abundance of these compounds was low (0.56% to 4.10%) compared to other CHO-S compounds in the water samples. Relative abundance is the relative intensity of each mass to charge (m/z) peak compared with

the most abundant peak (which is assigned 100%). The absence of these most of these $C_8 - C_{13}$ compounds in the deep lake water sample suggests these CHO-S compounds are degraded rather rapidly.

Table 21: Unique compounds containing carbon, hydrogen, oxygen and sulphur (CHO-S) with high H:C and O:C ratios located only in the Matukituki River (River), in the Matukituki River and shallow water site in Lake Wanaka (River and Lake (20 m)), and at all three sites. $C_8 - C_{13}$ compounds are in bold font. Relative abundance gives the relative intensity of each mass to charge (m/z) peak compared with the most abundant peak (which is assigned 1.0 (or 100%)).

Location	Formula	Neutral Mass	Relative abundance	O:C	H:C
River	$\mathbf{C_8H_{18}O_5S}$	226.09	0.56	0.63	2.25
	$\mathbf{C_{10}H_{22}O_6S}$	270.11	0.79	0.6	2.20
	$\mathbf{C_{11}H_{22}O_8S}$	314.10	1.79	0.73	2.00
	$C_{19}H_{38}O_{18}S$	586.18	0.75		2.00
River and Lake (20 m)	$\mathbf{C_8H_{16}O_5S}$	224.07	0.56	0.63	2.00
	$\mathbf{C_8H_{16}O_6S}$	240.07	1.56	0.75	2.00
	$\mathbf{C_9H_{18}O_6S}$	254.48	1.62	0.67	2.00
	$\mathbf{C_9H_{18}O_7S}$	270.08	0.90	0.78	2.00
	$\mathbf{C_{10}H_{20}O_6S}$	268.10	4.10	0.60	2.00
	$\mathbf{C_{10}H_{20}O_7S}$	284.09	2.12	0.70	2.00
	$\mathbf{C_{11}H_{22}O_7S}$	298.11	0.65	0.64	2.00
All three sites	$\mathbf{C_{11}H_{22}O_9S}$	330.10	1.70	0.82	2.00

The river also contained 394 different CHO-S formulae with low O:C (≤ 0.50) but high H:C (> 1.5) ratios. Ninety-eight different CHO-S compounds with low O:C and high H:C ratios were found at 20 m depth in the lake, and 73 different CHO-S compounds were found at 100 m. Molecules with low O:C (< 0.4) and high H:C (> 2.1) ratios fell within the range found in sulfonates commonly used as surfactants (see bold font, Table 22). A highly saturated (DBE = 0, H:C > 2) compound with a chemical formula similar to lauryl sulphate (dodecyl sulphate) ($C_{12}H_{26}O_4S$) made up a significant portion of DOM characterised in both the river (67.72%) and the lake at 100 m (47.76%). Other surfactant-like compounds were abundant at all three sites (Table 22).

Table 22: Formulae containing carbon, hydrogen, oxygen and sulphur (CHO-S) in Lake Wanaka and/or the Matukituki River with low O:C (< 0.5) and high H:C (> 1.5) ratios and a high (> 25) relative abundance. Sulfonate-like compounds are highlighted in bold font. Relative abundance gives the relative intensity of each mass to charge (m/z) peak compared with the most abundant peak (which is assigned 1.0 (or 100%)). (R) Matukituki River, (20) 20 m depth in Lake Wanaka, (100) 100 m depth in Lake Wanaka.

Location	Sites	Relative abundance	Formula	Mass	O:C	H:C
River	R	31.29	$C_{16}H_{26}O_8S$	378.13	0.50	1.63
	R	29.99	$C_{16}H_{28}O_8S$	380.15	0.50	1.75
	R	**29.66**	**$C_{19}H_{30}O_5S$**	**370.18**	**0.26**	**1.58**
	R	27.01	$C_{20}H_{32}O_{10}S$	464.17	0.50	1.67
River and 20 m	R	**67.72**	**$C_{12}H_{26}O_4S$**	**266.16**	**0.33**	**2.17**
	20	**47.76**				
All three sites	100	1.58	$C_{12}H_{20}O_6S$	292.10	0.5	1.67
	20	3.49				
	R	26.22				
	100	**1.35**	**$C_{14}H_{30}O_3S$**	**278.19**	**0.21**	**2.14**
	20	**13.73**				
	R	**32.13**				
	100	**25.45**	**$C_{14}H_{30}O_4S$**	**294.19**	**0.29**	**2.14**
	20	**22.80**				
	R	**29.2**				
	100	**22.73**	**$C_{14}H_{30}O_5S$**	**310.18**	**0.36**	**2.14**
	20	**24.91**				
	R	**26.71**				
	100	4.97	$C_{15}H_{24}O_7S$	348.12	0.20	1.60
	20	7.18				
	R	27.89				
	100	**1.47**	**$C_{15}H_{32}O_3S$**	**291.88**	**0.20**	**2.13**
	20	**11.88**				
	R	**27.66**				
	100	**19.93**	**$C_{16}H_{34}O_6S$**	**354.21**	**0.37**	**2.12**
	20	**24.09**				
	R	**28.38**				
	100	1.32	$C_{17}H_{26}O_3S$	326.15	0.23	1.53
	20	4.99				
	R	36.94				
	100	2.14	$C_{18}H_{28}O_4S$	340.17	0.22	1.55
	20	11.64				
	R	69.49				
	100	20.78	$C_{19}H_{30}O_3S$	338.19	0.16	1.58
	20	22.81				
	R	31.57				
	100	50.98	$C_{20}H_{34}O_3S$	354.22	0.15	1.7
	20	38.70				
	R	29.65				

Figure 24: Van Krevelen plots of H:C and O:C molar ratios in formulae containing carbon, hydrogen and oxygen (CHO) (A., C., E.) and CHO and sulphur (CHO-S) (B., D., F.) obtained from Lake Wanaka at 20 m and 100m depth and from the Matukituki River, respectively. Aliphatic compounds are represented by (●), aromatic compounds (compounds with a modified aromaticity index (AI_{mod}) ≥ 0.5 are represented by (Δ). Compounds with AI_{mod} > 0.67 are represented by (■).

An androsterone sulphate-like formula ($C_{19}H_{30}O_5S$) was detected in the Matukituki River water (Table 22). This does not mean androsterone sulphate was present in the water, as a large number of isomers are possible for any given exact molecular

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formula. As sulfonic acids are strong ionizers in negative mode electrospray (Gonsior, personal communication), trace amounts would be visible in our samples. While sulfonic acid signals that were present in all samples cannot be ruled out to be contaminants, sulfonic acids are also frequently used in land applications and may reflect land use practices in the catchment (Jensen 1999, Sablayrolles *et al.* 2009, Gonsior *et al.* 2011).

The river also contained a higher number of compounds with relatively low H:C (H:C < 1.5) and O:C (O:C ≤ 0.50) ratios compared with either lake site, with 243 unique compounds present in the river, while only 73 compounds were found at 20 m depth and 64 were present at 100 m depth (data not shown). While these compounds do not fall within the threshold of the aromaticity index, they indicate the river contained not only a greater number of CHO-S formulae than the lake, but also a more diverse range of CHO-S compounds than the lake.

5.4 Discussion
5.4.1 Aromaticity of Riverine and Lake DOM
The data presented above provide a snapshot of the DOM signature of the Matukituki River and Lake Wanaka on one sampling date, and do not account for variability in the DOM signature over time.

In support of my hypothesis that deep water in Lake Wanaka would contain more refractory DOM than shallow waters, the greatest number of aromatic structures was found in deep-sourced (100 m depth) lake water. This was reflected by the aromaticity index as well as by comparing average double bond equivalents and double bond equivalents normalised to carbon (DBE/C) between sites. Using the latter method, the highest average DBE and DBE/C occurred in the deep water of the lake. Deep lake water also had the lowest average H:C values, possibly due to photobleaching of aromatic compounds in surface waters (Catalán *et al.* 2013). This result is consistent with data from the open ocean, where DOM in deep waters and surface waters are spectroscopically and chemically distinct. In the open ocean, surface DOM is predominantly composed of recently produced, labile biological substrates while DOM from deep waters consists of both biologically usable material (e.g. amino acids, simple sugars) as well as old (3000 to 4300 y.o.) biologically refractory humic material (30-80%) (Mopper and Schultz 1993).

Contrary to my second hypothesis, the Matukituki River did not contain more aromatic compounds than upper waters in Lake Wanaka. The low number of aromatic structures in the River water may reflect weather conditions preceding sampling, as weather conditions can influence the level of aromaticity in soil organic matter. In their 1989 study, Zech *et al.*, reported that soil aromaticity increased as the temperature:precipitation ratio increased, and other studies have shown that the number of in-stream aromatic compounds increases with increasing flow rate (Wilson and Xenopoulos 2009, Minor *et al.* 2012). In the Wanaka region, one could speculate that aromaticity of soil organic matter in the Matukituki Valley would be higher during dry spells in the summer, as warmer temperatures combine with low rainfall. In my study, water samples were collected in the winter when temperatures would be fairly low, which may have affected the aromaticity of the SOM. Precipitation and river flow rates were also low in the weeks prior to sampling, making it less likely aromatic compounds in SOM were transported to the river. The low flow rate of the Matukituki River at the time of sampling (29 m^3 s^{-1}), may have further affected the DOM signature of the River water, as small streams, with more variable DOM composition, can disproportionately impact on the DOM signature in larger downstream systems during periods of low flow (Wagner *et al.* 2015).

Aromaticity of SOM can also vary depending on the mineral content of the soil and the chemical structure of organic matter (Zech *et al.* 1997). The Matukituki Valley is made up of brown soils (previously called yellow-brown earths) along the steep glacially-formed valley walls, and recent fluvial deposits along the valley floor. The brown allophanic and orthic soils in the Matukituki Valley are capable of adsorbing and stabilising DOC (Martin and Haider 1986, Zech *et al.* 1997), reducing its potential for transport to nearby surface waters (Lin *et al.* 2012). Aromatic polyphenols have been shown to adsorb well to mineral phases (Gonsior *et al.* 2014), including when mixed with high sediment loads in rivers (Gonsior *et al.* 2016), which would remove this material from the DOM pool.

The low number of aromatic structures in the River water may also reflect landscape characteristics of the catchment. Steep valley walls tend to have less productive plant cover and less soil organic matter than soils on gentle slopes that support highly productive vegetation (Lambert *et al.* 2000). The combination of severe erosion potential along the Matukituki Valley hillsides (LINZ 2005), thin hillside soil organic

layers, and adsorption of aromatic structures to minerals in the soils could reduce the amount aromatic tDOM being transported to stream and downstream water bodies. Consistent with this conclusion, Wagner et al. (2015) reported few aromatic humic-like structures in the DOM signature from the Ganges-Brahmaputra River, which they attribute to low amounts of terrestrially-derived DOM entering the upper reaches of the river due to thin soil organic layers on the steeply-sided, sparsely vegetated terrain (Wagner *et al.* 2015). Catchment vegetation can also affect the amount of aromatic DOM supplied to the river. Aromatic DOM concentrations are higher in soils beneath patches of woody vegetation than beneath open areas (Cerdán *et al.* 2016). While indigenous beech forests are present up the Matukituki Valley, the catchment is predominantly covered in native tussocks and grassland, which may not supply much aromatic DOM.

5.4.2 Diversity of the CHO-S signature

In support of my third hypothesis, the Matukituki River DOM was distinct from both lake sites by having the greatest number of formulae containing carbon, hydrogen, oxygen and sulphur (CHO-S). Overall, CHO-S formulae made up 28.7% of compounds in the Matukituki River and 12.9 to 16.6% of compounds in the Lake Wanaka. However, CHO-S was the only heteroelement-containing DOM signature that was significantly increased in the Matukituki River. No CHO-P formulae were detected by mass spectrometry, which suggests particulate phosphorus (see Chapter 4) or dissolved inorganic phosphorus (See Figure 6 in Chapter 2) make up the majority of P in the Matukituki River and Lake Wanaka. The greatest CHO-N diversity occurred at the deep water (100 m) site in the lake, although the relative abundance of CHO-N compounds at all three sites was very low (< 6%). As CHO-N in deep lake water also had the highest average DBE of the three sites and the lowest average H:C values, increased diversity of dissolved organic N may reflect the presence of refractory aromatic structures that are resistant to biodegradation and are too deep to undergo photodegradation. Chemically distinct deep waters dominated by long-lasting humic substances that, while photoreactive, are biologically refractory, has been reported in the open ocean (Mopper and Schultz 1993).

The reason Matukituki River water had a higher proportion of sulphur-containing DOM than the lake is unclear. Potentially, the high number of CHO-S formulae reflects agricultural practices in the Matukituki Valley. Soils in Central Otago are

considered sulphur-deficient (Leamy *et al.* 1974, Brash and Beecroft 1987), and sulphur-containing fertilizers are frequently applied to soils in the Matukituki Valley. While inorganic sulphur is not necessarily a good proxy for organically-bound sulphur, it may indicate that organic sulphur is also elevated due to land use practices. Plants cannot use elemental sulphur until it is converted to sulphate by soil bacteria. Sulphate-sulphur that is taken up by plants can help increase plant productivity, which results in increased incorporation of S into organic matter and the eventual flushing of decomposing plant matter into the river and lake. The link between agricultural activity and a more diverse, aliphatic CHO-S (and CHO-N) signature has been reported in a study of large river systems from around the world (Wagner *et al.* 2015). Other studies have also reported more diverse CHO-S signatures in relation to different anthropogenic activities (Gonsior *et al.* 2011, Tseng *et al.* 2013). While the Matukituki River did not have a more diverse CHO-N signature than the Lake, this may reflect the fact that sulfonic acids are much better ionizers than organic nitrogen compounds, and are detected much more easily in FT-ICR-MS.

Potential sulfonates in the Matukituki River water could stem from agricultural activity in the catchment. Sulfonates are surfactants found in detergents, sewage sludge, fertilizers and pesticides (Jensen 1999, Sablayrolles *et al.* 2009, Gonsior *et al.* 2011). Sulfonates are used in pesticide and fertilizer formulations as they make good emulsifiers and dispersal agents (Jensen 1999, Sablayrolles *et al.* 2009). In the United States, lignin sulfonate (a synthetic product) can be used as a plant or soil amendment in farming practices in the United States, including on organic farms. Sulfonates have been recorded in undisturbed soils, possibly as a result of wind drift following aerial application of pesticides that contain linear alkylbenzene sulfonate (LAS) (Carlsen *et al.* 2002).

Sulfonates are generally highly biodegradable under aerobic conditions (Scott and Jones 2000, Jurado *et al.* 2013), with a > 90% biodegradation rate of LAS in laboratory tests (Jurado *et al.* 2013) and in wastewater treatment plants that employ aerobic treatment processes (Scott and Jones 2000). Sulfonate-like substances were found in water samples from the Matukituki River and both sites in Lake Wanaka (Table 22). However, the lowest diversity of CHO-S formulae were found at the deep water (100 m) site (Table 17), which could indicate these molecules are rapidly

biodegraded in the lake. It could also indicate they are removed through binding to other particles, uptake by biota or sequestration in the sediment.

Sulphur bound to organic compounds in the Matukituki River water could also be geologic in origin. Sulphur entering freshwater lakes is primarily derived from the weathering of rocks in the catchment and oxidation from organic sources (Girdano *et al.* 2008). In the Wanaka region, the primary basement rock types (quartzofeldspathic schist (greyschist) and greenschist (Craw 1984a, Rosen and Jones 1998) contain minor amounts of pyrite (FeS_2) (Craw 1984b). The large amounts of fresh rock in the Matukituki catchment can lead to fairly rapid weathering, resulting in the flushing of pyrite into streams and the Matukituki River. While weathering processes could oxidise the pyrite to sulphate and ferrous iron ($2FeS_2 + 7\ O_2 + 2\ H_2O \rightarrow 2Fe^{2+} + SO_4^{2-} + 4H^+$), this SO_4^{2-} would not bind with DOM in the water column, as DOM is negatively charged. Sulphate oxidised from pyrite could become part of the DOM pool through assimilation by plants. However, high rainfall and rapid erosion rates in the Valley means eroded pyrite or SO_4^{2-} is likely rapidly flushed into streams and downstream water bodies, reducing the amount of time available for incorporation into plant tissue.

5.5 Conclusions

Bulk concentrations of DOM in the Matukituki River and the water column of Lake Wanaka were similar, but more aromatic DOM structures were present in the deep water of Lake Wanaka than in surface waters. The higher number of aromatic structures in deep lake water may reflect more rapid removal of labile, aliphatic material by lake bacteria. Contrary to predictions, the diversity of aromatic DOM compounds was relatively low in the river, which could result from landscape characteristics and vegetation cover in the catchment, or possibly reflect the dominance of compounds derived from agricultural production, or even in-stream production.

The Matukituki River contained not only a greater number of sulphur-containing OM compounds than the lake, but also a more diverse range of CHO-S compounds than the lake. As my study provides only a snapshot of the DOM signature of these water bodies, a temporal analysis of DOM character in the River and Lake is needed to confirm my results. Some of this S is probably geologic in origin, but some may

reflect application of fertilizers with high SO_4-S content in the River catchment. Future work with sulphur stable isotopes could help elucidate the source material of the S, and quantify whether (or how much) CHO-S in the Matukituki River is a result of agricultural development in the catchment.

6 Bacterioplankton metabolism of dissolved organic matter in Lake Wanaka

6.1 Introduction

Land use influences delivery of dissolved organic and inorganic nutrients to streams and lakes, affecting microbial cycling of macronutrients and carbon (Findlay *et al.* 2001, Burns and Galbraith 2007, Galbraith and Burns 2007). The metabolism of dissolved organic matter (DOM) is generally attributed to heterotrophic microorganisms such as bacteria and other protists, which metabolise dissolved organic carbon (DOC) as an energy source (Johnson 2008). The rate of bacterial mineralisation of DOM is related to both the quantity and the "quality" of the DOM (Findlay *et al.* 2001). The "quality" of the DOM can be characterised by the molecular weight and the N- and P- richness of the DOM. DOM of high quality should be high in N and P content and of low molecular weight, to facilitate transport across membranes and enzymatic transformation (Freese *et al.* 2007).

"Native" bacteria residing in the water column metabolise DOM, either taking up available dissolved organic nitrogen (DON) and dissolved organic phosphorus (DOP) into microbial biomass, or mineralising it into inorganic forms that are available to algae (Bernhardt and Likens 2002, Bernhardt *et al.* 2005). Under conditions of N and P scarcity, bacteria can also compete with phytoplankton for inorganic nutrients (Thingstad *et al.* , Mindl *et al.* 2005, Cunha and Almeida 2009), and this ability to take up both organic and inorganic dissolved forms of N and P allows the bacteria to better cope with changes in nutrient availability in their environment (Cunha and Almeida 2009).

Seasonal changes in water column stratification can affect the supply of DOM and macronutrients to bacterial communities in lake systems. Increased water temperature, solar radiation and the development of a relatively shallower mixed layer in the spring can instigate phytoplankton blooms and the associated release of low molecular weight (LMW) DOM compounds for bacterial uptake (Ducklow *et al.* 1993, Kirchman 1994). Stratification can restrict the movement of this photosynthetically-produced DOM into the hypolimnion (Ducklow *et al.* 1993, Kirchman 1994) trapping deep water bacterial populations and resulting in reduced bacterial productivity (BP) and fewer numbers of actively respiring cells (Lovejoy *et*

al. 1996). Labile DOM associated with phytoplankton biomass (chl *a*) has been linked with bacterial productivity both in spring blooms (e.g. Cole et al. 1988), and during winter mixing (Lovejoy *et al.* 1996). Seasonal changes in DOM source and lability (associated with phytoplankton biomass and terrestrially-derived labile OM) have also been associated with taxonomic changes in the bacterial community (Dominik and Höfle 2002, Crump *et al.* 2003), while seasonal changes in physico-chemical variables in an estuarine system have been associated with changes in bacterial physiological diversity (Davis and Benner 2005). Bacterial productivity fuels the microbial food web and supplies carbon to higher trophic levels (Farooq and Long 2001). Therefore, it is important to understand the response of bacterioplankton communities to changes in DOM and macronutrient supply.

In Lake Wanaka, primary productivity and organic matter concentrations in the water column are low, and nutrient scarcity often limits phytoplankton growth (Bayer 2013). P-limitation is particularly apparent in the surface waters during the summer months when the lake is thermally stratified. However, Lake Wanaka experiences a winter/spring chl *a* maximum after turnover replenishes surface nitrate concentrations from the deeper waters (Bayer 2013). As chl *a* concentration is a biological indicator of phytoplankton biomass (Ducklow *et al.* 1993), more labile DOM (L-DOM) compounds may be present in the water column during these periods for bacterial metabolism. The effect this increase in chl *a* concentration has on microbial physiological diversity is unknown. The availability of phytoplankton-derived DOM may also correlate to the production of enzymes by the bacterial communities (bacterial physiological diversity; BPD) to break down a diverse array of organic substrates. Thus, I hypothesised that:

Hypothesis (i): BPD will increase during periods of high phytoplankton biomass (chl *a*) in Lake Wanaka.

Tributaries to the lake can also supply L-DOM to lake bacterioplankton communities. In general, DOM sourced from agriculturally modified catchments tends to be less structurally complex than DOM derived from forested or wetland-dominated catchments (Wilson and Xenopoulos 2009), and is therefore easier to metabolise. Currently, agricultural development in the Lake Wanaka catchment is dominated by low-intensity farming (Rosen and Jones 1998). In the Matukituki River catchment

(one of the two main inflows to Lake Wanaka) farms are primarily stocked with sheep, beef cattle and deer. As agricultural practices within the Matukituki River catchment may enhance the export of low molecular weight dissolved organic matter (LMW-DOM) to the lake, I hypothesised that:

Hypothesis (ii): Bacterial activity will be enhanced by the availability of terrestrially-derived labile DOM

In oligotrophic systems, macronutrient scarcity can affect bacterial biomass production (del Giorgio and Cole 1998). While conditions of nutrient scarcity, combined with low concentrations of organic matter (OM) in the water column may be sufficient for planktonic bacterial metabolism, growth may be restricted (Caron 1994, Stets and Cotner 2008). Under these conditions, bacterial respiration rates (BR) may remain high in order to keep metabolic pathways primed to take advantage of increases in nutrient concentrations (Smith and Prairie 2004, Berggren *et al.* 2010). The availability of L-DOM in the absence of N and P may result in the secretion of excess microbial carbon as organic exudate, or as respired CO_2 (Berggren *et al.* 2010). However, the availability of both labile organic matter and inorganic nutrients can strengthen the coupling between bacterial biomass synthesis and energy uptake. As nutrient concentrations (particularly P) are low in Lake Wanaka (Bayer 2013), I hypothesised that:

Hypothesis (iii): Microbial production will be stimulated by increasing P availability, while the increased availability of labile DOC will stimulate bacterial respiration (BR) (reference Hypothesis 2).

6.2 Methods

In June and October 2012 and February 2013, 20 litres of water were collected from a) the open water of Lake Wanaka in Aspiring Basin (44º35.702 S 169º04.030 E) at depths of 20 m and 100 m, and b) from the mouth of the Matukituki River. River and lake water were stored in acid-washed polyethylene containers, rinsed thoroughly with Milli-Q water, and transported on ice in the dark back to the laboratory. In the laboratory, all water was stored in the dark at 4°C, and experiments were set up within 24 hours of collection. Five litres of river water and 5 litres of lake water were filtered through Milli-Q-rinsed 0.22 µm polycarbonate filters to remove biota. The remaining lake water and river water was filtered through Milli-Q-rinsed 2.0-µm-pore

size polycarbonate filters which removed most phytoplankton, large heterotrophs and particle-attached bacteria, leaving free-living bacteria, small protists, pico-phytoplankton and picoeukaryotes.

6.2.1 Microbial Physiological Community Structure/Diversity

Biolog™ ecoplates (Biolog, Inc. 21124 Cabot Blvd., Hayward, CA 94545) were used to determine changes in bacterial physiological community structure in the lake and the river. Biolog™ ecoplates contain 31 simple organic substrates, including polymers, carbohydrates, amino acids, carboxylic acids and amines. Inoculation of the ecoplates with sample water can give a metabolic "fingerprint" of the microbial community based on the carbon substrates used. Changes in the fingerprint pattern can indicate changes in bacterial physiological community structure over time and bacterial physiological community structure between sites.

The Biolog™ ecoplates contain three replicate wells for each substrate, as well as three control wells that do not contain a carbon substrate. One hundred and fifty μl of 2.0 μm-filtrate from the lake (20 m or 100m) or the river were used to inoculate each well. Plates were inoculated within 24 hours of sample collection and incubated at 15°C in the dark for 7 days, with readings taken daily starting at time zero (immediately after inoculation). A temperature of 15°C was chosen as an incubation temperature to allow for rapid bacterial response while maintaining a temperature within the natural range for surface waters of the lake during summer (14.3 °C - 15.2 °C at 20 m depth). *In situ* temperatures at 100 m depth (9°C – 9.5°C) and the Matukituki River (7.3°C - 11°C) were consistently lower than incubation temperatures. At 20 m depth, *in situ* temperatures were lower than incubation temperatures in June (11.5 °C) and October 2012 (9.5°C). The ability of the bacterial community to metabolise a substrate was indicated by the formation of coloured tetrazolium salts. Colour development was measured at optical density OD590 on a FLUOStar Omega plate reader (BNG Labtech, Ortenberg, Germany) at the University of Otago, Dunedin. Plate readings made on day 5 were used to assess bacterial physiological diversity as the number of wells exhibiting color development plateaued by day 5 on all plates. In all incubations, the background signal in the control wells increased over the course of the incubation. To eliminate false positive readings as a result of background noise, a significant colour development detection limit was

established as the mean colour development of control wells plus two times the standard deviation of colour development in those wells. Optical density responses were converted to binary representations (where, 0 = no or negative response and 1 = positive response).

6.2.2 Bioavailability experiment

Bioavailability experiments were run in a pilot experiment in March, followed by experiments in June and October 2012, and February 2013. Treatments consisted of lake water controls, lake water + saturating concentrations of N and P (Lake+Nutrients), lake water + river water (Lake+River) and lake water + river water + nutrients (Lake+River+Nutrients) (Table 23). Lake water used in the experiments was sourced from 20 m depth at the Aspiring Basin site (44º35.702 S 169º04.030 E). For each treatment, 150 ml of 0.22-µm-filtered stream or lake water and 150 ml of 2.0-µm-filtered lake water were added to three replicate 300-ml BOD bottles. The BOD bottles were wrapped repeatedly in black plastic to prevent light penetration and loaded onto a plankton wheel running at 4 RPM to provide a constant rate of turbulence throughout the experiment. Room temperature was maintained at 12°C ± 1.0°C for all experiments, reflecting the average annual water temperature of Lake Wanaka. To determine whether nutrient enrichment stimulated metabolism of DOM in the treatments, parallel treatments were spiked with a high concentration of inorganic nutrients (1200 µg NO_3-N l^{-1} and 140 µg PO_4-P l^{-1} final concentration) (Table 23).

Table 23: Treatments used in the bioavailability bioassays in June and October 2012. In February 2013, only the Lake and Lake+Nutrients treatments were run.

Treatment	Inoculum and Exudate proportions	Nutrients
Lake	½ 0.22-μm filtered lake water ½ 2.0-μm filtered lake water	
Lake + Nutrients	½ 0.22-μm filtered lake water ½ 2.0-μm filtered lake water	1200 μg NO_3-N l^{-1} 140 μg PO_4-P l^{-1}
Lake + River	½ 0.22-μm filtered river water ½ 2.0-μm filtered lake water	
Lake + River + Nutrients	½ 0.22-μm filtered river water ½ 2.0-μm filtered lake water	1200 μg NO_3-N l^{-1} 140 μg PO_4-P l^{-1}

The experiment lasted for 15 days with dissolved oxygen (DO), dissolved organic carbon (DOC), total organic carbon (TOC), nitrate (NO_3-N), dissolved reactive phosphorus (DRP), total nitrogen (TN), total phosphorus (TP) and total dissolved nutrients (TDN) concentrations measured on day 0 and day 15. Dissolved oxygen was measured using a YSI 58 oxygen meter (YSI Inc., Yellow Springs, OH). Two

BOD bottles containing Milli-Q water were run alongside the treatments to account for drift in the oxygen meter between the start and end of the experiment. On day 0 and day 15, DO was measured twice in three randomly chosen bottles to also account for drift in the oxygen meter. Dissolved N, P and DOC concentrations were obtained by filtering 50-ml aliquots from each bottle through acid-washed pre-combusted Whatman, glass fibre GF/F filters (0.7 μm nominal pore size) that were pre-rinsed with 200 ml of Milli-Q water under low vacuum pressure (< 100 mmHg). Milli-Q water held in a pre-sterilised polyethylene container was filtered alongside stream water for quality control purposes. All water samples were then frozen at − 20°C prior to analysis. DOC was measured on a Shimadzu Total Carbon Analyser TOC-V CSH (Shimadzu, Kyoto, Japan) using potassium hydrogen phthalate as a standard. Samples were thawed, and then purged with ultra-pure oxygen to remove dissolved inorganic carbon (DIC). Four injections were run for each sample, with the three closest concentrations averaged to give the DOC value.

Dissolved and total nutrient samples were processed on a Skalar Auto-analyser (Skalar, Breda, the Netherlands) using standard colorimetric methods. TN and TP samples were digested using potassium peroxidisulphate, boric acid and sodium hydroxide and autoclaved for 30 minutes prior to analysis. To minimise sample contamination, all filtration equipment and plastic ware were acid-washed and rinsed with Milli-Q water, and Milli-Q water was filtered alongside water samples as laboratory blanks. Laboratory blanks and test tubes containing Milli-Q water were interspersed with field samples for quality control purposes and to ensure carryover between samples was negligible. Randomly chosen samples were re-run to account for drift in the instrument. Blank values were subtracted from treatment samples before analysis.

Bacterial respiration was calculated in two ways: as mg of carbon mineralised per m^3 per day (mg C m^{-3} day^{-1}) and the overall change in CO_2 (ΔCO_2) over the course of the 15-day experiment. ΔCO_2 was calculated indirectly using ΔDO as a proxy, assuming a respiration quotient (RQ) of 1 (Wetzel and Likens 2001). As the BOD bottles are a closed system, the movement of carbon should be traceable by the change in concentrations of TOC (measured as the non-purgeable organic carbon (NPOC) in 2.0 μm filtrate by the Schimadzu TOC analyser), DOC, POC and CO_2. In closed

systems, ΔTOC should be negatively associated with ΔCO_2 as the organic carbon source is broken down and mineralised. Therefore, if ΔTOC and ΔCO_2 are negatively correlated, then ΔPOC can be calculated as $= -1 * (\Delta DOC + \Delta CO_2)$ (Carlson *et al.* 1999). Change in POC was used to infer microbial biomass production.

6.2.3 Statistical Analysis

All statistical analyses were carried out using Canoco (v. 4.5) and SPSS (v. 21.1, IBM) software. Microbial physiological diversity was compared using correspondence analysis (CA), an ordination technique in which the main patterns of substrate metabolism by bacterial communities were statistically summarised on two independent axes. A Categorical Principal Components Analysis was also run to graphically display the relationship between carbon substrate usage, season and depth in Lake Wanaka. For each site and sampling date, organic substrates were grouped as carbon, hydrogen, oxygen (CHO) compounds, CHO plus nitrogen (CHO-N) or CHO plus phosphorus (CHO-P). Bacterial utilisation of substrates in these groups was compared using Chi-square (χ^2) tests. Physico-chemical parameters were compared with the total number of substrates utilised by day 5 using Pearson's product-moment correlation. Day 5 was chosen for comparison as the number of substrates being metabolised plateaued after this point in all sites. Comparisons of mean concentrations between sites were carried out using one-way ANOVA. If assumptions of normality or homoscedasticity were violated, Kruskal-Wallis non-parametric analyses were undertaken.

In the bioavailability experiments, one-way ANOVA followed by Tukey's *post hoc* test was used to compare treatment effects in June and October. In February, two-sample t-tests were performed to compare lake controls and the Lake+Nutrients treatment. In instances where the assumptions of normality or homoscedasticity were violated, comparisons were made using the Kruskal-Wallis non-parametric test. Potential correlations between variables (BR, ΔDOC, ΔPOC, ΔDRP, ΔNO_3-N) were made using Pearson's product-moment correlation. The strength of relationships between variables was tested using linear regression. In all analyses, the statistical threshold for significance was $\alpha = 0.05$.

6.3 Results

6.3.1 Bacterial physiological diversity and phytoplankton biomass

Table 24: Seasonal differences in organic substrates metabolised (X) by bacterioplankton communities from the Matukituki River and Lake Wanaka at 20 m and 100 m. MW = molecular weight (g). type = the substrate group (e.g. AN = amine, AA = amino acids, CHO = carbohydrates, PM = polymers, CA = carboxylic acids, CP = orthophosphates). 'N,P' represents whether the compound contained nitrogen or phosphorus. Total = the total number of organic substrates utilised from each site at each sampling date. A = Autumn (March), W = Winter (June), Sp = Spring (October), and S = Summer (February).

	MW	Type	N,P	20 m A	W	Sp	S	100 m A	W	Sp	S	River A	W	Sp	S
Putrescine	88.1	AN	N	X	X	X				X	X	X	X	X	X
Phenyl ethylamine	121.2	AN	N		X	X				X	X	X			X
l-Serine	105.1	AA	N	X	X	X				X	X	X	X	X	X
l-Threonine	119.1	AA	N							X					X
l-Asparagine	132.1	AA	N	X	X	X		X		X	X	X	X	X	X
Phenylalanine	165.2	AA	N				X			X	X				X
l-Arginine	174	AA	N		X	X				X	X	X	X	X	X
Glycyl-glutamic acid	204.1	CA	N		X	X				X	X	X	X		X
i-Erythritol	122.1	CHO			X	X			X	X	X	X	X	X	X
d-Xylose	150.1	CHO				X				X		X	X		
d-Mannitol	182.2	CHO		X	X	X	X	X	X	X	X	X	X	X	X
b-Methyl glucoside	194.2	CHO										X	X	X	X
N-Acetyl-Glucosaminic acid	237	CA	N	X	X	X		X	X	X	X	X	X	X	X
d-Cellobiose	342.3	CHO			X	X	X		X	X		X	X	X	X
α-D-Lactose	360.3	CHO				X			X	X		X	X	X	X
Glycogen	666.6	PM		X	X	X			X	X	X	X	X	X	X
α–Cyclodextrin	972.8	PM				X			X	X	X	X	X	X	X
Tween 40	1277	PM		X	X	X	X	X	X	X	X	X	X	X	X
Tween 80	1310	PM		X	X	X	X		X	X	X	X	X	X	
α-ketobutyric acid	102	CA							X	X			X		X
Pyruvic acid methyl ester	102.1	CA		X	X	X	X	X	X	X	X	X	X	X	X
γ hydroxybutyric acid	104.1	CA			X				X		X	X	X	X	X
Itaconic acid	130	CA			X	X			X	X	X	X	X	X	X
D-Malic acid	134.1	CA			X	X				X	X	X	X	X	X
4-Hydroxybenzoic acid	138.1	CA			X	X				X	X	X	X	X	X
2-Hydroxybenzoic acid	138.1	CA									X				
d-Galacturonic acid	194.1	CA		X	X	X	X	X	X	X	X	X	X	X	X
d-Glucosaminic acid	195.2	CA	N	X	X	X			X	X	X	X	X	X	X
d-Galactonic acid	196	CA		X	X	X	X		X	X	X	X	X	X	X
D-l-α-Glycerol phosphate	172.1	CP	P		X	X			X	X	X	X	X	X	X
Glucose-1-phosphate	260.1	CP	P	X					X	X		X	X	X	X
Total				**13**	**22**	**25**	**7**	**6**	**15**	**28**	**24**	**27**	**27**	**24**	**28**

At 20 m depth, the bacterial community metabolised fewer amine, amino acid and orthophosphate substrates in February (summer) and March (autumn) than in the June (winter) and October (spring) (Table 24). A similar pattern occurred at 100 m, although the pattern lagged behind by one season (Figure 25A and B). At 100 m, the bacterial community metabolised fewer amine and amino acid substrates in March and June than in the October and February. The ability of bacteria to metabolise substrates was not correlated with the molecular weight of the compounds at either depth or during any season.
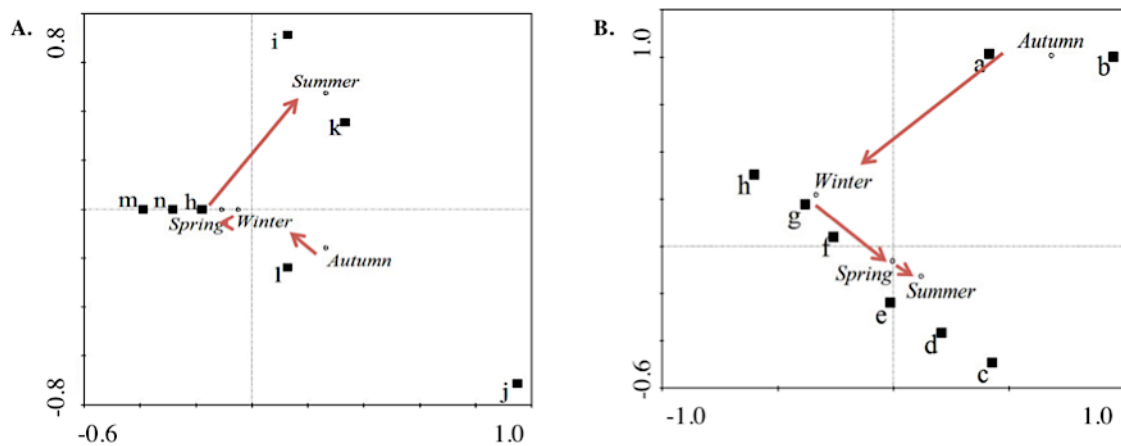
Figure 25: Ordination plots of seasonal differences in the number and type of organic substrates being metabolised by the microbial community at A. 20 m depth and B. 100 m depth. In plot A., Axis 1 (45%) represents the difference in substrate utilisation in the summer compared with the other seasons, while Axis 2 (36%) represents the difference in substrate utilisation in March (autumn) compared with June (winter) and October (spring). In plot B., Axis 1 (43.4%) is interpreted as the difference in substrate utilisation in the autumn compared with other times of the year; while Axis 2 (100m: 39.9%) represents the difference in substrate utilisation in June (winter) and October (spring) compared with February (summer). (○) represents the season the sample was taken, while (■) represent the organic substrates metabolised by the bacterial communities. As there were a limited number of patterns of organic substrate utilisation, substrates often overlapped in ordination space. In A., letters represent: a = N-acetyl-glucosaminic acid, b = l-asparagine; c = 2-hydroxybenzoic acid; d = 4-hydroxybenzoic acid; a-cyclodextrin; D-l-a-glycerol phosphate; d-mallic acid; glycyl-glutamic acid; l-arginine; l-serine; phenylalanine, phenylethyamine; putrescine; e = d-xylose, l-threonine; f = d-glucosaminic acid, itaconic acid, i-erythritol, glycogen, tween 80; g = a-D-lactose, a-ketobutyric acid, d-cellobiose, glucose-1 phosphate h = γ-hydroxybenzoic acid. In plot B, letters represent: i = d-cellobiose; j = glucose-1-phosphate (used only in the autumn); k = d-galactonic acid, tween 80 (used during every season); l = d-glucosaminic acid, glycogen, l-asparagine, l-serine, n-acetyl-glucosaminic acid, putrescine (used June, October, March); m = a-cyclodextrin, a-D-lactose, d-xylose, phenylalanine (used only in October); n = 4-hydroxybenzoic acid, D-l-a-glycerol phosphate, D-mallic acid, glycyl-glutamic acid, i-erythritol, itaconic acid, l-arginine, phenylethylamine (used in June and October); h= γ-hydroxybenzoic acid.

Binary substrate utilisation data were plotted in a correspondence analysis. While no correlations among substrates were observed in the ordination plots, seasonal patterns

in substrate use were observed. At 20 m (Figure 25A), Axis 1 accounts for 45% of total variance in the model, and represents the difference in substrate utilisation pattern in the summer compared with other times of the year. Axis 2 (36% of total variance in the model) represents the difference in substrate utilisation pattern in June (winter) and October (spring) compared with March (autumn). Bacterial metabolic activity at 100 m depth followed a similar pattern, (where Axis 1 = 43.4% and Axis 2 = 39.9% of total variance) although it lagged behind patterns observed at 20 m by one season (Figure 25B). This lag is apparent in a component loadings plot showing seasonal differences in organic substrate metabolism at both depths (Appendix E, Figure E1). The ordination plots showed that seasonal changes in bacterial physiological community structure were reflected the overall number of substrates used by the bacterial community. Thus, the diversity of organic substrates utilised per plate was compared with chl a concentrations and physico-chemical parameters measured at the time of sampling. Chlorophyll a, DOC and nutrient concentration ($\pm$ 1 standard deviation) for each sampling date are shown in Table 25.

Table 25: Mean values $\pm$ 1 standard deviation of physico-chemical and biological parameters in Lake Wanaka and the Matukituki River at the time water samples were taken for analysis of microbial physiological diversity. T = water temperature (°C), DOC = dissolved organic carbon (mg l^{-1}), NO$_3$-N = nitrate (μg l^{-1}), DRP = dissolved reactive phosphorus (μg l^{-1}), chl a (mg m^{-3} at 20 m depth), total substrates = the total number of carbon substrates used by day 5 following incubation on Biolog Ecoplates, * = no data. b.d. = below detection

Site	Month	T	DOC	NO$_3$-N	DRP	chl a	Total substrates
River	March	10.9	2.47	46.34	2.41	*	27
	June	7.9	3.32 ± 0.49	49.17	1.94	*	27
	October	7.3	8.50 ± 0.20	48.2 ± 3.3	1.0 ± 0.3	*	24
	February	*	2.28 ± 0.43	21 ± 1.47	1.12 ± 0.12	*	28
Lake - 20 m	March	15.1	2.36 ± 0.34	14.37	b.d.	1.2	13
	June	11.1	3.05 ± 0.24	30.3 ± 0.18	0.45, 0.28	1	22
	October	9.6	6.77 ± 0.98	34.9 ± 1.15	b.d.	1.61	25
	February	14.3	2.12 ± 0.33	22.7 ± 0.66	1.02, < 1.0	0.41	7
Lake - 100 m	March	10.6	1.87 ± 0.06	49.62	b.d.	0.29	6
	June	11	3.25 ± 0.004	*	*	0.82	15
	October	9.4	6.95	*	*	1.55	28
	February	9.5	2.55 ± 0.27	39.2 ± 0.44	0.97, < 1.0	*	24

Bacterial physiological diversity (total substrates used) was strongly positively associated with chl a concentration (r = 0.901, R^2 = 0.812, p = 0.006) in the lake

(Figure 26). DOC concentration was also positively correlated with both chl a ($r = 0.850$, $p = 0.015$, $n = 7$) and the total number of substrates used ($r = 0.744$, $R^2 = 0.554$, $p = 0.034$) in the lake (Figure 26). Although a negative trend between BPD and temperature was apparent, the relationship was not significant ($r = -0.656$, $R^2 = 0.430$, $p = 0.077$, $n = 7$).
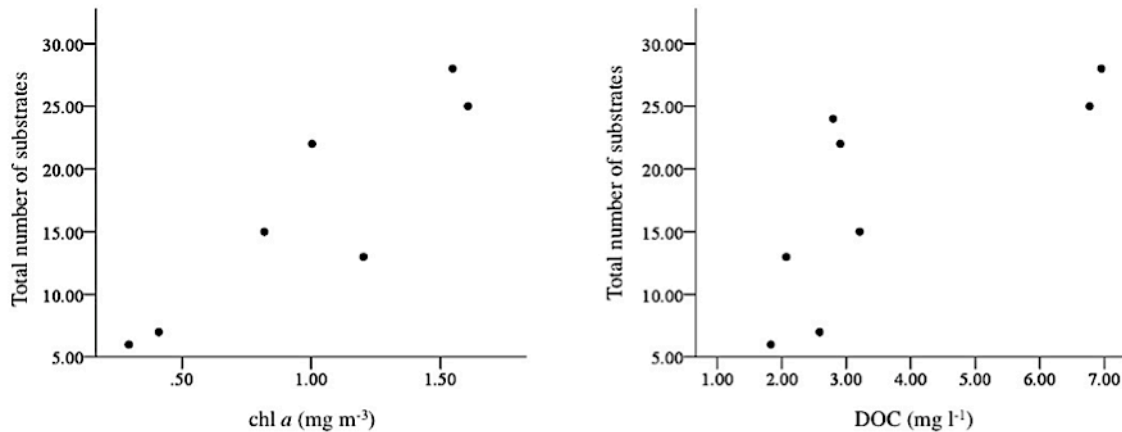
Figure 26: Scatterplot of the total number of organic substrates utilised by the bacterial community in Lake Wanaka and (left panel) chl a concentration (mg m^{-3}) or (right panel) dissolved organic carbon (DOC) concentration (mg l^{-1}) at the time of sampling.

6.3.2 DOC lability and bacterial activity

The relationship between bacterial activity and the availability of terrestrially-derived DOM was examined using i) BPD, ii) bacterial respiration and iii) DOC uptake rates. In the bioassays, lake water was amended with river water from the Matukituki River catchment in order to determine the quality of the riverine DOM, based on the responses of the microbial parameters. In both the October (spring) and June (winter) experiments, DOC concentrations decreased while CO_2 concentrations (estimated by changes in DO) increased, reflecting the respiration of DOC. However, this relationship was only statistically significant during the October experiment (October: $r = -0.737$, $p = 0.015$, $n = 10$; June: $r = -0.693$, $p = 0.057$, $n = 8$). As all experiments were conducted at the annual mean temperature of 12°C, these relationships reflect differences in DOM lability, not in seasonal temperatures.

While BR rates were not significantly different between treatments in June, respiration rates ($F_{(1,4)} = 43.920$, $p = 0.003$) were significantly higher in the Lake+River treatment than in the control in October. In June, significantly more DOC ($F_{(1,3)} = 23.372$, $p = 0.017$) was taken up in the Lake+River treatment than in the

Lake treatment. This difference was not apparent in October, probably because of high within-treatment variability in DOC uptake (Lake+River = -0.24 ± 0.16, Lake = -0.09 ± 0.28 mg DOC l^{-1}).

The ecoplate incubations showed bacterial communities in the Matukituki River were capable of metabolising a wide variety of substrates regardless of season (Figure 27). The strong variability in carbon substrate uptake in the lake compared to the river meant the ordination plots could be interpreted in the same way as described in Figure 25. In a correspondence analysis using combined data from the river and the lake, the first two axes explained 56.2% (using data from 20 m depth) to 58.6% (using data from 100 m depth) of total variance (Figure 27). Using water from 20 m, Axis 1 (35.2% of total variance) represents the difference in substrate utilisation in the February (summer) compared with other times of the year while Axis 2 (21% of total variance) represents the difference in substrate utilisation in June (winter) and October (spring) compared with March (autumn).
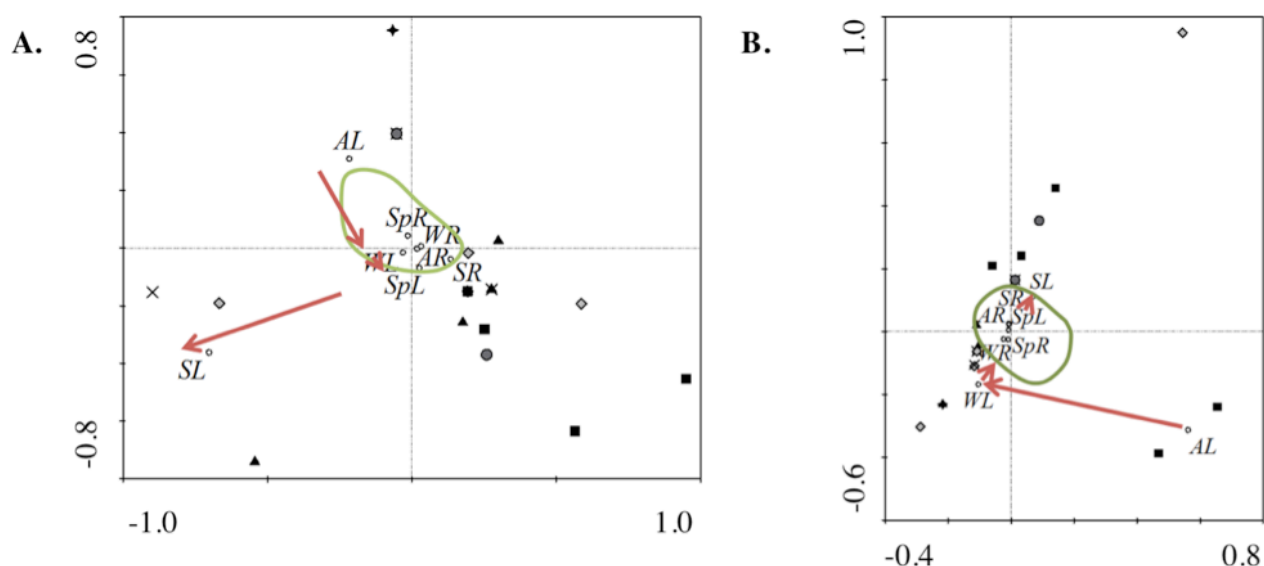
Figure 27: Ordination plots of the seasonal differences in bacterial physiological diversity in the Matukituki River and Lake Wanaka at A., 20 m depth and B., 100 m depth. In A., Axis 1 explains 35.2% of total variance, while Axis 2 explains 21% of total variance. In B., Axis 1 explains 30.2% of total variance, while Axis 2 explains 28.4% of total variance. Site codes: SL = Summer-Lake; SpL = Spring-Lake; WL = Winter-Lake; AL = Autumn-Lake; SR = Summer-River; SpR = Spring – River; WR = Winter- River; AR = Autumn – River. Symbols represent organic substrate groups: (■) amino acids; (●) amines; (▲) CHO; (x) polymers; (✦) orthophosphates; (◆) carboxylic acids. Clustering of Matukituki River samples is indicated by the green circle. Red arrows indicate seasonal changes in carbon substrate use in the lake.

Bacterial metabolic activity at 100 m depth followed a similar pattern, although it lagged behind patterns at 20 m by one season (Figure 27). In (B) Axis 1 (30.2% of total variance) represents the difference in substrate utilisation in March (autumn) compared with other times of the year; while Axis 2 (28.4% of total variance) represents the difference in substrate utilisation in June (winter) and October (spring) compared with February (summer). Samples from the Matukituki River tended to cluster together as organic substrate uptake that was consistently high regardless of season (Figure 27). The number of substrates used in the lake was most similar to those in the river in October (spring).

Within the river samples, DOC positively correlated with BPD ($r = 0.978$, $R^2 = 0.986$, $p = 0.022$, $n = 4$). This relationship between DOC and microbial physiological diversity was not apparent when the River and Lake data were combined, likely because of the consistently higher number of substrates used in the river. A Kruskal-Wallis comparison confirmed the riverine microbial population was capable of metabolising more carbon substrates than the lake bacterial population at 20 m ($\chi^2 = 4.133$, $p = 0.042$). Significantly higher DRP concentrations were recorded in the Matukituki River than the Lake ($F_{(2, 7)} = 32.114$, $p < 0.001$), and when the Lake and River data were combined, bacterial physiological diversity was positively associated with DRP concentration ($r = 0.643$, $p = 0.013$, $n = 9$).

6.3.3 P availability, bacterial activity and bacterial production

Nutrient enrichment did not increase microbial biomass production (as measured by the change in particulate organic carbon concentration (ΔPOC)). Instead, POC concentrations decreased in almost all experiment bottles regardless of season or nutrient enrichment. This decrease in POC may be related to limitations of my experimental design (see the Discussion, section 6.4.3.). Nutrient enrichment increased BR in Lake controls and Lake+River treatments, although the difference between treatments was only significant in October (Figure 28). In October, respiration rates increased in lake water when amended with either river water or saturating concentrations of N and P ($F_{(3,8)} = 29.415$, $p < 0.001$), indicating that N- and P-limitation may be affecting uptake of DOM at this time of year.

Dissolved oxygen concentration changed very little in the Lake Control treatment during the October experiment. While DO concentration decreased in all the Lake

Control BOD bottles over the course of the October experiment, the decrease in one of the replicates could be accounted for by drift in the oxygen meter. After correcting for drift, inclusion of this replicate reduced the mean respiration rate of the Lake Control. In the other two Lake Control BOD bottles, respiration rates were 1.3 and 1.8 g C m^{-3} day^{-1} after correcting for drift.
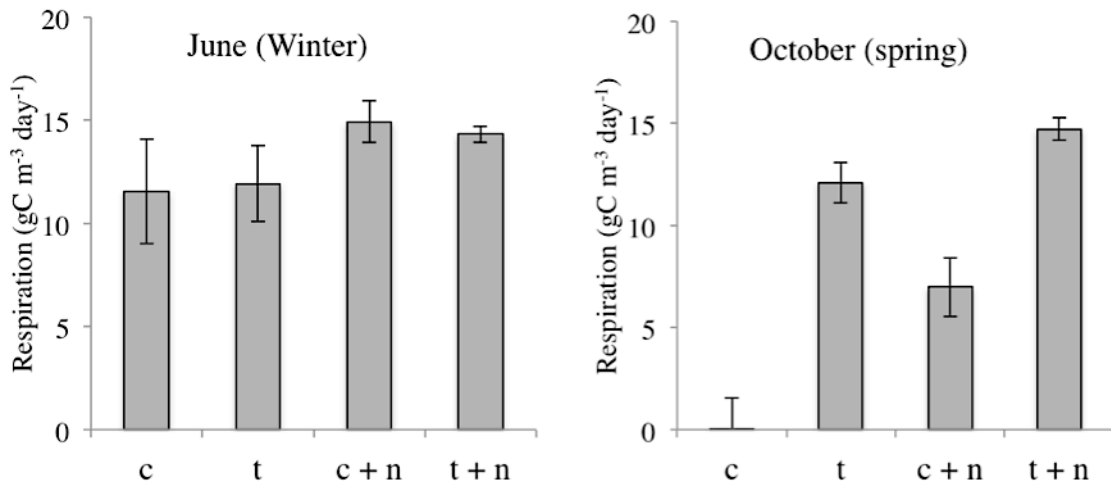
Figure 28: Mean respiration rates for treatments from the winter (June) and spring (October) bioavailability experiments. Any dissolved oxygen (DO) change less than 0.26 mg l^{-1} in June, or 0.2 mg l^{-1} in October and March can be attributed to instrument error, equivalent to 6.5 and 5 g C m^{-3} day^{-1}, respectively. Error bars represent ± 1 standard error. c = Lake water control, t = Lake water + Matukituki River water, c+ n = Lake water + nutrients, t+n = Lake water + Matukituki River water + nutrients.

DOC and DRP uptake increased with nutrient enrichment (Figure 29). In June (winter), October (spring) and February (summer), DRP uptake was higher in nutrient-enriched treatments than in unamended treatments (Appendix E Table E1). Dissolved organic carbon uptake also increased in nutrient-enriched treatments compared with unamended treatments in February and October. While DOC uptake was highest in the Lake+River treatment in June, the difference between treatments was not significant (Appendix E, Table E1). Nitrate-nitrogen uptake increased in nutrient-enriched treatments compared with unamended treatments in October and June. When data from the nutrient-enriched treatments were pooled, DOC uptake was not significantly associated with DRP uptake in nutrient-enriched treatments (Lake+River+Nutrients: $r = 0.104$, $r^2 = 0.011$, $p = 0.760$; Lake+Nutrients: $r = 0.525$, $r^2 = 0.276$, $p = 0.226$).
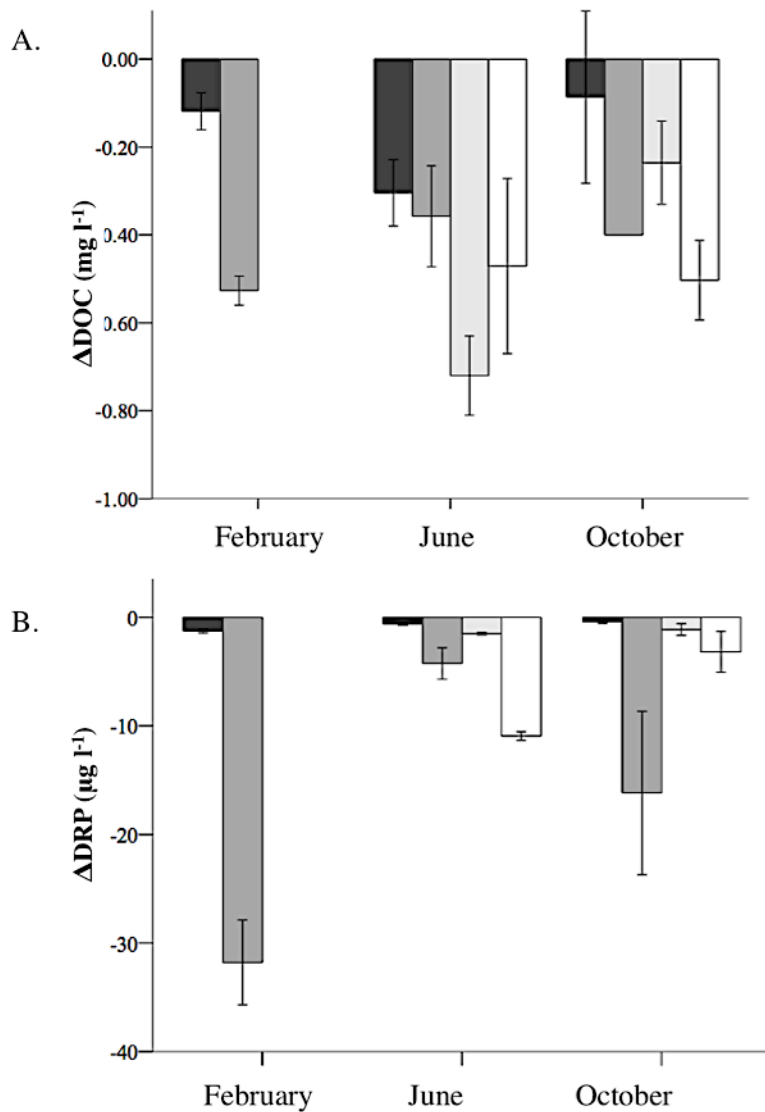
Figure 29: Mean values of the A., change in dissolved organic carbon (DOC) (ΔDOC) and B., dissolved reactive phosphorus (ΔDRP) over the 15-day incubation period in the February, June and October bioavailability experiments. Lake Controls (■); Lake+Nutrients (■); Lake+River (■); Lake+River+Nutrients (□). Bars represent ± 1 standard error.

6.4 Discussion

6.4.1 Bacterial physiological diversity and phytoplankton biomass

In support of my hypothesis that bacterial physiological diversity (BPD) would be postively related to phytoplankton biomass, the microbial community utilised a more diverse range of substrates as chl *a* concentrations increased. In Lake Wanaka, seasonal patterns in substrate use differed by depth, and substrate use phenology in the hypolimnion lagged behind shallow water patterns by one season (Figure 30, Appendix E, Figure E1). While taxonomic bacterial community structure has been reported to vary vertically and temporally in lake systems (Hofle *et al.* 1999, Dominik and Höfle 2002, Nelson 2008), my study appears to be the only reported instance of

vertical and temporal variations in physiological bacterial community structure in a freshwater lake.

Nelson (2008) noted bacterial communities were most taxonomically similar when the water column was well-mixed, while thermal stratification produced distinct communities, "with hypolimnetic communities generally grouping with samples from the previous date" (Nelson 2008). Dominik and Hofle (2002) also reported that epilimnetic and hypolimnetic bacterial communities were distinctive during periods of thermal stratification in a small (14.3 ha, 29.2 m deep) eutrophic lake. In their study, bacterial taxonomic diversity decreased with depth, except at the deepest hypolimnetic site in the spring when the thermocline was newly established. At that time, the bacterial community diversity increased, which Dominik and Hofle (2002) attributed to bacterial degradation of senescent phytoplankton from the previous autumn. While bacterial taxonomic diversity does not necessarily reflect physiological diversity (Langenheder *et al.* 2005), the influence of thermal stratification on substrate use patterns in my study agrees with temporal changes in bacterial taxonomic diversity reported by Nelson (2008) and Dominik and Hofle (2002).

It is not surprising that seasonal variations in biological, chemical and physical parameters within lake systems influence bacterial physiological and phylogenetic community structure. Phytoplankton blooms can release highly labile DOM into the water column for bacterial uptake (Dominik and Höfle 2002, Crump *et al.* 2003, McCallister and del Giorgio 2008), increasing bacterial activity through the release of dissolved free amino acids (DFAAs) and other low molecular weight DOM (Ducklow *et al.* 1993, Kirchman 1994, Davis and Benner 2005). Seasonal variations in thermal stratification can affect the settling and advection of phytoplankton- and DOM-rich waters into deeper waters, affecting vertical patterns of bacterial respiration and growth (Ducklow *et al.* 1993). In the open water of Lake Wanaka, the distribution of chl *a* was strongly influenced by thermal stratification in the lake. During winter mixing, chl *a* was uniformly distributed throughout the water column, while during the summer/early autumn, when thermal stratification was well-established, the highest concentrations of chl *a* were confined to the upper 50 m of the water column (see Chapter 3). In March 2012, maximum chl *a* concentrations occurred above 42 m, with a chlorophyll maximum occurring at 26 m.
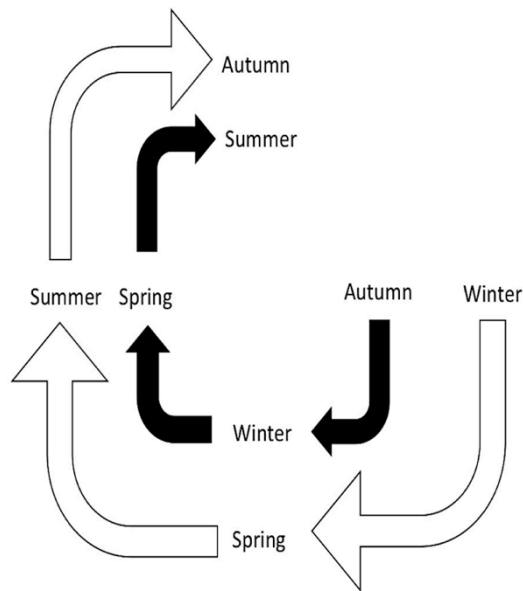
Figure 30: Schematic diagram showing the delayed effect of thermal stratification in Lake Wanaka on organic substrate uptake by hypolimnetic bacterioplankton (100 m depth) (white arrows) compared to epilimnetic bacterioplankton (20 m depth) (black arrows).

Previous research suggests Lake Wanaka experiences annual chl *a* maxima in the winter/early spring when the lake is mixed (Bayer 2013), and the relatively high concentrations of chl *a* recorded in the winter (June 2012: 0.63 – 1.1 µg l^{-1}), and early spring (October 2012: 1.40 – 1.92 µg l^{-1}, depth > 5 m) during my study support this conclusion. Winter chl *a* maxima are unusual in monomictic lakes as algal biomass and productivity traditionally decrease due to cool water temperatures and reduced light availability (Vincent 1983). However, winter peaks in phytoplankton biomass have been reported in other New Zealand lakes, including Lake Coleridge and Lake Taupo (White *et al.* 1980, Vincent 1983, Schallenberg and Burns 1997, James *et al.* 2001). In Lake Wanaka, winter phytoplankton growth is likely enabled by good water transparency (Bayer 2013) (2009 and 2011: Secchi depth ranged from 8 to 14.2 m (Weaver, unpublished data); 2006 to 2007 Secchi depth ranged from 9 to 19.2 m (Otago Regional Council 2009)) combined with replenishment of nutrients from the hypolimnion (Bayer *et al.* 2015).
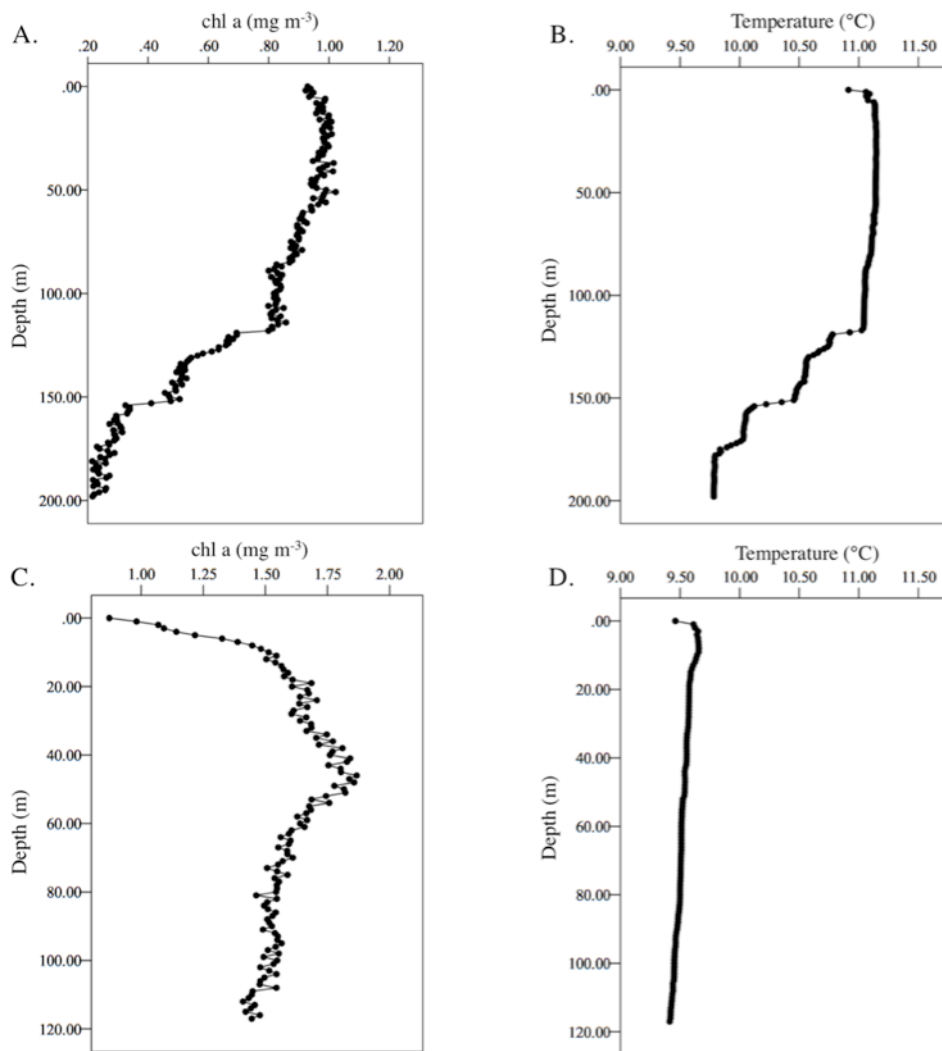
Figure 31: Chlorophyll *a* and water temperature profiles from the open water of Lake Wanaka in June and October 2012. A., shows chl *a* and B., shows water temperature in June 2012. C., shows chl *a* and D., shows water temperature in October 2012.

Although organic substrate uptake was not significantly linked to temperature, temperature may be an indirect driver of bacterial physiological diversity via thermal stratification and mixing. In March 2012, when thermal stratification in the lake was well-established, few organic substrates were metabolised in shallow (20 m) or deep (100 m) waters. As the thermocline broke down in June 2012 (Figure 31), the number of substrates used by the bacterial community increased at 20 m, but not at 100 m. The increase in the number of organic substrates used corresponded to increased chl *a* concentration in shallow waters (Figure 31). However, a weak thermocline was still present (1.2 °C change over 70 m) at 100 m, which may have restricted the availability of labile DOM to deep water bacterial communities. In October 2012, when the lake was completely isothermal, chl *a* concentrations were high and

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distributed fairly uniformly between 20 m and 100 m depth. Under these conditions, bacterial communities from 20 m and 100 m in Lake Wanaka were functionally capable of metabolising a diverse array of organic substrates. In February 2013, the lake was thermally stratified and algal biomass at 20 m was low. Few organic substrates were utilised in shallow waters, but microbial communities in deep waters were still capable of metabolising a variety of organic substrates.

6.4.2 DOC lability and bacterial activity

In River and Lake ecoplate incubations, riverine bacterial populations were able to consisently metabolise a wide range of carbon substrates regardless of season (Table 25), suggesting riverine microbial communities were 'acclimatised' to a variety of DOM sources (Stutter and Cains 2016). The greater variety of heteroelement DOM signatures in the Matukituki River compared with Lake Wanaka (see Chapter 5) supports the suggestion that riverine microbial communities are acclimatised to a more diverse DOM pool than communities in the lake. The ability of the bacterial community to break down a wide variety of substrates may also reflect consistent exposure to a labile supply of DOM, as well as a more more consistent nutrient supply, as DRP concentrations were higher in the river than the lake.

While allochthonous DOC has frequently been considered recalcitrant (Tranvik 1992, Jaffe *et al.* 2008), recent studies have shown terrestrially-derived DOM can alter bacterial community composition and stimulate bacterial activity and productivity (Findlay *et al.* 2001, Crump *et al.* 2003, Catalán *et al.* 2013). Catalán et al. (2013) reported increased DOC uptake, bacterial growth efficiency and cell-specific growth when bacteria from a coastal lagoon were exposed to terrestrially-derived labile DOC. They found that DOC inputs into this large, shallow lagoon (78 ha, mean depth1.37 m) were less aromatic than autochthonous DOM, and could be rapidly degraded into labile material through photooxidation and bacterial interaction (Catalán *et al.* 2013). Crump et al. (2003) reported that seasonal shifts in bacterioplankton community composition and bacterial productivity were related to shifts in labile DOM sources in Toolik Lake, Alaska, with pulsed inputs of terrestrially-derived labile DOM from snowmelt triggering transient but significant shifts in bacterial community composition (Crump *et al.* 2003). As Toolik Lake is small and shallow (1.5 km^2, 25 m maximum depth, 7 m mean depth), changes in bacterial community composition

were influenced in part by the influx of riverine bacterial communities during these pulsed flows.

In the bioavailability experiment, the increase in bacterial respiration and DOC uptake rates in treatments amended with Matukituki River water also supports my hypothesis that bacterial activity will increase in response to increased availability of terrestrially-derived labile DOM. In these experiments, the measures of bacterial activity increased in response to additions of Matukituki River water. In October, bacterial respiration rates were significantly higher in Lake+River treatments than in the Lake Controls. In June, bacterial respiration rates were high in both the Lake and Lake+River treatments, but more DOC was taken up in the Lake+River treatment than in the Lake Control. Increased uptake of DOC in the Lake+River treatment may reflect the quality of the riverine DOM. As DRP concentrations were significantly higher in the Lake+River treatment than the Lake Control, increased uptake of DOC in the Lake+River treatment may also reflect the effect of nutrient limitation on bacterial activity in the Lake Controls.

6.4.3 Nutrient enrichment and bacterial activity

Nutrient enrichment did not produce a substantial increase in POC concentration in any of the treatments during any experimental run. This result contradicts my hypothesis that bacterial production would increase with increasing availability of P, and contrasts other studies of temperate lake systems (Vidal *et al.* 2011 Smith and Prairie 2004, and others). As there are difficulties associated with using POC as an estimate of bacterial productivity, the results of my study may reflect limitations of my experimental design.

Frequently, radiolabelled substrates (such as [^{14}C] leucine or [^{3}H] thymidine) are used to measure bacterial productivity (BP) in short-term experiments (< 36 hours) (Tibbles and Harris 1996). In these experiments, replicate samples and a kill control are taken from each treatment at established time intervals, a pre-determined amount of [^{14}C] leucine or [^{3}H] thymidine is added, and the samples are incubated for 0.5 to 3 hours (del Giorgio and Cole 1998). Incubation times are short as longer incubations can reflect substrate uptake by organisms other than bacteria, or the movement of radiolabelled material to other parts of the bacterial cell (Moriarty 1990). While these techniques are very sensitive measures of BP, the short incubation time makes it

difficult to compare BP with BR, as measures of oxygen uptake require longer time scales (> 24 hours) (del Giorgio and Cole 1998).

Long-term experiments are advantageous in this respect, as one can measure changes in DOC, POC and respiration rates over the same time scale. However, there are several drawbacks to this experimental design. First, bacteria may adhere to walls of the treatment vessel, confounding abundance measures (Zobell and Anderson 1936, del Giorgio and Cole 1998). Second, filtration does not isolate bacteria from other picoplankton (del Giorgio and Cole 1998), and some predators, such as heterotrophic nanoflagellates (HNF) can also pass into the filtrate. Predator grazing during the experiment can affect bacterial abundance, productivity and respiration (del Giorgio and Cole 1998). In one study, the presence of HNF in incubation bottles reduced bacterial biomass accumulation by more than half, and increased respiration rates in these bottles were attributable to grazer activity (Johnson and Ward 1997).

A third drawback of the experimental design is that some planktonic organisms (including some bacterial species) do not respond well to confinement in bottles. Auto-inhibitory metabolites can form during microbial growth in bottle experiments, resulting in a decrease in biomass of some bacterial species (e.g. *Escherichia coli*) (Landwall and Holme 1977). Bottle incubations can also negatively affect autotrophic picoplankton in the filtrate. In their 2011 study, Calvo-Diaz *et al*. reported a strong (> 50%) decrease in picophytoplankton biomass over a 24-hour period during bottle incubations. As this decrease was not apparent *in situ*, the authors suggest that their results reflect the adverse reaction of the picophytoplankton to bottle confinement (Calvo-Diaz *et al.* 2011).

Although nutrient enrichment was not associated with increased bacterial productivity, nutrient enrichment stimulated microbial respiration and uptake of DOC and DRP in Lake water. Nutrient concentrations and respiration rates were higher in all treatments compared with the Lake Control, which suggests low nutrient availability may inhibit bacterial activity in unamended lake water. As I did not quantify bacterial productivity (BP), I cannot directly compare DOC or P uptake with bacterial growth in my treatments. However, relationships between C and P availability or uptake and BR, BP and/or BGE have been reported in multiple studies (Smith and Prairie 2004, Hall and Cotner 2007, Stets and Cotner 2008, Berggren *et al.*

2010, Vidal *et al.* 2011). In a study of 20 boreal lakes in Canada with a range of DOC and P concentrations, Smith and Prairie (2004) reported that bacterial productivity was dependent not only on C, but also the P availability. They did not find a relationship between addition of a labile DOC source (glucose) alone and increased bacterial growth. Likewise, Vidal et al. (2011) reported that additions of C (in the form of glucose) alone produced no significant increases in any of the parameters measured (BA, BR, BP, SBR, ΔDOC, ΔP). Instead, they found that C+P added to water from four boreal lakes resulted in significant increases in BR (1.5 to 8.6 times) and BP (0.3 to 1.26 times). Stets and Cotner (2008) reported that DOC additions increased DRP uptake in oligotrophic lake water, although the same result did not occur in eutrophic lake water. Likewise, Carlson and Ducklow (1992) found that the addition of labile carbon in the form of amino acids or glucose increased bacterial growth efficiency (BGE). However, in treatments with added glucose, cells produced storage C and increased in mass rather than in abundance. It is possible that the combination of labile DOC and P stimulated both bacterial activity (as measured by BR) and bacterial growth in my treatments, but the limitations of the experimental design meant BP was not adequately measured. Future studies may consider using more sensitive measures of BP while also accounting for BR in this oligotrophic system.

6.5 Conclusions

Land use intensification is increasing DOC and N loads to Lake Wanaka (Chapter 2). While in vitro experiments do not reflect *in situ* conditions and cannot be extrapolated directly to the field, my results show that labile DOC and P are currently entering the lake via the Matukituki River, and that this material is capable of stimulating bacterioplankton metabolic activity. The phenology of substrate utilisation in the epilimnion and hypolimnion of the lake suggest that microbes are strongly coupled to autochthonous OM. As microbial activity also increased with additions of river water and nutrients, lake bacterioplankton are likely opportunistic and flexible. Increased inputs of nutrients and DOC to the lake from development can stimulate microbial uptake of P, resulting in these microbes outcompeting phytoplankton for this key nutrient. The implication of this change on trophic interactions in the lake warrants further study.

7 General Discussion

7.1 Importance of freshwater resources

Fresh water provides habitats for almost 6% of all vertebrate and invertebrate species on Earth (Shiklomanov 1993, Dudgeon *et al.* 2006, Carrizo *et al.* 2013) and is important for human survival. Despite the importance of freshwater in many facets of human life, many anthropogenic activities lead to water pollution, habitat degradation and biodiversity loss. Over the past 40 years, water quality in New Zealand has been declining, mainly due to the increased influx of diffuse source pollutants as land development, particularly agricultural development, increases (McColl 1972, Vincent *et al.* 1984, Davies−Colley 2013, Larned *et al.* 2016).

On the South Island of New Zealand, water quality in upland catchments is generally good (Davies−Colley 2013) and land use activities are relatively low-intensity. Land use intensification in these upland catchments can substantially increase in-stream nutrient concentrations (Niyogi *et al.* 2007), resulting in increased nutrient export (Johnson 2008) to downstream lakes. While nutrient and organic matter loading to receiving lakes may initially be diluted by lake volume, the continual input of external material can cause shifts towards degraded water quality over time (Edmondson 1994, Jassby *et al.* 2003, Scavia *et al.* 2014). Large lakes are no exception, with instances of eutrophication documented in large lakes from New Zealand and abroad (Edmondson 1994, Schanz 1994, Edgar 1999, Caruso 2000, Rutherford 2003).

7.2 Contributions of my research project

In the examples cited above, large lakes underwent significant changes as a result of intensive anthropogenic activity. However, when urban or agricultural development in a catchment is relatively low-intensity, determining the effect of land use on in-lake processes requires very precise study. My research project contributed to our knowledge of how land use practices impact on aquatic ecosystems by examining the effect of low-intensity development of high country grassland catchments on nutrient dynamics and microbial activity in a large, low-productivity lake system.

A key finding of my work is that there is a strong correlation between the proportion of pasture in the catchment and dissolved organic carbon (DOC) in streams during periods of low to moderate flow, but that air temperature and soil moisture can mitigate the influence of pasture cover on surface water DOC concentration under wet

or very dry conditions. While no known studies of DOC dynamics in New Zealand currently include soil moisture as a fixed effect, the mediating effect of soil moisture on the relationship between landscape characteristics and in-stream DOC concentration has been reported overseas (Wilson and Xenopoulos 2008). As the predictive strength of landscape variables can decrease as soil conditions deviate from 'normal' (Wilson and Xenopoulos 2008), inclusion of soil moisture in predictive models can be useful in helping explain variations in DOC. When sampling streams with flashy hydrographs, soil moisture may serve as a more useful predictor variable of DOC concentration than rainfall, particularly if intensive sampling is not feasible.

I also found a relationship between the proportion of pasture in the catchment and increased concentrations of inorganic nitrogen (N) in stream water in Chapter 2, but no relationship between vegetation cover and phosphorus (P). The lack of a relationship between P and pasture cover could reflect the fact that farming in the catchment is low-intensity. Alternatively, it could reflect the ability of the minerogenic soil in the catchment to effectively bind P, or the fact that high rainfall events were not captured. The latter explanation would mean particulate (and potentially) dissolved inorganic P fluxes from the catchment were likely underestimated, as high flow events can significantly increase P influx to stream water through runoff and subsurface flow, and particulate P bound to river bed sediments may be re-suspended by increased stream flow rates (House *et al.* 1998, Correll *et al.* 1999, Kolpin *et al.* 2000). The influence increased influx of particle-bound P would have on phytoplankton in Lake Wanaka is unclear, as particulate P is often considered less bioavailable than PO_4-P (Hatch *et al.* 1999). However, microbial mineralisation in the sediments would eventually release particle-bound P into the water column (Hupfer and Lewandowski 2008).

Transparent exopolymer particle (TEP) formation did not increase following addition of catchment-derived nutrients and DOC in laboratory treatments. The lack of an association between these variables may reflect the hydrological condition of the streams (Chateauvert *et al.* 2012) at the time of water collection, or interactions between bacteria, TEP and DOC (Arnous *et al.* 2010) in the treatment bottles. However, enrichment of lake water with N and P facilitated transparent exopolymer formation (TEP) in laboratory treatments. Nutrient enrichment also stimulated algal growth, chl *a* concentration and diatom abundance. The relationship between diatom

blooms, TEP production and organic aggregate formation is consistent with previous research in marine and freshwater systems (Alldredge *et al.* 1995, Passow and Alldredge 1995a, Grossart *et al.* 1997). TEP formation was not associated with *Lindavia intermedia* abundance, but this may reflect removal of large predators and a shift in community structure due to bottle effects (C. Burns, personal communication). The relevance of the findings concerning the production of TEP and nutrient availability should be tested further.

While increased nutrient export from the smaller streams analysed in Chapter 2 and Chapter 3 probably has localised impacts on the lake around the stream mouths, land use intensification in the catchments of large tributaries could potentially influence processes in the open waters of the lake. Thus in Chapter 4, I analysed how the main inflowing tributaries to Lake Wanaka, the Matukituki River and the Makarora River, influence phytoplankton activity and distribution in the lake. Cold river temperatures and high suspended solid loads produced a traceable plume outside the Matukituki River mouth, but these variables were not useful in tracking the Makarora River plume.

Plume profiles at entrance-mixing sites were complex, and the river plume frequently interflowed as several distinct layers. The complex layers of lighter and denser water at this site probably result from the steep drop off from the delta into the lake (Spigel *et al.* 2005, McCullough *et al.* 2007, Mackay *et al.* 2011). Often, a thin layer of dense water also plunged along the bottom at this site. As it extended out into the lake, the direction of the plume varied, similar to plume patterns from other large, deep lakes regionally (Pickrill and Irwin 1982) and overseas (McCullough *et al.* 2007). When the lake was isothermal or weakly stratified, the plume flowed along the bottom, interflowed as a density current or occasionally flowed near the surface of the lake. When thermal stratification was well-established, the river plume plunged in nearfield waters then consistently interflowed as a density current above the thermocline.

Chlorophyll *a* concentrations were generally lower at the entrance-mixing site than further out in the lake. This may reflect light attenuation (Smith and Demaster 1996) as the turbid plume was initially entrained near the surface, but is also likely related to initial dilution by the river plume (Mackay *et al.* 2011). Once past the initial zone of turbulent mixing, chl *a* was often higher between the layers of the plume, and

occasionally above or below the plume, suggesting algae were increasingly able to utilise inflowing nutrient concentrations as light penetration improved and initial riverine dilution decreased (Kimmel *et al.* 1990, Mackay *et al.* 2011). The highest chl *a* concentrations occurred at mid-basin sampling sites, where the influence of the river plume was not otherwise measurable. As chl *a* profiles were not corrected for non-photochemical quenching, fluorescence readings from shallow nearshore sites may not represent actual phytoplankton abundance near the lake surface (Fennel and Boss 2003, Sackmann *et al.* 2008).

Dissolved organic carbon concentrations in the Matukituki River and the lake were generally similar, but the composition of dissolved organic matter (DOM) differed on at least one occasion. In June 2012, DOM signatures from deep (100 m) and shallow (20 m) water in Lake Wanaka showed that more aromatic DOM structures were present in the deep water of Lake Wanaka than in shallow waters, possibly reflecting the importance of DOM degradation and polymerisation by lake native bacteria. Matukituki River did not contain more aromatic structures than shallow waters of Lake Wanaka, despite a significant proportion of woody vegetation (20%) in the Matukituki Valley. The low number of aromatic structures in the River DOM may reflect the capability of allophanic brown soils in the Matukituki Valley to adsorb and stabilise DOM (Martin and Haider 1986, Zech *et al.* 1997), reducing its potential for transport to nearby surface waters (Lin *et al.* 2012). The high number of aliphatic compounds in the Matukituki River water could also reflect the dominance of compounds derived from agricultural production (15% of catchment land use) or even in-stream production.

The Matukituki River water contained 28.7% more formulae containing carbon, hydrogen, oxygen and sulphur (CHO-S) than the lake in June. The source of the S bound to these OM compounds is unknown. It may be geologic in origin, as basement rock types in the catchment contain minor amounts of pyrite (Craw 1984b). It may also be related to agricultural activity and the application of S-containing fertilizers in the catchment. Studies have shown that anthropogenic activity can increase the abundance of highly unsaturated aliphatic CHO-S and carbon, hydrogen, oxygen and nitrogen- (CHO-N) containing formulae in large river systems (Gonsior *et al.* 2011, Tseng *et al.* 2013, Wagner *et al.* 2015). While CHO-S diversity was higher in the Matukituki River compared with Lake Wanaka, the River did not contain a very

diverse CHO-N signature. Possibly this is because some organic sulphur compounds are better ionizers than organic nitrogen compounds, and are detected more easily by FT-ICR-MS (Fourier transform ion cyclotron mass spectrometry). However, the lack of a similar increase in CHO-N diversity in the River water weakens the supposition that agricultural activity is the source of the S.

The composition of the DOM in the River and Lake (Chapter 5) may have influenced patterns in microbial metabolic diversity recorded in June 2012. On this date, DOM from the Matukituki River and from 20 m depth in Lake Wanaka contained a greater proportion of allophanic compounds than water collected from 100 m depth in the Lake. Microbial communities from both the river and shallow lake site (20 m) were capable of breaking down a more diverse array of organic substrates than the deep water (100 m) microbial community.

In June, the Riverine bacterial community was capable of breaking down the greatest number of organic substrates, which may reflect exposure to a more diverse (and potentially more biodegradable) DOM signature. Studies have shown that microbial phylogenetic community structure (Dominik and Hofle 2002, Nelson 2008) can vary spatially and temporally in response to DOM quality and quantity. Changes in DOM supply can select for some taxa over others, leading to shifts in bacterioplankton community structure or growth (Dinasquet *et al.* 2013, Blanchet 2015), and additions of highly labile DOM can increase bacterial taxonomic diversity (Landa *et al.* 2013). As bacterial taxonomic diversity does not necessarily reflect physiological diversity (Langenheder *et al.* 2005), the link between diveristy of the DOM signature and microbial physiological diversity in these systems needs to be explored further.

Chapter 6 followed on from Chapter 5 by examining whether DOM and P currently entering the lake via the Matukituki River were capable of stimulating bacterioplankton metabolic activity in the lake. As riverine bacterial communities were consistently able to breakdown a diverse array of organic substances, they may experience consistent exposure to a labile supply of DOM, and potentially a more consistent supply of P. In the lake, seasonal patterns in organic substrate use differed by depth, which may reflect variations in thermal stratification affecting the movement of DOM into deeper waters. While phylogenetic community structure has been reported to vary vertically and temporally in lake systems (Hofle *et al.* 1999,

Dominik and Höfle 2002, Nelson 2008), my study appears to be the only reported instance of vertical and temporal variations in physiological community structure in a freshwater lake.

In Lake Wanaka, organic substrate utilisation increased with increasing chl *a* concentration, and organic substrate use patterns in deep water lagged behind shallow water patterns by one season. As Lake Wanaka experiences a chl *a* maxima during the winter and early spring when the lake is mixed (Bayer *et al.* 2015) temperature may indirectly drive bacterial physiological diversity via thermal stratification and mixing. The influence of thermal stratification on substrate use patterns in my study agrees with temporal changes in bacterial phylogenetic diversity reported in other studies (Dominik and Höfle 2002, Nelson 2008) where the release of highly labile DOM into the water column by phytoplankton (Ducklow *et al.* 1993, Dominik and Höfle 2002, Crump *et al.* 2003, Davis and Benner 2005) and the influence of the thermal structure of the water column on the movement of phytoplankton- and DOM-rich waters into deeper waters affects vertical patterns of bacterial community diversity (Dominik and Höfle 2002), respiration and growth (Ducklow *et al.* 1993).

In bioassays containing mixtures of Lake Wanaka and Matukituki River water, addition of river water to lake water stimulated microbial respiration and uptake of DOC. Changes in dissolved reactive phosphorus (DRP) concentration were not measureable in Lake Controls, as initial concentrations were frequently below detection. However, DRP uptake increased as availability increased, either through addition of river water, or amendment of treatments with N and P (Figure 29). Other studies of oligotrophic lakes also report increasing respiration rates in response to increased availability of labile DOM and P (Stets and Cotner 2008, Scott *et al.* 2012).

7.3 Avenues of Future Research

- My research project did not consider temporal changes in the structural complexity of DOC. Previous research suggests the DOC becomes less structurally complex during prolonged dry spells, while more complex, recalcitrant aromatic components becomes available during wetter conditions (Wilson and Xenopoulos 2009, Minor *et al.* 2012). As predicted climate changes in the Wanaka region include an increased frequency of extreme rainfall events (Bayer 2013), the potential for pulsed inputs of recalcitrant,

aromatic DOC into Lake Wanaka increases. During dry spells or periods of low flow, DOM composition would likely be more variable (Wagner *et al.* 2015). Future research could include determining the changing character of stream and lake DOM in relation to land use and weather patterns, and factoring in how climate change affects the loading and character of exogenous DOM entering Lake Wanaka.

- My project showed that nutrient enrichment increased TEP production in Lake Wanaka water *in vitro*, but we still do not know the mechanisms responsible for *in situ* TEP formation in this lake, or how TEP production/abundance varies spatially or temporally in relation to other water quality variables. Understanding mechanisms responsible for *in situ* TEP production is important, as organic aggregates provide nutrient-enriched micro-patches for colonisation by various microbial organisms (Berger *et al.* 1996), as well as a food source for larger components of the microbial food web (Simon *et al.* 2002).

- The settling of large phytoplankton-enriched organic aggregates can remove a source of P from surface waters (Li and Logan 1995, Logan *et al.* 1995, Grossart *et al.* 1997) that could then be released in deeper waters of the lake through microbial mineralisation in the water column or in the sediments (Hupfer and Lewandowski 2008). To date, no known work has attempted to quantify the phosphorus sorption capacity of the sediments in Lake Wanaka. Such research could provide valuable information about P cycling from the sediments, and the effect settling organic aggregate material is having on the lake bed.

- The Matukituki River had a more diverse CHO-S signature than Lake Wanaka in June 2012, but we do not know whether a high number of unique CHO-S compounds are consistently exported to Lake Wanaka. We also do not know the source of the S, or the degree to which diversity of the DOM signature influences microbial physiological diversity in the lake or its inflowing tributaries. We do not know if CHO-S exported to Lake Wanaka is used by lake phytoplankton, bacterioplankton or by benthic communities. Neither do

we know whether inputs of S are affecting chemical interactions in the water column or in sediments of the lake.

- Physical modeling of climate change impacts on Lake Wanaka suggest increased wind-mixing and water temperatures could affect the duration and depth of thermal stratification in the lake (Bayer 2013). As Lake Wanaka lies along a roughly north-south axis, increased shear forces from wind energy could affect mixing depth, which in turn could affect the coupling between chl *a* and bacterial physiological diversity (BPD). The impact that a decoupling between chl *a* and BPD would have on carbon movement within the lake is unknown.

- Currently, Lake Wanaka is oligotrophic. Farmers and regional government scientists are working together to individually tailor farm management tools (e.g. OVERSEER) to catchments in order to farm more efficiently (Aspinall, personal communication). Such tailored management practices are key to helping control diffuse influx of nutrients to the lake. However, models such as CLUES and OVERSEER do not take into account DOM, and (as shown in my study and others (Findlay *et al.* 2001, Wilson and Xenopoulos 2009, Wagner *et al.* 2015)), land use practices can alter DOM character and influence bacterioplankton activity.

- Little work has been carried out in the littoral zone ecology of Lake Wanaka, and more information is needed on benthic processes and the coupling between benthic and pelagic pathways for nutrient movement in large lake systems (Vadeboncoeur *et al.* 2002) in order to more comprehensively understand whole-lake responses to agricultural intensification. Thus, future research could include tracing the influence and flow of externally-derived energy through littoral food webs in Lake Wanaka.

7.4 Conclusions

In the Lake Wanaka catchment, nitrogen (N) and dissolved organic carbon (DOC) concentrations are higher in tributaries that have more pasture cover in their catchments. While nutrient and DOC loads exported from small tributaries probably have localised impacts on the lake, the main tributaries to the Lake can deliver external material much further from shore. One of the main inflows, the Matukituki

River, has similar bulk DOC, N and P concentrations to the Lake. However, these two water bodies differ in proportions of particulate and dissolved organic and inorganic nutrients, as well as in DOM composition. External material brought in by the Matukituki River is capable of stimulating phytoplankton growth in nearshore waters. Riverine DOM is also capable of influencing microbial metabolic activity and respiration in Lake Wanaka water. Respiration rates and DOC uptake increase under nutrient enrichment, and increased availability of N and P stimulate algal growth and facilitate organic aggregate formation in laboratory experiments.

Increased macronutrient and DOC loading to the Lake has the potential to stimulate changes in the microbial population, affect phytoplankton productivity and result in changes in food webs and community structure. While the manner in which such changes in matter and energy flow would manifest themselves in higher trophic levels is beyond the scope of this study, my results provide a foundation for future research concerning land development and microbial dynamics in large, oligotrophic lake systems.

Table A1: Mean and range of raw data for physico-chemical variables measured over the course of the sampling period. Abbreviated variable titles and stream names include: (C) conductivity, (DO) dissolved oxygen, (NO_3-N) nitrate-nitrogen, (DRP) dissolved reactive phosphorus, (SM) virtual soil moisture conditions; (A) Alpha Burn, (F) Fern Burn, (B) Bullock Creek, (SC) Stoney Creek, (BC) Boundary Creek, (W) Wharf Creek, (MT) Matukituki River, and (N) Neck Creek..

Stream		A	F	B	SC	BC	W	MT	N
T (°C)	Mean	11.6	14.5	12.1	11.9	10.1	10.3	11.8	11
	Min-Max	9.3-12.7	11.1-17.9	10.5-16	8.5-14.3	8.0-14.4	8.5-14.3	8.5-14.0	9.1-15
Rainfall (mm/7days)	Mean	16.7		10.7		23.3		19.3	25.2
	Min-Max								
SM (%)	Mean	47.9		39.1		59.5		49.9	54.7
	Min-Max	13.1-82.8		7.9-78.5		19.1-97.9		14.1-86.5	28.7-80.3
pH	Mean	7.8	8.0	7.7	8.0	8.0	8.2	7.8	8.2
	Min-Max	7.2-8.2	7.6-8.4	7.3-8.0	7.5-8.9	7.9-8.2	8.0-8.4	7.4-8.2	7.9-8.4
C (μS/cm)	Mean	67.9	78.2	81.2	40.6	33.0	53.1	67.1	63.8
	Min-Max	*1.0-11.6	*1.0-121.6	45.5-92.6	<*1.0-105.5	4.9-57.4	4.2-77.8	61.8-79.6	55.8-80.9
DO (mg/l)	Mean	9.8	9.3	10.1	10.8	10.8	10.3	10.1	10.2
	Min-Max	7.6-11.1	7.6-10.3	8.0-11.2	8.4-15.2	8.9-12.1	8.6-11.5	8.0-11.5	8.8-11.3
DOC (mg/l)	Mean	3.9	3.3	3.0	4.9	2.1	2.8	2.3	2.0
	Min-Max	2.96-4.75	2.30-4.25	1.93-4.29	4.01-5.69	1.14-5.41	1.88-3.82	1.27-3.81	1.57-2.27
TN (μg/l)	Mean	419	126	652	437	27.7	13.2	73	13.6
	Min-Max	262-581	71-199	539-753	153-635	3.5-79	2.2-22.5	40.1-166	6.3-28.0
NO_3-N (μg/l)	Mean	387	76	478	288	11	10.3	45.7	2
	Min-Max	217-515	19.4-134	230-624	39.2-522	5.9-18.5	5.1-17.5	31.2 - 62	<1.0-8.3
TP (μg/l)	Mean	8.2	5.3	3.5	20.4	14.0	17.9	11.8	3.2
	Min-Max	2.6-17.4	2.5-13.6	1.4-7	9-25.7	1.4-87	6.0-55	5.1-31.8	1.6-5
DRP (μg/l)	Mean	1.8	1.0	1.0	3.0	1.8	5.6	2.0	1.3
	Min-Max	<1.0-5.2	<1.0-3.9	<1.0-4.5	1.1-4.0	<1.0-3.7	3.3-8.9	<1.0-5.3	<1.0-2.3

[1] An outlier was removed from each chemical parameter measured. These outliers were recorded in Stoney Creek in February 2012. The stream was nearly stagnant at the time of sampling.

* Instances when conductivity was less than 5 μS/cm occurred mainly on one sampling date (February 4, 2012) during low flow conditions, and in one stream with consistently low flows.

Table A2: Comparison of linear mixed-effects regression models using Bayesian Information Criterion (BIC). Lower BIC values indicate a better the model.

Model	BIC
DOC = Fixed(rain) + Random(pasture cover) + error	119.695
DOC = Fixed(soil moisture) + Random(pasture cover) + error	108.554
DOC = Fixed(air temp) + Random(pasture cover) + error	86.554
DOC = Fixed(rain) + Fixed(Soil moisture) + Random(pasture cover) + error	109.621
DOC = Fixed(rain) + Fixed(air temp) + Random(pasture cover) + error	84.453
DOC = Fixed(air temp) + Fixed(Soil moisture) + Random(pasture cover) + error	84.69
DOC = Fixed(air temp) + Fixed(rain) + Fixed(air temp*rain) + Random(pasture cover) + error	80.138
DOC = Fixed(air temp) + Fixed(Soil moisture) + Fixed(air temp*soil moisture) + Random(pasture cover) + error	76.181

Appendix B

Table B1: Average pH values (1 ± standard deviation) in each treatment during the 12-day February 2013 experiment. Columns denote the number of days from the start of the experiment.

Treatment	Day 0	2	4	6	8	10	12
Control	7.27	7.42 ± 0.12	7.74 ± 0.09	7.61 ± 0.09	7.41 ± 0.14	7.41 ± 0.06	7.59 ± 0.09
Pasture	7.53	7.39 ± 0.01	7.55 ± 0.04	7.51 ± 0.04	7.37 ± 0.00	7.33 ± 0.04	7.50 ± 0.18
Pasture + Urban	7.48	7.33 ± 0.02	7.41± 0.09	7.38 ± 0.05	7.30 ± 0.01	7.31 ± 0.00	7.46 ± 0.02
Tussock	7.69	7.33 ± 0.02	7.42 ± 0.01	7.40 ± 0.02	7.34 ± 0.01	7.34 ± 0.01	7.44 ± 0.03
Control + N	7.27	7.49 ± 0.07	7.48 ± 0.08	7.48 ± 0.09	7.53 ± 0.06	7.79 ± 0.08	8.47 ± 0.25
Pasture + Urban + N	7.53	7.43 ± 0.01	7.44 ± 0.01	7.46 ± 0.02	7.50 ± 0.02	8.00 ± 0.08	9.14 ± 0.04
Urban + N	7.48	7.18 ± 0.04	7.15 ± 0.05	7.17 ± 0.03	7.23 ± 0.05	7.64 ± 0.11	9.29 ± 0.25
Tussock + N	7.69	7.17 ± 0.01	7.21 ± 0.03	7.19 ± 0.01	7.33 ± 0.05	8.31 ± 0.27	9.23 ± 0.10

Table B2: ANOVA comparison of cell numbers in nutrient-enriched and unamended mixture treatments in February 2013. Results for the total number of cells and for dominant algal classes are given. d.f. = degrees of freedom

	df	F	p
Total cells ml^{-1}	2, 22	89.449	<0.001
Chlorophytes ml^{-1}	2, 22	20.715	<0.001
Lindavia ml^{-1}	2, 22	14.420	<0.001
Diatoms ml^{-1}	2, 22	56.775	<0.001
Nitzschia ml^{-1}	2, 22	55.010	<0.001

Table B3: Dissolved organic carbon (DOC) concentrations (mg C l^{-1}) in stream water used in the February 2013 12-day experiment. Experiment bottles contained mixtures of 50 μm-filtered Lake Wanaka water and 0.22 μm-filtered stream exudate from streams draining pastoral land cover (Pasture), pastoral and urban cover (Pasture + Urban) and tussock land cover (Tussock).

Stream	Treatment	DOC (mg C l^{-1})
Bullock Creek	Pasture + Urban	28.66 ± 0.89
Alpha Burn	Pasture	6.65 ± 0.36
Boundary Creek	Tussock	12.65 ± 2.36

Appendix C

Table C1: Comparison of dissolved oxygen concentrations (% saturation) between the Matukituki River and two depths (20 m and 100 m) at the Aspiring Basin site (44°35.702S 169°04.030E) (max depth 220 m) in Lake Wanaka.

Date	River DO (%)	Lake at 20 m DO (%)	Lake at 100 m DO (%)
September 2009	103.1	92.4	91.7
November 2009		94.5	91.0
March 2010	102.6	96.1	93.9
March 2011	101.1	95.1	91.6
May 2011		93.1	91.0
November 2011	99.5	96.2	94.5
January 2012	98.9	97.7	93.2
March 2012	90.3	96.7	90.3
June 2012	92.4	89.9	90.6
October 2012	111.6	91.1	91.8
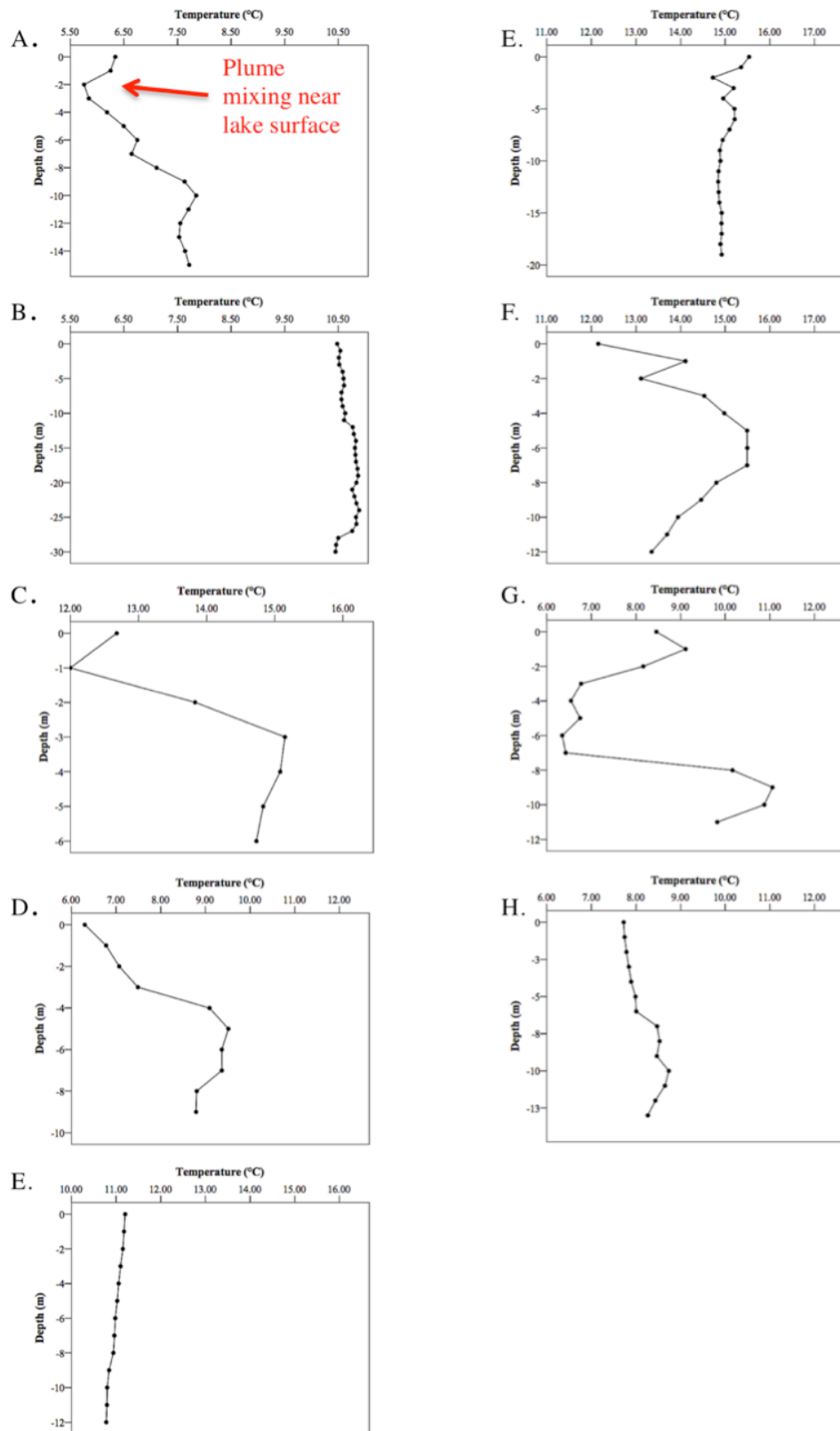
Figure C1: Temperature profiles at sampling sites directly outside the Matukituki River mouth in, A. September 2009, B. November 2009, C. March 2011, D. March 2011 E. May 2011, F. November 2011, G. January 2012, H. March 2012, I. June 2012. The red arrow(s) indicate the cooler water of the river plume.
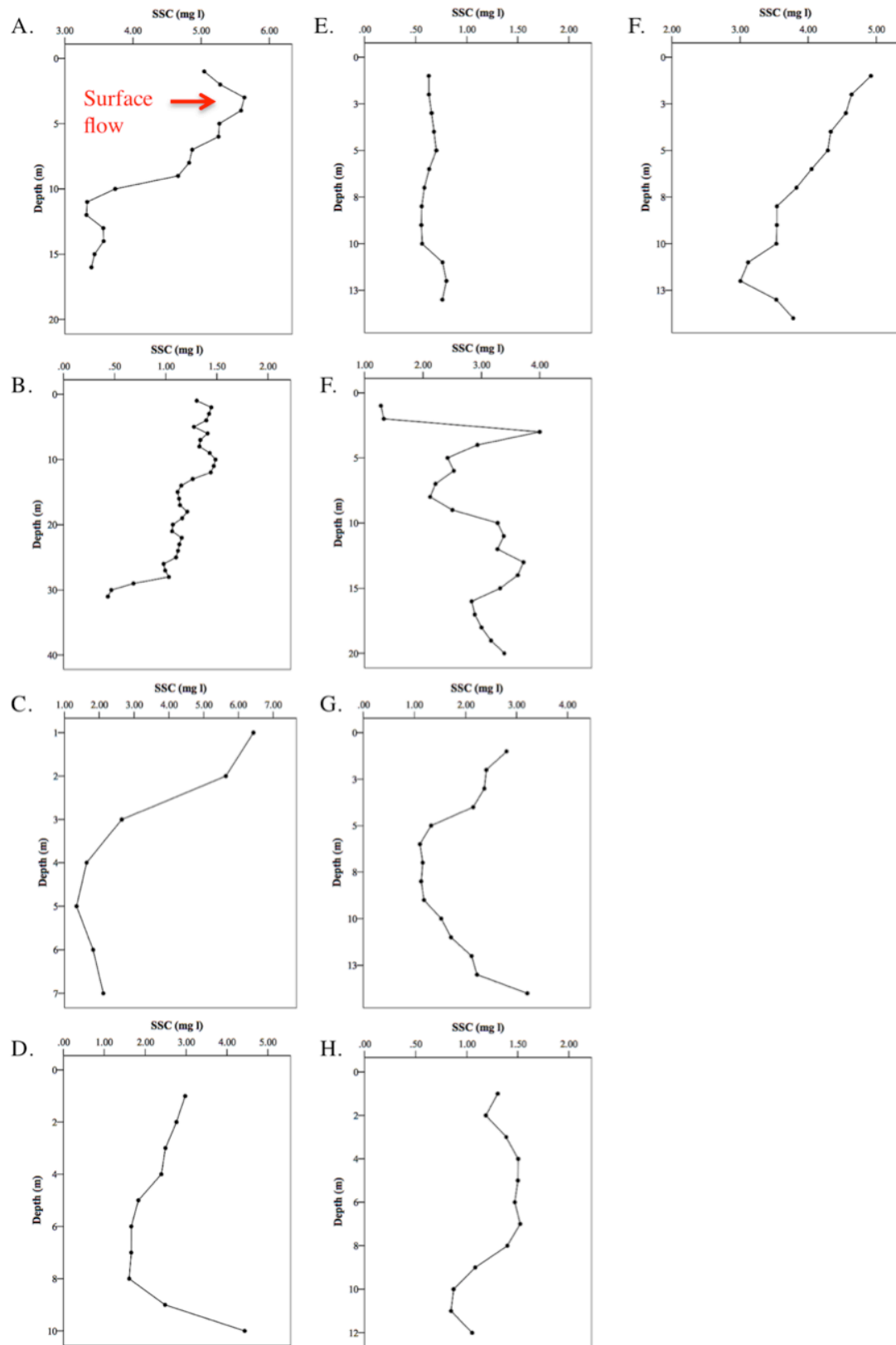
Figure C2: Suspended solid profiles at sampling sites directly outside the Matukituki River mouth in, A. September 2009, B. November 2009, C. March 2011, D. March 2011 E. May 2011, F. November 2011, G. January 2012, H. March 2012, I. June 2012. The red arrow(s) indicate the cooler water of the river plume.

September 2009

Figure C3: (top) Total suspended solids (mg l^{-1}) and (bottom) temperature (°C) profiles from the (left to right) Matukituki River mouth out into Lake Wanaka in September 2009. Distance from the river mouth is given for each profile.

October 2012

Figure C4: (top) Total suspended solids (mg l^{-1}) and (bottom) temperature (°C) profiles from the (left to right) Matukituki River mouth out into Lake Wanaka in October 2012. Distance from the river mouth is given for each profile.

November 2011

Figure C5: (top) Total suspended solids (mg l^{-1}) and (bottom) temperature (°C) profiles from the (left to right) Matukituki River mouth out into Lake Wanaka in November 2011. Distance from the river mouth is given for each profile
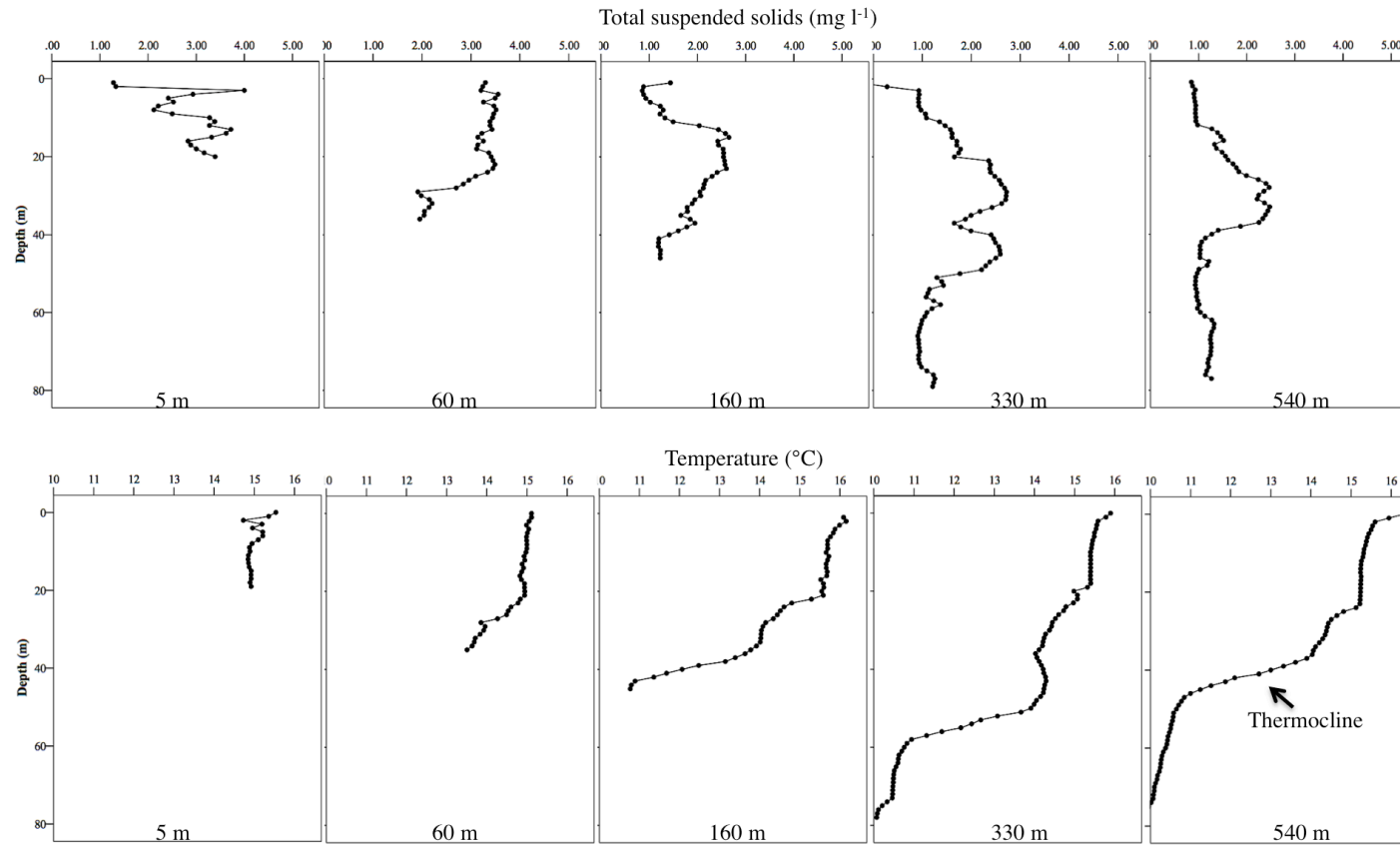
January 2012

Figure C6: (top) Total suspended solids (mg l^{-1}) and (bottom) temperature (°C) profiles from the (left to right) Matukituki River mouth out into Lake Wanaka in January 2012. Distance from the river mouth is given for each profile.

March 2012
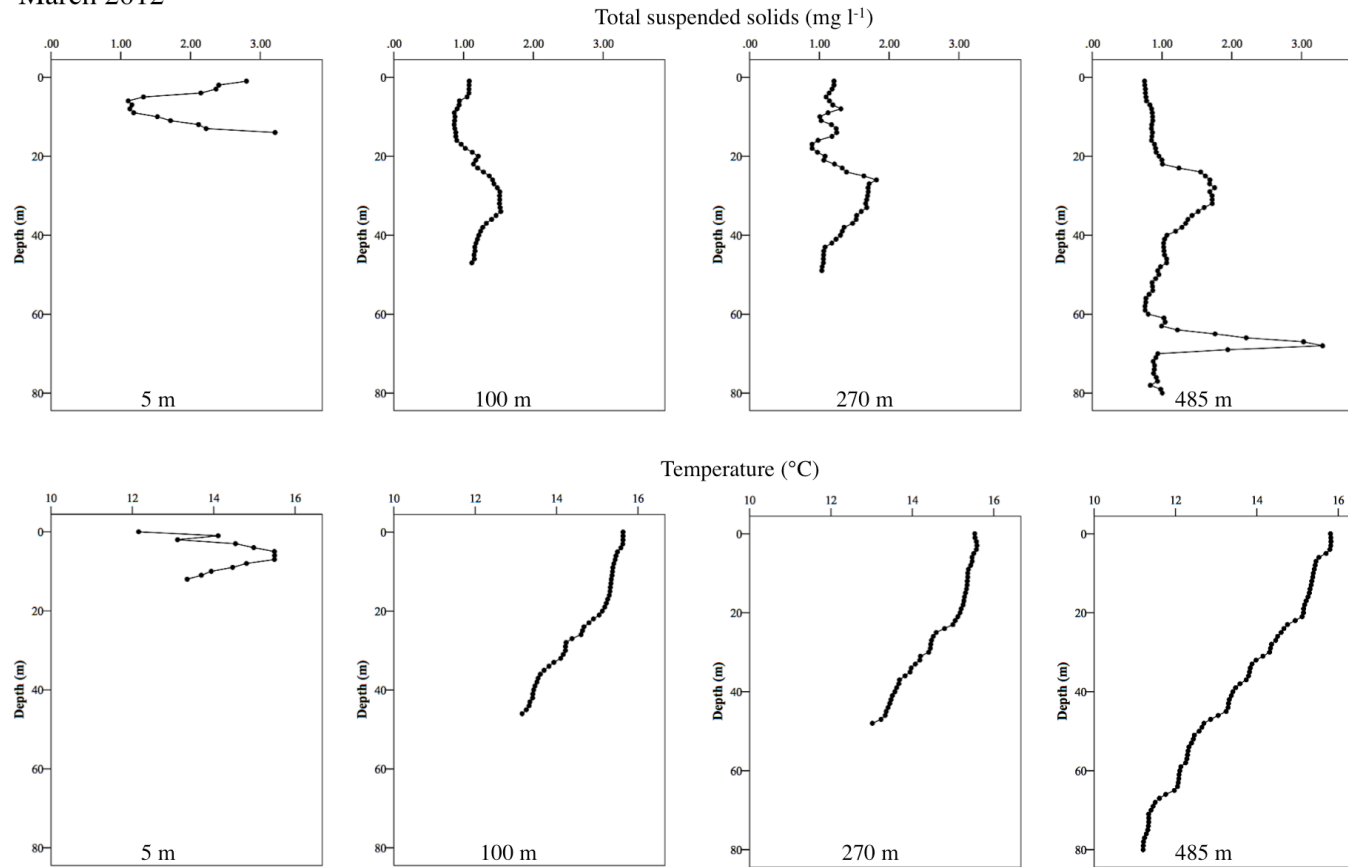
Figure C7: (top) Total suspended solids (mg l⁻¹) and (bottom) temperature (°C) profiles from the (left to right) Matukituki River mouth out into Lake Wanaka in March 2012. Distance from the river mouth is given for each profile.

May 2011
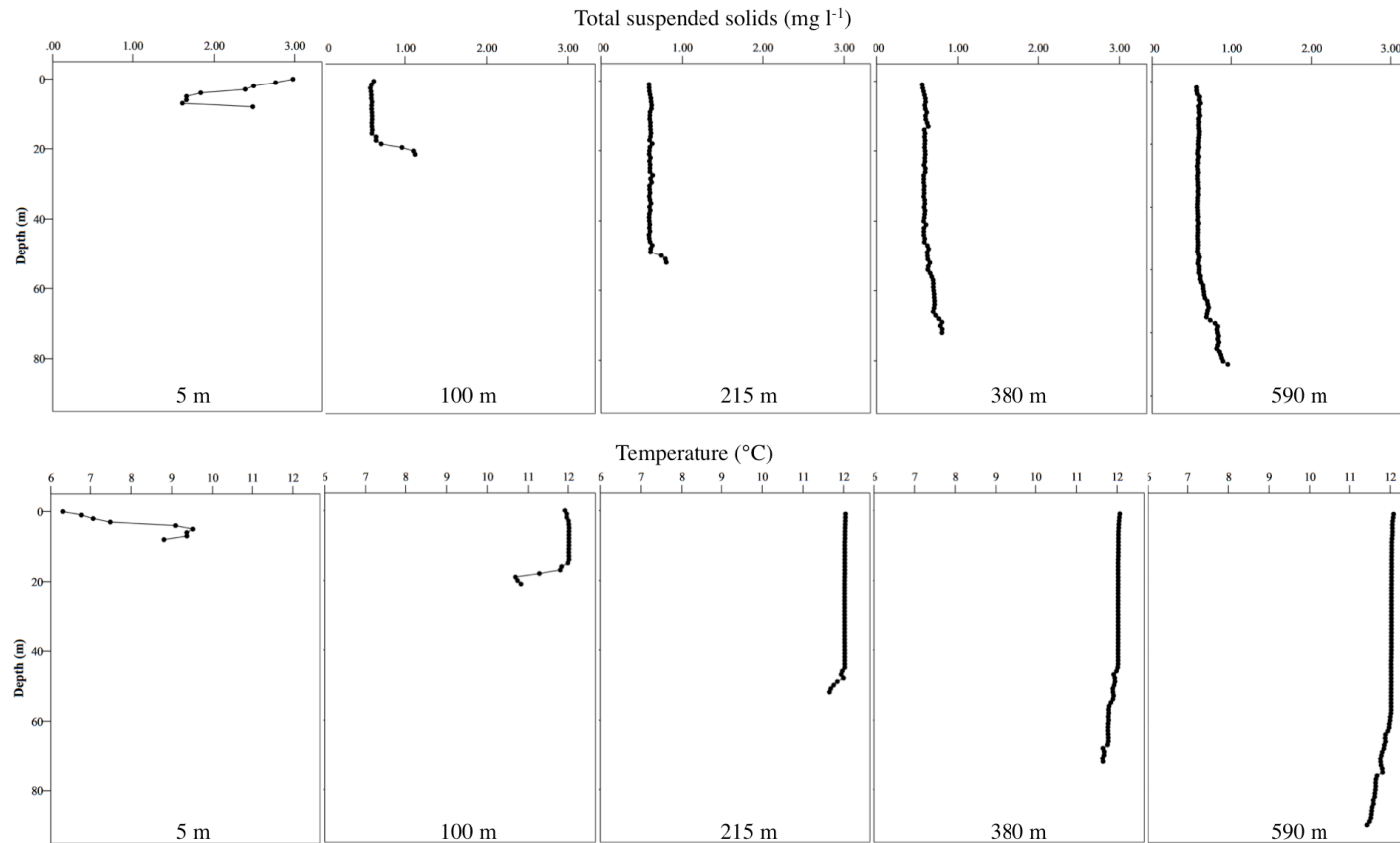
Figure C8: (top) Total suspended solids (mg l^{-1}) and (bottom) temperature (°C) profiles from the (left to right) Matukituki River mouth out into Lake Wanaka in May 2011. Distance from the river mouth is given for each profile.

June 2012

Total suspended solids (mg l^{-1})
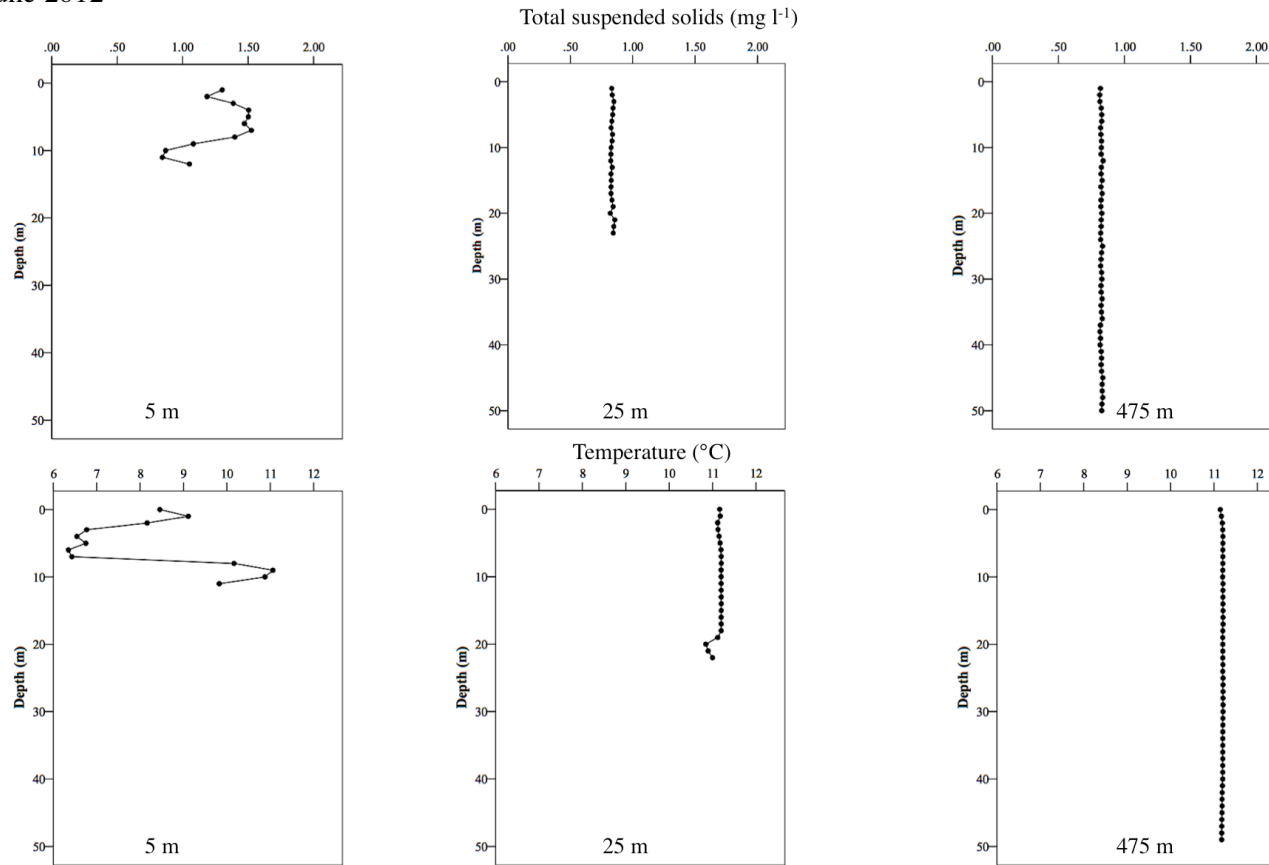
Temperature (°C)
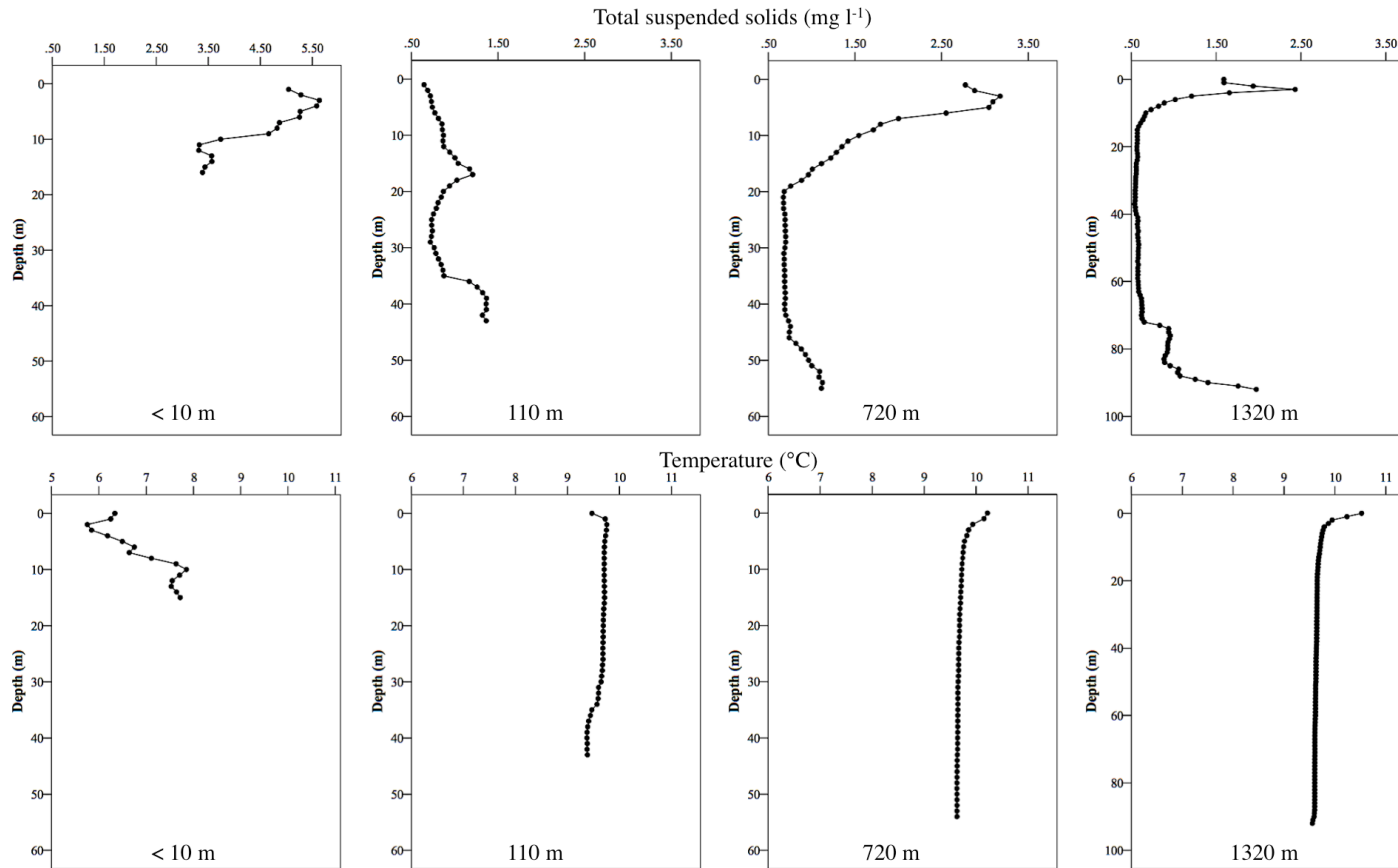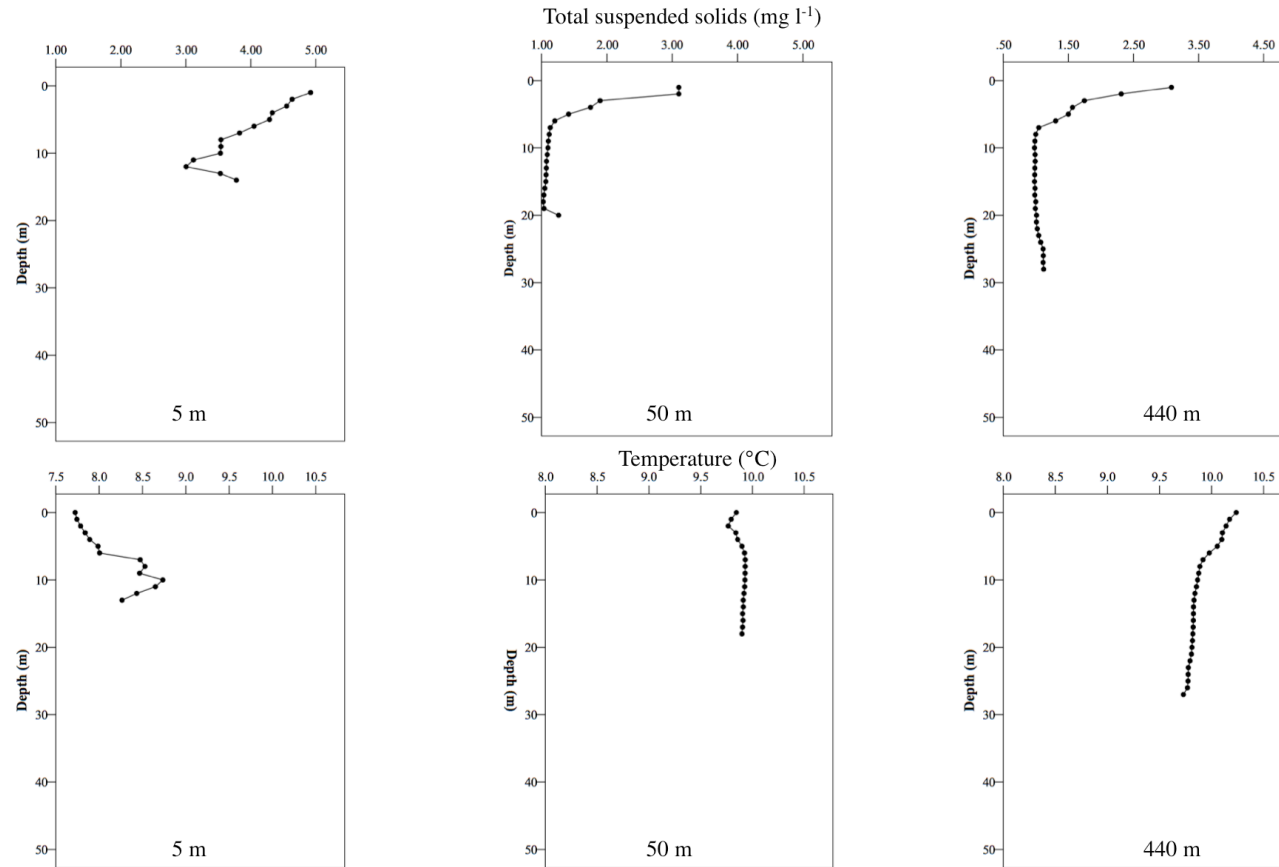
Figure C9: (top) Total suspended solids (mg l^{-1}) and (bottom) temperature (°C) profiles from the (left to right) Matukituki River mouth out into Lake Wanaka in June 2012. Distance from the river mouth is given for each profile.
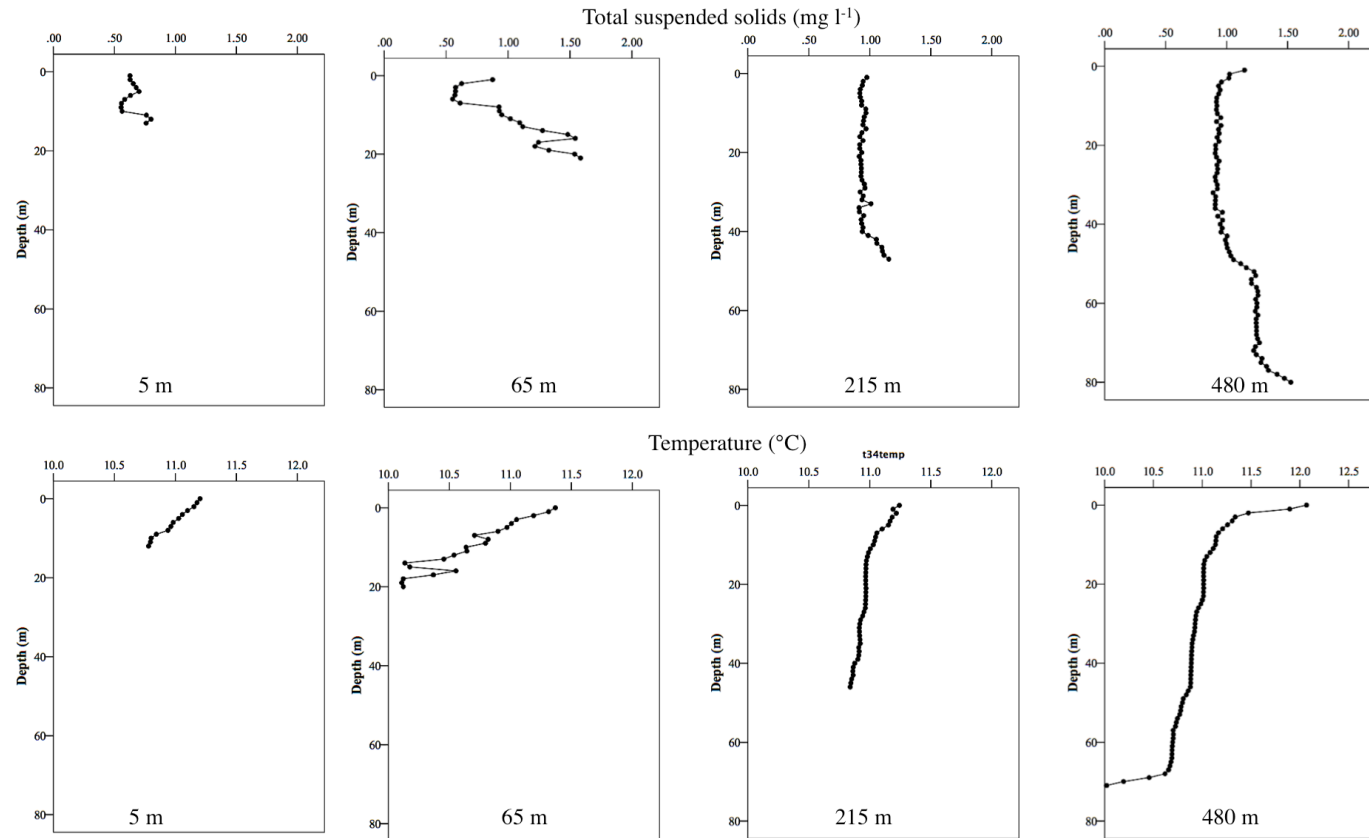
January 2012

Chl a (mg m⁻³)

Density (kg l⁻¹)

March 2012
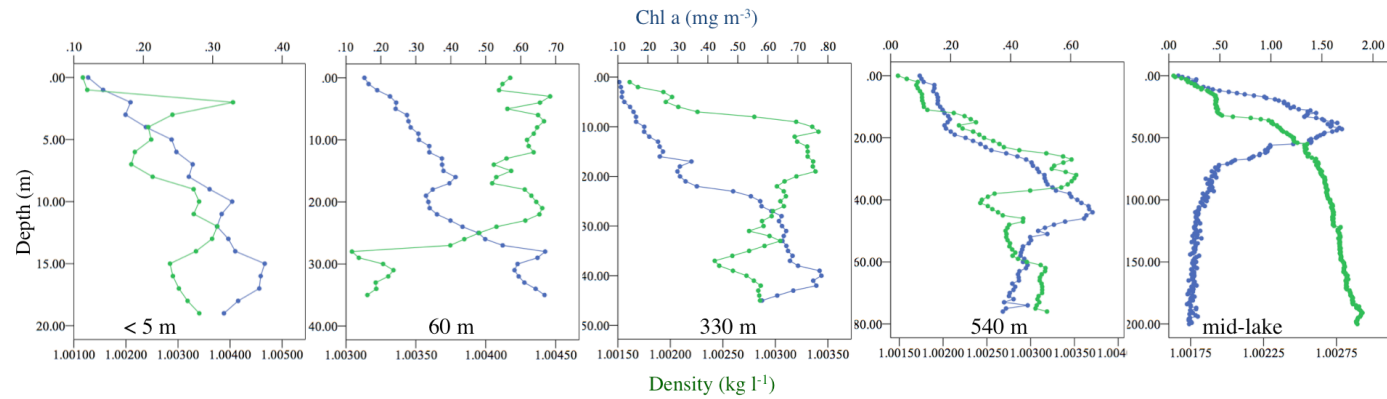
Chl a (mg m⁻³)
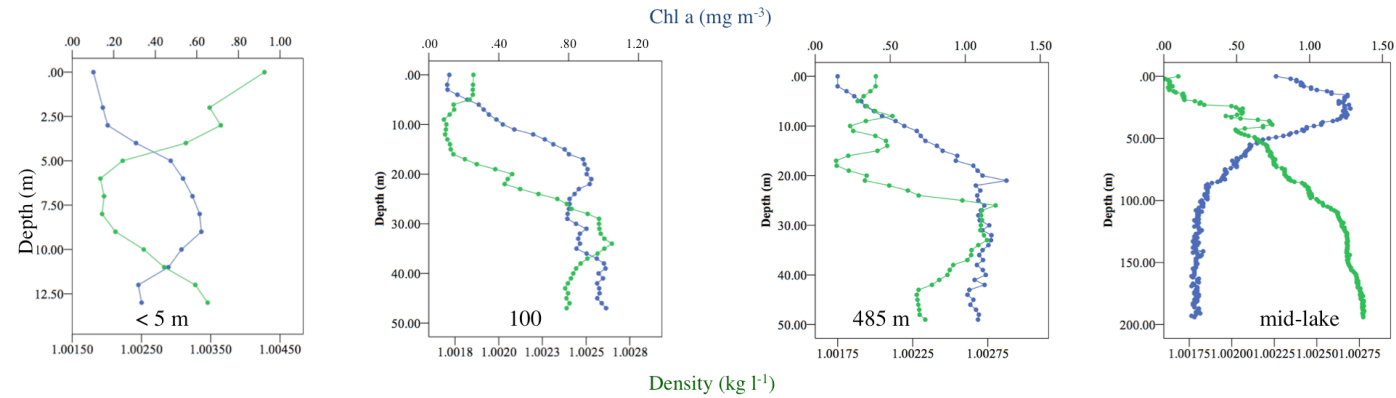
Density (kg l⁻¹)

Figure C10: Density (kg l⁻¹) and chlorophyll *a* (mg m⁻³) profiles from the Matukituki River mouth out into Lake Wanaka in (top) January 2012 and (bottom) March 2012. The blue line represents chl *a*, the green line represents density. The scale for chl *a* is given on the top axis of each graph. The scale for density is given on the bottom axis of each graph. Distance from the river mouth is given for each profile.

September 2009
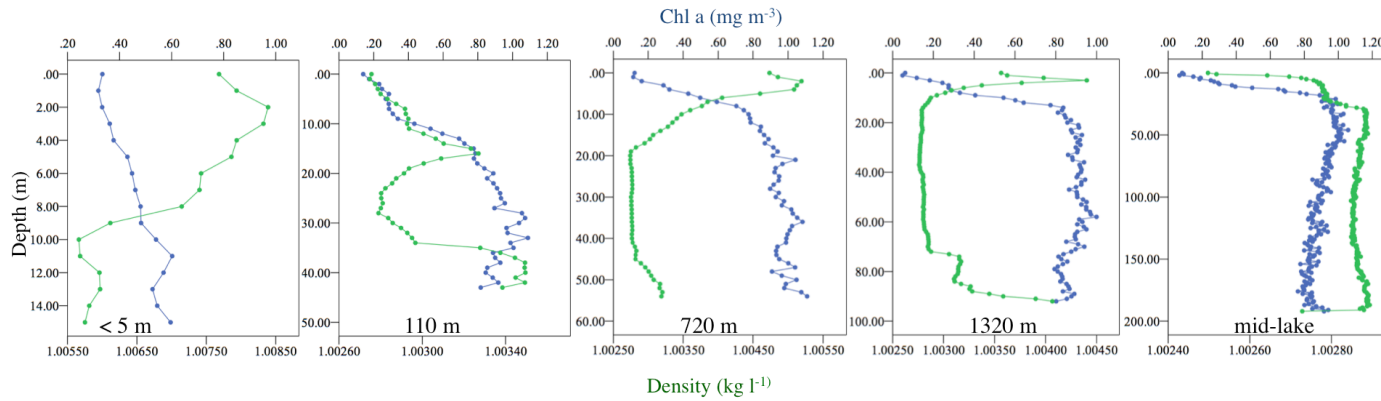
May 2011
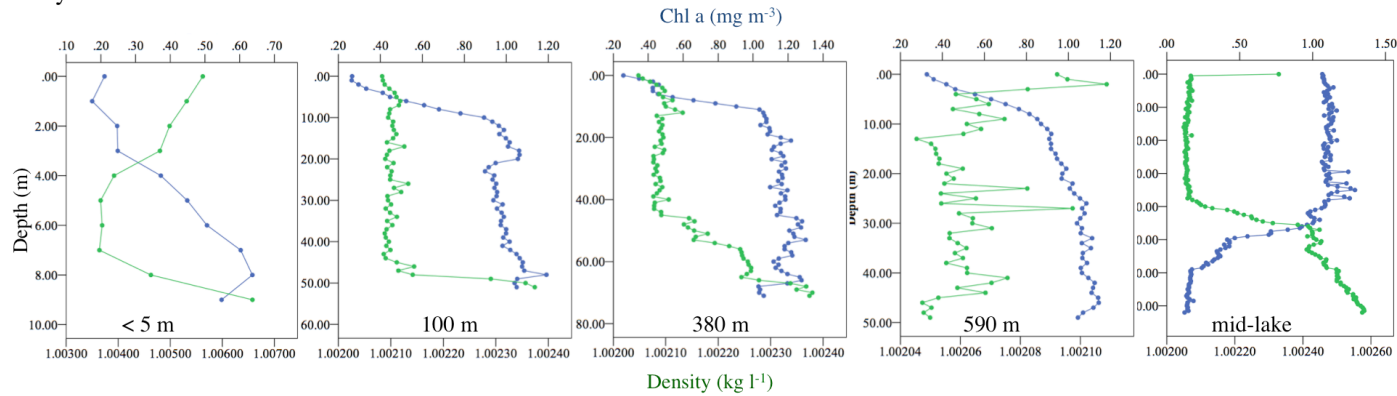
Figure C11: Density (kg l⁻¹) and chlorophyll *a* (mg m⁻³) profiles from the Matukituki River mouth out into Lake Wanaka in (top) September 2009 and (bottom) May 2011. The blue line represents chl *a*, the green line represents density. The scale for chl *a* is given on the top axis of each graph. The scale for density is given on the bottom axis of each graph. Distance from the river mouth is given for each profile.
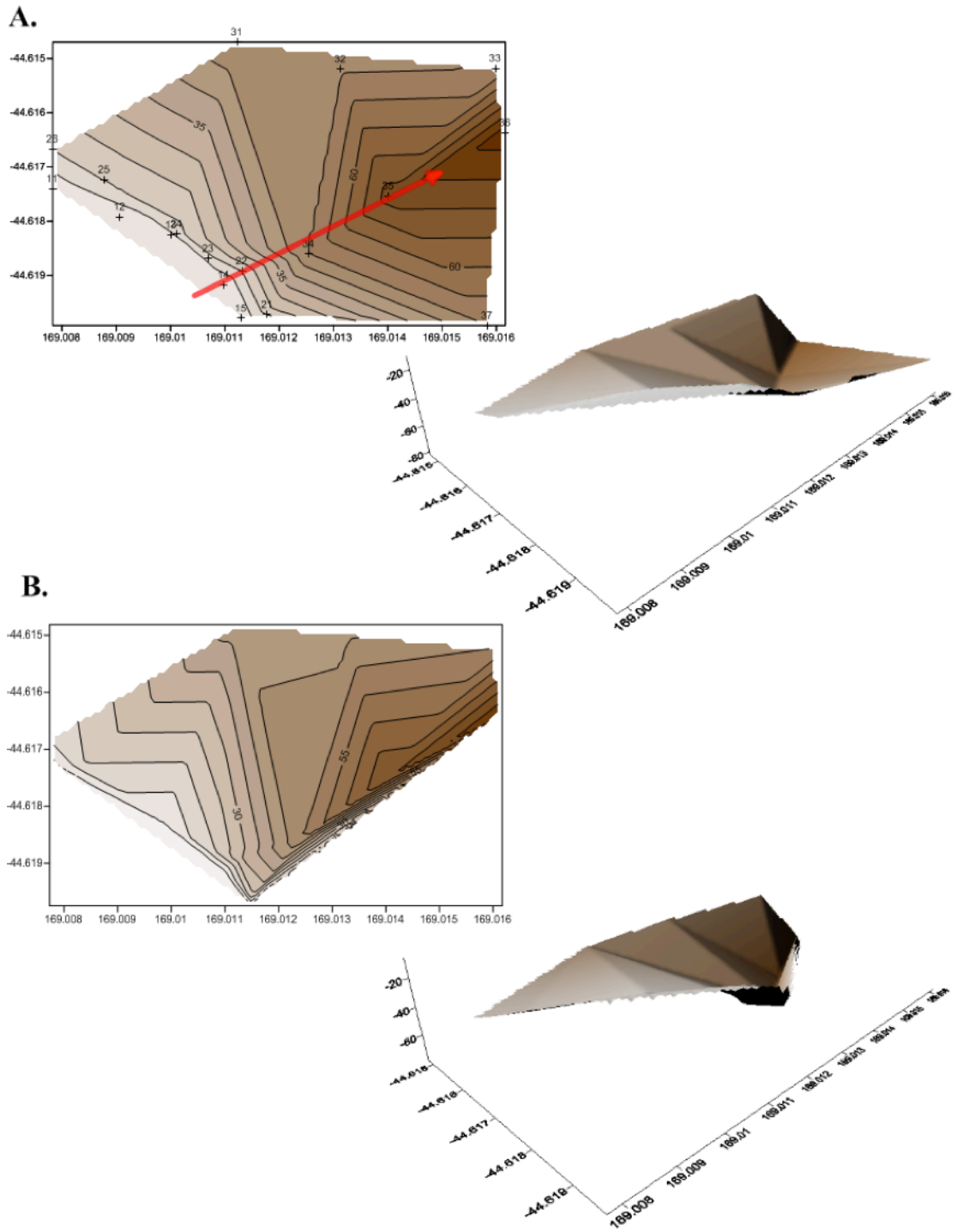
Figure C12: Bathymetry of West Wanaka Bay (left: plan view, right: 3-D image) in A. May 2011, and B. January 2012. Site 37 (labeled and marked by (+) on bottom right corner of plan view Map A.) was not sampled in January, which affected placement of the interpolation lines. Red arrow in A. shows the direction of the river plume and possible sublacustrine channel. Contour lines = 5 m.
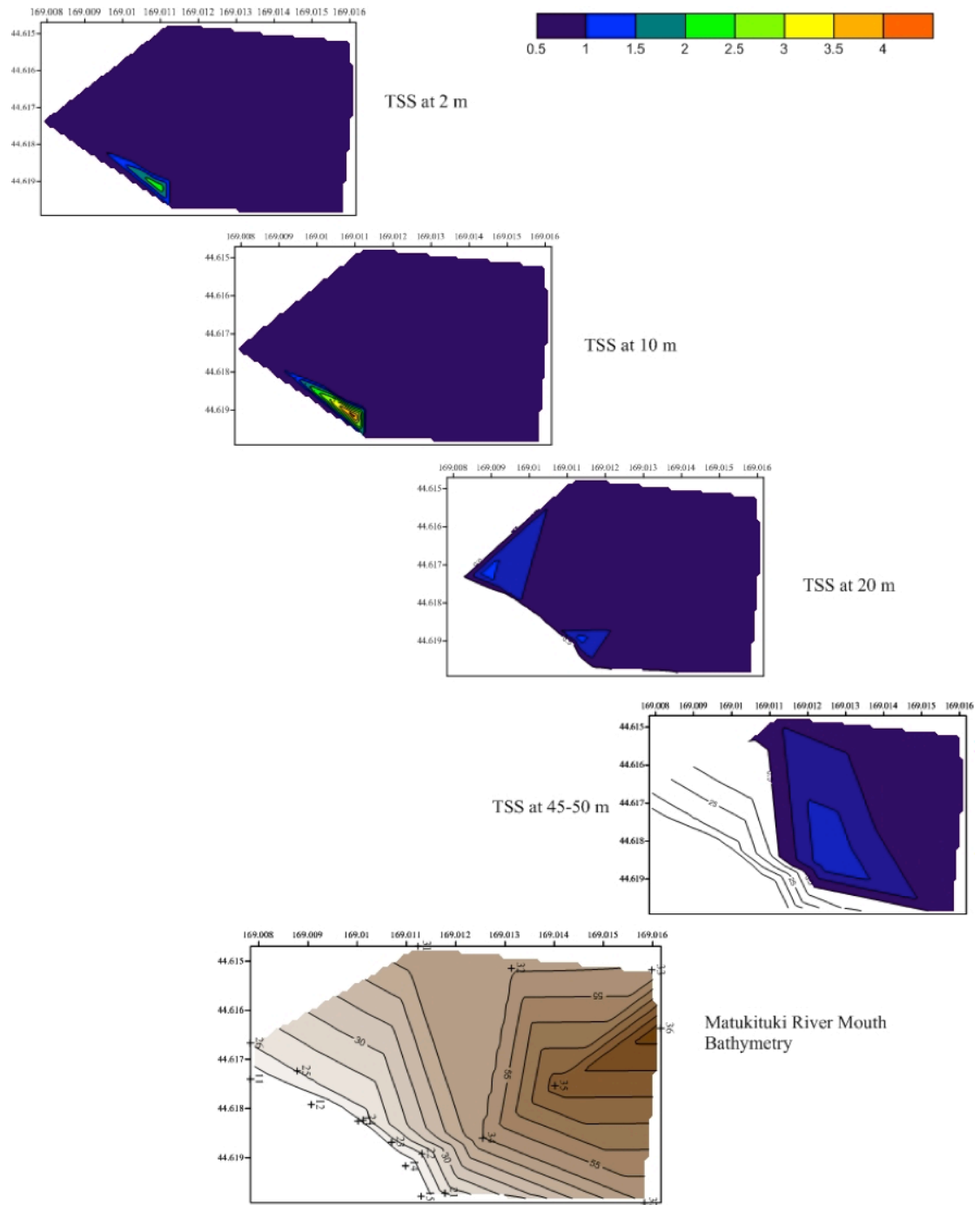
Figure C13: Plan view showing the river plume (denoted by suspended solid concentration) flowing into Lake Wanaka in May 2011. Blue graphs show suspended solid concentration extending out into the lake with increasing depth. The brown graph (bottom center) shows bathymetry of the Lake at the time casts were taken. Sampling sites are noted (+) on the bottom graph.
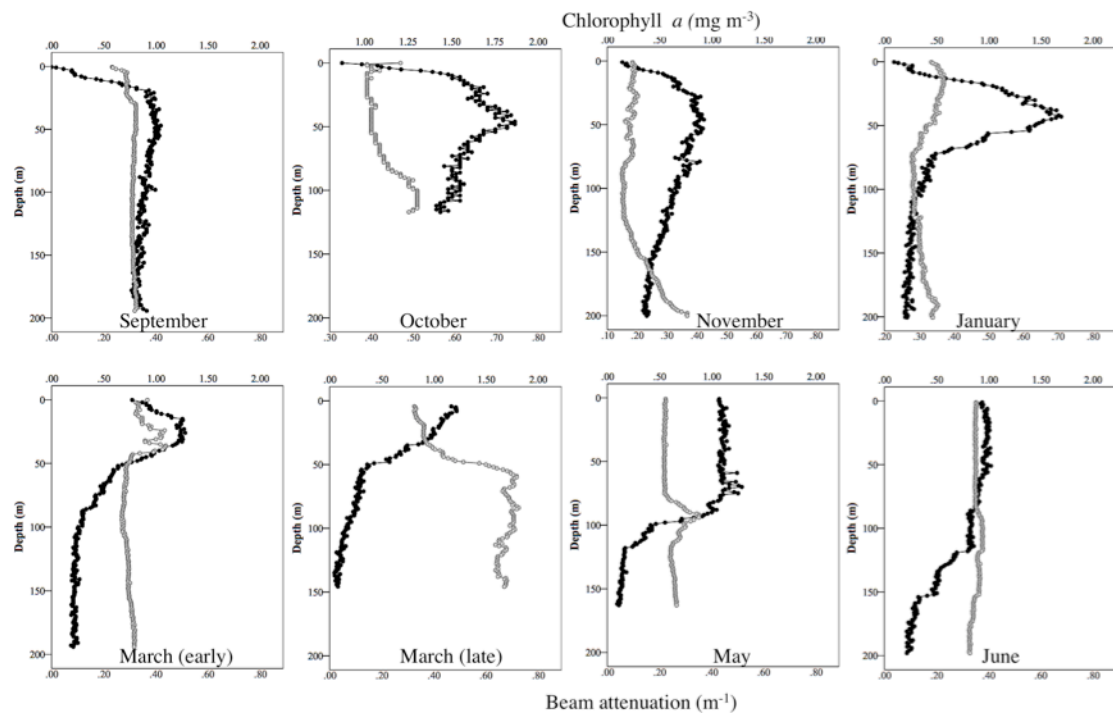
Figure C14: Chlorophyll *a* (mg m⁻³) (black circle) and beam attenuation (m⁻¹) (gray circle) profiles at the Aspiring Basin site at different times of the year. On each graph, the top axis denotes chl *a*, while the bottom axis denotes beam attenuation. Sampling dates are: September: September 19, 2009; October: October 12, 2012; November: November 19, 2011; January: January 7, 2012; March(early): March 10, 2012; March(late): March 26, 2011; May: May 21, 2011; June: June 9, 2012.

Appendix D

Table D1: The number of unique formulae containing only carbon, hydrogen and oxygen (CHO), carbon, hydrogen, oxygen and nitrogen (CHO-N) and carbon, hydrogen, oxygen and sulphur (CHO-S) in the Matukituki River (R) and Lake Wanaka at 20 m (20) and 100 m (100) depth in June 2012. Average mass (kDa), O:C and H:O ratio (± 1 standard deviation) in each sample are also given for CHO, CHO-N and CHO-S. The relatively large number of different CHO-S formulae in the river water is indicated in bold.

		T1	*T2*	*E1*	*E2*	*H*
Unique CHO		1515*	1740*	1704*	1434*	1521*
CHOS		879*	923*	223*	571*	170*
CHON		11*	7*	89*	22*	44*
CHO	Mean O/C	0.47	0.48	0.49	0.49	0.48
	Mean H/C	1.20	1.19	1.16	1.16	1.13
CHOS	Mean O/C	0.40	0.46	0.45	0.37	0.40
	Mean H/C	1.55	1.51	1.50	1.51	1.43
CHON	Mean O/C	0.45	0.47	0.46	0.45	0.44
	Mean H/C	1.16	1.13	1.10	1.17	1.10
Mean MW (CHO)		447.83	454.80	469.71	451.85	447.47
Mean MW (CHOS)		497.65	460.31	443.51	525.38	481.47
Mean MW (CHON)		353.57	359.25	340.87	323.51	360.68

* Duplicate samples were averaged before the formula assignments (intensities of each m/z ion), resulting in more peaks over the relative abundance threshold, and an overall increase in the number of assigned formulae for the averaged spectra. Averaging the duplicates allowed for removal of many noise peaks that were not present in both samples, and the inclusion of more low abundance m/z peaks that were present in both samples. The inclusion of these low abundance peaks resulted in an increase in the total number of formulae in the averaged samples compared with teach duplicate sample.

Table D2: Chi-squared contingency table comparing the number of aliphatic and aromatic carbon, hydrogen and oxygen (CHO) containing formulae in Lake Wanaka at 20 m and 100 m depth. Aliphatic and aromatic formulae are determined using the Aromaticity Index (AI) and modified Aromaticity Index (AI_{mod}), where AI <=0.5 denotes aliphatic compounds, while AI > 0.5 denotes aromatic compounds. Observed (Obs), Expected (Exp) and cell-specific χ^2 values are given.

		20 m	*100 m*	*Total*
AI <=0.5	Obs	2175	2431	4606
	Exp	2145.88	2460.12	
	χ^2	0.40	0.34	
AI >0.5	Obs	99	176	275
	Exp	128.12	146.88	
	χ^2	6.62	5.77	
Total		2274	2607	4881

Table D3: Between-sample comparisons of mean hydrogen to carbon ratios H:C, mean oxygen to carbon ratios (O:C), mean double-bond equivalents (DBE), and mean double-bond equivalents normalised to the number of carbons (DBE/C). K-W = Kruskal Wallis test, d.f. = degrees of freedom

Variable	Test	d.f.	F	χ^2	Sig
H:C	ANOVA	2, 7183	4.386		0.012
O:C	K-W	2		8.949	0.011
DBE	K-W	2		36.033	<0.001
DBE/C	ANOVA	2, 7183	16.457		<0.001

Table D4: Chi-squared contingency table comparing the number of aliphatic and aromatic carbon, hydrogen and oxygen (CHO) containing formulae from each site. Aliphatic and aromatic formulae are determined using the Aromaticity Index (AI) and modified Aromaticity Index (AI_{mod}), where AI <=0.5 denotes aliphatic compounds, while AI > 0.5 denotes aromatic compounds. Observed (Obs), Expected (Exp) and cell-specific χ^2 values are given.

		River	20 m	100 m	Total
AI <=0.5	Obs	2206	2175	2431	6812
	Exp	2185.03	2155.85	2471.32	
	χ^2	0.20	0.17	0.66	
AI >0.5	Obs	99	99	176	374
	Exp	119.97	118.35	135.68	
	χ^2	3.66	3.16	11.98	
Total		2305	2274	2607	7186
		River	20 m	100 m	Total
AI_{mod} <=0.5	Obs	1867	1823	2023	5713
	Exp	1832.52	1807.87	2072.61	
	χ^2	0.65	0.13	1.19	
AI_{mod} >0.5	Obs	438	451	584	1473
	Exp	472.48	466.13	534.39	
	χ^2	2.52	0.49	4.61	
Total		2305	2274	2607	7186
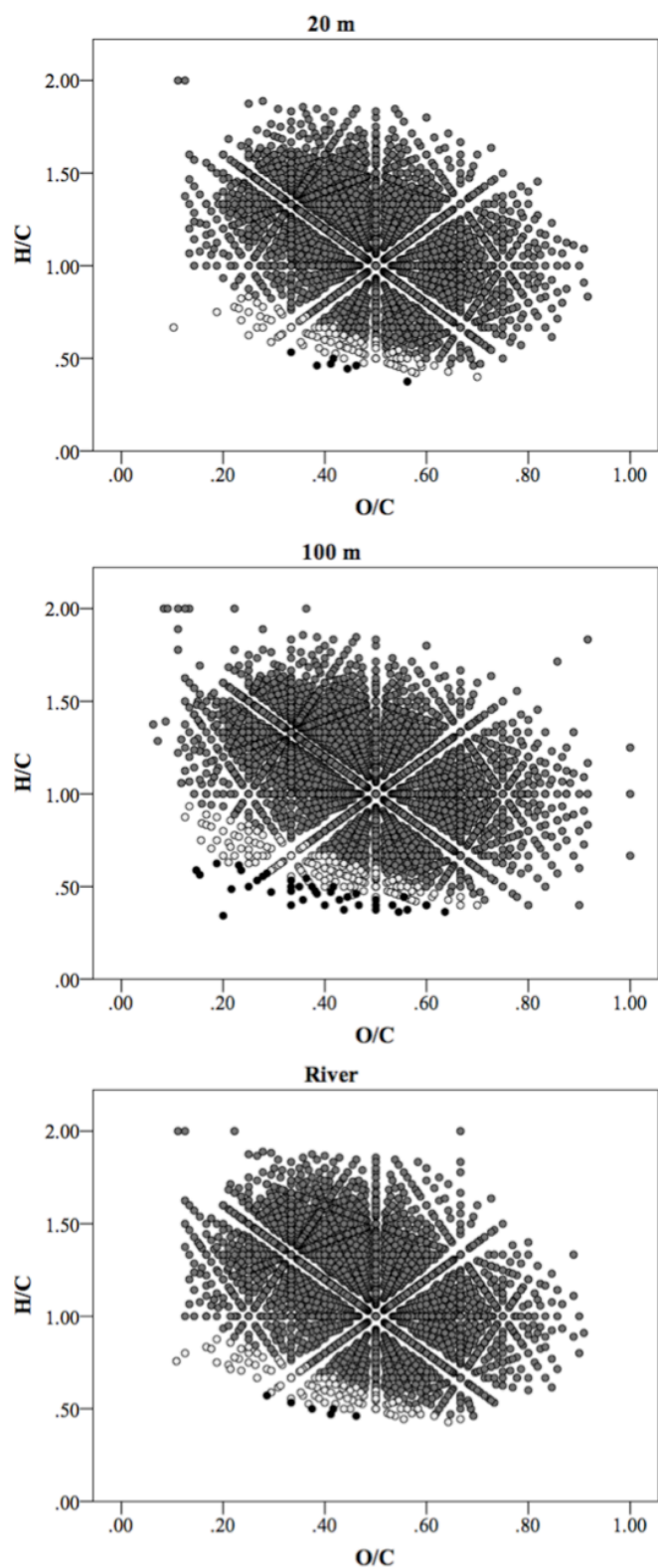
Figure D1: Van Krevelen plots of H:C and O:C molar ratios in formulae containing carbon, hydrogen and oxygen (CHO) obtained from Lake Wanaka at 20 m and 100m depths and from the Matukituki River, respectively. Aliphatic compounds are represented by (●), aromatic compounds (compounds with a modified aromaticity index (AI_{mod}) ≥ 0.5 are represented by (○). Compounds with AI_{mod} > 0.67 are represented by (●).

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Appendix E
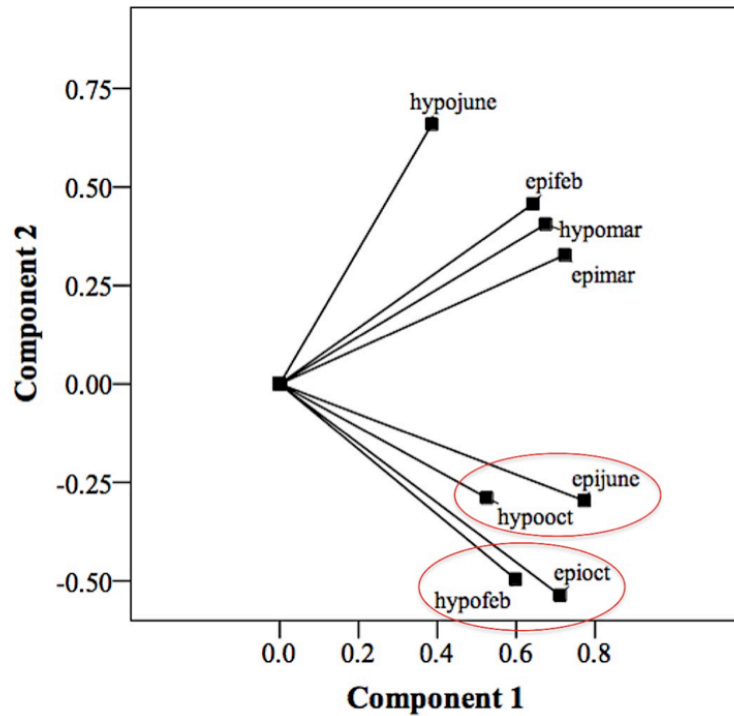
Figure E1: Component loadings plot showing seasonal differences in the number of organic substrates metabolised by the microbial community at 20 m and 100 m depth in Lake Wanaka. hypojune = 100 m depth in June 2012, epifeb = 20 m depth in February 2013, hypomar = 100 m depth in March 2012, epimar = 20 m depth in March 2012, epijune = 20 m depth in June 2012, hypooct = 100 m depth in October 2012, epioct = 20 m depth in October 2012, hypofeb = 100 m depth in February 2013. Red circles highlight where microbial physiological diversity (MPD) appears to lag behind MPD in shallow (20 m) waters by one season. As sampling ran from March 2012 to February 2013, MPD from 20 m depth in February 2013 (epifeb) is not comparable with MPD from hypolimnetic water in March 2012 (hypomar).

Table E1: Between-treatment comparisons of nutrient and DOC uptake during the June (winter), October (spring) and February (summer) bioassay experiments. DOC: dissolved organic carbon (mg l^{-1}), DRP: dissolved reactive phosphorus (μg l^{-1}); NO^3-N: nitrate nitrogen (μg l^{-1}), K-W: Kruskal-Wallis test, d.f.: degrees of freedom, χ^2: chi-square value.

Experiment	solute	Test	d.f.	F	χ^2	Sig.
June	DOC	ANOVA	3, 7	4.053		0.058
	DRP	K-W			4.860	0.027
	NO_3-N	K-W			5.333	0.021
October	DOC	ANOVA	1, 7	6.613		0.037
	DRP	K-W			5.771	0.016
	NO_3-N	K-W			4.500	0.034
February	DOC	ANOVA	1, 4	58.561		0.002
	DRP	K-W			3.857	0.050

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