Genomics and eDNA provide a holistic understanding of microbial communities and zoonoses in Aotearoa's waters

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Preface to Thesis

"The most important things are the hardest things to say. They are the things you get ashamed of, because words diminish them — words shrink things that seemed limitless when they were in your head to no more than living size when they're brought out."

The Body – Stephen King

Abstract

In Aotearoa, water quality and freshwater ecosystem health is declining. Much of that decline has been blamed on livestock farming and is quantified by using invertebrate communities or *Escherichia coli* levels. However, the wider microbial community, particularly archaea and eukaryotes, have been overlooked in the current ecosystem health measures. Some of the more well-known impacts of microorganisms on waterways are sporadically measured (e.g., algae blooms, drinking water contamination by faeces, and shellfish toxins) but the drivers of their community structure, both individually and as a whole, in Aotearoa is poorly understood. This thesis is a start at rectifying this knowledge gap.

The studies in this thesis were approached with a One Health perspective and provide a quantitative, holistic analysis of microbial communities by investigating three significant challenges facing Aotearoa's freshwater ecosystems. Those challenges - the spread of waterborne disease, eutrophication, and microbial biogeochemical cycling - were investigated using microbiological cultures and environmental DNA, analysed with metagenomic and other molecular methods.

I was able to determine that targeted testing for genetic loci associated with antimicrobial resistance and Shiga toxin-producing *E. coli* virulence was a useful in monitoring three Canterbury waterways for human health. Furthermore, enteropathogenic human and bovine strains of *E. coli* appeared unresponsive to in-stream nitrate-nitrogen concentrations of 0, 1, and 3 mg/L and native in-stream biota in microcosms. However, environmentally sourced *E. coli* imported as part of the in-stream biota survived longer in NO_3-N concentrations of 1 and 3 mg /L than at 0 mg/L.

Microorganism groups (e.g., archaea, bacteria, and microbial eukaryotes) responded to different environmental, spatio-temporal, and physico-chemical drivers depending on taxonomic level. As a group, lotic pressures and dispersal outweighed other drivers in community structuring. Archaeal communities were highly correlated with Austral season and the most abundant functional groups reflected a likely response to common agricultural pollutants found the Waiotahe catchment and in many rural rivers across Aotearoa (e.g., nitrogen pollution and livestock waste/effluents). The drivers commonly associated with bacterial survival (e.g., conductivity and temperature) were less important than dispersal and lotic pressures, particularly at lower taxonomic levels. Cultured *E. coli* concentrations from sediment and/or the water column were poorly indicators of *Campylobacter*, *Enterobacter*, and *Enterococcus* relative abundances. Additionally, neither *Enterobacter* nor *Enterococcus*

relative abundances were correlated with *E. coli*/*E. cloacae* group concentrations or *Campylobacter* relative abundances.

These findings have important implications for water quality monitoring and recreational human health risk assessments in Aotearoa. Currently, microbiological water testing is limited to bathing season (i.e., late spring to early autumn) and to culturing either *Enterococcus* in saline/brackish water or *E. coli* in freshwater. Effective water quality monitoring must include both water and benthic substrates to accurately portray the entire riverscape. Genetic loci associated with zoonotic human pathogens are present in some of Aotearoa's waterways and they are likely a result of catchment land use, livestock farming, and effluent contamination. Additionally, genetic loci can be detected with collection methods similar to those employed for current water quality monitoring using *Escherichia coli* and some molecular methods are more specific (i.e., not proxies).

Metagenomic methods allowed for the discernment of microbial communities and core biomes from genetic information extracted from environmental samples. Microbial communities were affected by many different in-stream conditions; however, dispersal and the pressures associated with lotic systems proved to be more important than adjacent land use, precipitation levels, or season. In contrast, archaeal communities were better explained by season.

It is clear that water monitoring in Aotearoa needs an overhaul and to incorporate new technology in a thoughtful and ecologically informed manner. A review of the current methods and new technologies should be undertaken by a multi-disciplinary group of experts in the fields of microbiology, epidemiology, and freshwater and microbial ecology. Community buy in and the inclusion of Māori values and indigenous rights should be at the forefront of any proposed changes to freshwater restoration and conservation.

List of Publications

1. Davis, M.T., Canning, A.D., Midwinter, A.C., and Death, R.G. Nitrate enrichment does not affect the persistence of enteropathogenic *Escherichia coli* in aquatic microcosms. *PeerJ*, published September 27, 2022.

2. Davis, M.T., Midwinter, A.C., Cosgrove, R. and Death, R.G., 2020. Detecting Genes Associated with Pathogenicity and Antimicrobial Resistance in Three New Zealand Waterways. *PeerJ*, published December 3, 2021.

3. Phiri, B.J., Pita, A.B., Hayman, D.T., Biggs, P.J., Davis, M.T., Fayaz, A., Canning, A.D., French, N.P. and Death, R.G., 2020. Does land use affect pathogen presence in New Zealand drinking water supplies?. *Water Research*, *185*, p.116229.

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Terms and Abbreviations

AMR	Antimicrobial resistance, a genetic tolerance/resistance to antibiotic(s)
Antibiotic	A chemical that kills or restricts bacterial growth
Community	Organisms residing in the same place at the same time
Ecosystem	Defined areas where the interactions between living beings and their physical environment are viewed as a system
eDNA	DNA acquired from the environmental sources, not from a single organism
Enrichment	Enhanced, typically with a particular nutrient(s) (e.g., nitrogen)
Eutrophication	An overabundance of nutrients, typically used in reference to aquatic systems
Host	The environment (living or not) in which a living organism resides (e.g., earth is host to many species, humans host many bacteria)
Horizontal gene transfer	The acquisition of genetic elements through transfer
Interface	The common boundary shared between systems, organisms, or phases
Macroinvertebrate	An invertebrate that is visible without magnification
Macrophyte	Aquatic plants excluding algae
MALDI-TOF	Matrix Assisted Laser Desorption/Ionisation-Time of Flight, a culture free method where samples are ionised and identified using time of flight mass spectrometry
Multi-host	Microorganisms that can be hosted by more than two species
Novel host	A new host species for a microorganism, indicative of a spillover event
Pathogen	Describes the relationship an infecting agent has with a particular host, identifying it as the causative agent of a disease process in that host species
Pathogenesis	How a disease process develops
PCR	Polymerase chain reaction, a culture free method to identify targeted genetic element(s) using paired primers (specific DNA sequences)

Periphyton	Heterotrophic microbes including algae and cyanobacteria, attached to submerged aquatic surfaces
Pollution	Substances discharged into the receiving environment faster than it can be used (e.g., nutrients, plastics, or gasses)
Reach	The length of a river between two defined points
Reservoir host	The environment in which a microorganism grows and multiplies without causing a disease process
Shotgun Metagenomics	A method for using all of the DNA within a given sample by breaking it into random, small fragments, which are then sequenced and used to identify community members and/or assemble entire genomes
Silo	The isolation of people or research from external influences
Spillover	The movement from one host species into a novel host species
STEC	Shiga-toxin-producing *Escherichia coli*, a ruminant hosted zoonoses
Strain	Genetic variant of a microorganism
Virulence	A microorganism ability to initiate and maintain a disease process in a host
Virulence genes	Genetic elements coding for toxin or other genetic elements related to virulence (e.g., *stx* - Shiga-toxin production, *eae* - facilitates attachment and effacement of *E. coli* to intestinal cells)
Zoonotic	Animal hosted microorganism that can cause a disease process in humans

Preface to Chapter 1

"Remake our world into a place where we may raise our families on happiness amidst an abundance of water… is this our goal?"

Dune – Frank Herbert

"Ocean is more ancient than the mountains and freighted with the memories and the dreams of Time"

The White Ship – H.P Lovecraft

1 Introduction

This is a multi-disciplinary thesis covering molecular, microbial, and freshwater ecology. It is focused on improving the management of freshwater ecosystems in Aotearoa. However, much of the background information needed to understand each chapter is not conventionally understood between disciplines, or even within a single discipline. Therefore, a significant amount of background information necessary to understanding the methods and study findings is presented in Chapters 1 and 2. The information is presented from a big picture perspective to a more localized view.

1.1 Part One – Introducing Freshwater Ecosystems: Ecological Health, Human Health, and Global Care

1.1.1 Global Freshwater Ecological Health

Although ~70% of the Earth's surface is covered in water, just 0.01% of that water is freshwater (Gleick, 1996). This equates to freshwater covering less than 1% of Earth's 510,100,000 km^2 surface (Dudgeon et al., 2007; NASA, 2020). However, more than 150-200,000 aquatic species rely on healthy freshwater habitats, above and below ground, for survival (Cazzolla Gatti, 2016). The exact number of species - both terrestrial and aquatic - reliant on freshwater as a resource is unknown. This is likely because most studies on terrestrial and aquatic biodiversity treat these habitats as separate entities, not interconnected and interdependent (Ward & Tockner, 2001; Astorga et al., 2011; Allen et al., 2012; Knapp et al., 2017). For this reason, the effects of freshwater pollution reach further than many studies estimate and may ultimately result in biodiversity losses across all habitat types.

Ecosystems are defined areas where the interactions between living beings and their physical environment are viewed as a system (Rapport et al., 1998). We measure the health of ecosystems by assessing how they are performing relative to the ideal range of functions expected from them (Costanza, Norton & Haskell, 1992; Shrader-Frechette, 1994). Some of the services provided by freshwater ecosystems include: drinking water; power generation; irrigation; producing aquatic foods (e.g., fish, shellfish, waterfowl); flood control; recreational activities; wildlife habitat; and soil fertilisation (Postel & Carpenter, 1997; Dodds, Perkin & Gerken, 2013; Higgins et al., 2021). Ecosystems are often viewed only in terms of the services they provide humans; however, human manipulation of land and water

is undermining freshwater ecosystem functioning resulting in a reduction in those services (Levin, 2009; Hayes et al., 2014; Sayers et al., 2015; Hjalten et al., 2016).

The declining health of freshwater ecosystems and the organisms living in them is well documented (Saunders, Meeuwig & Vincent, 2002; Pereira et al., 2010; Turak et al., 2017; Tickner et al., 2020). In Aotearoa, the majority of freshwater ecosystem degradation has been tied to changes in land use and the increase in intensive farming, specifically dairy farming, over the last few decades (Foote, Joy & Death, 2015; Julian et al., 2017). One reason for the continued decline is the misplaced perception that, no matter how polluted, water can be made drinkable with technology (Cervantes, 2009; Vasudevan & Oturan, 2014; Xia et al., 2020). However, that view is discordant with reality, as more than 30% of the human population and millions of other organisms do not have access to clean freshwater, either because technology is unable to keep up with pollution levels, the technology is inaccessible to communities needing it, or the water is no longer there (World Health Organization, 2020). Additionally, this reductionist view ignores the right of non-human organisms to clean water (Alkemade et al., 2009; de Vries et al., 2011; Kok et al., 2018; Kraus, 2019). Technology is a powerful tool, but the belief that it can remediate continuous pollution is unrealistic, unsustainable, and unaffordable (Foote, Joy & Death, 2015; Culhane, Robinson & Lillebø, 2020; Lupi et al., 2020; Hyytiäinen et al., 2021). Other reasons for the global decline of freshwater ecosystem health include the economics of pollution prevention and remediation (Baskaran, Cullen & Colombo, 2009; Stoeckl et al., 2013), the inability to see chemical and bacterial pollutants with casual observation (Sirés & Brillas, 2012; Shah et al., 2020), and a lack of education around what healthy waterways look like and how they function (Lindemann-Matthies & Bose, 2008; Flotemersch et al., 2019). Freshwater ecosystems are currently under more stress than any other ecosystem and this stress will continue to increase as human demand escalates with growing populations and climatic changes (Reid et al., 2019).

1.1.2 Aquatic Systems – Neglected Vectors of Human Pathogens

Terrestrial aquatic ecosystems (e.g., lakes, rivers, wetlands, and estuaries) function as a network, receiving, carrying, remediating, and evacuating waste from the catchments they drain (Davies et al., 2010; Larned et al., 2020; Polvi et al., 2020). Physical filtration and the metabolic processes of the communities residing in soils (e.g., plants, invertebrates, and microorganisms) are the primary methods of remediation for chemical and biological pollutants applied to land (Reddy, Khaleel & Overcash, 1981; Almaganbetov et al., 2008;

Abatenh et al., 2017). However, like any filter, soil has a saturation point (e.g., with microorganisms, nutrients, water) and excess is lost as leachate, entering adjacent aquatic systems with the flow of water red (Reddy, Khaleel & Overcash, 1981; Mirsal, 2008). Through the movement from land to water or by direct contamination, rivers may become passive vectors and aquatic stores for human, animal, and plant pathogens they host, transport, and transmit (Reddy, Khaleel & Overcash, 1981; Dangendorf, 2004; Semenza et al., 2012; Mori & Smith, 2019).

1.1.3 Eutrophication of Freshwater Ecosystems

Eutrophication (an overabundance of nutrients) is a common, human driven pollutant and a threat to aquatic ecosystem health and functioning worldwide (Selman & Greenhalgh, 2010; Lürling et al., 2016; Srivastav, 2020). The nutrients primarily associated with eutrophication in freshwater systems are nitrogen and phosphorus; of the two, nitrogen is a greater issue in Aotearoa's rivers (Conley et al., 2009). Most nitrogen mobilisation into aquatic systems is a direct result of fertiliser overuse and agricultural practices and land management, primarily pasture raised livestock farming reliant on intensively cultivated feed crops (e.g., grasses, palm kernel, and maize) and the waste produced by livestock (Tilman et al., 2002; Potter et al., 2010). The negative ecological responses to increased nitrogen in aquatic systems are well documented, namely, a loss of biodiversity through habitat changes and/or direct toxicity to aquatic life (Figure 1.1) (Vorosmarty et al., 2010; Vilmin et al., 2018). This has led to many countries establishing nitrogen limits to protect the ecological health of their rivers (Camargo & Alonso, 2006; Heiskary & Bouchard Jr., 2015; Poikane et al., 2019). The national bottom line for annual median nitrate in Aotearoa's rivers is 2.4 mg NO_3-N/L, set to limit the effects of nitrate toxicity on in-stream organisms, not trophic state (e.g., biological productivity) (Ministry for the Environment, 2020).

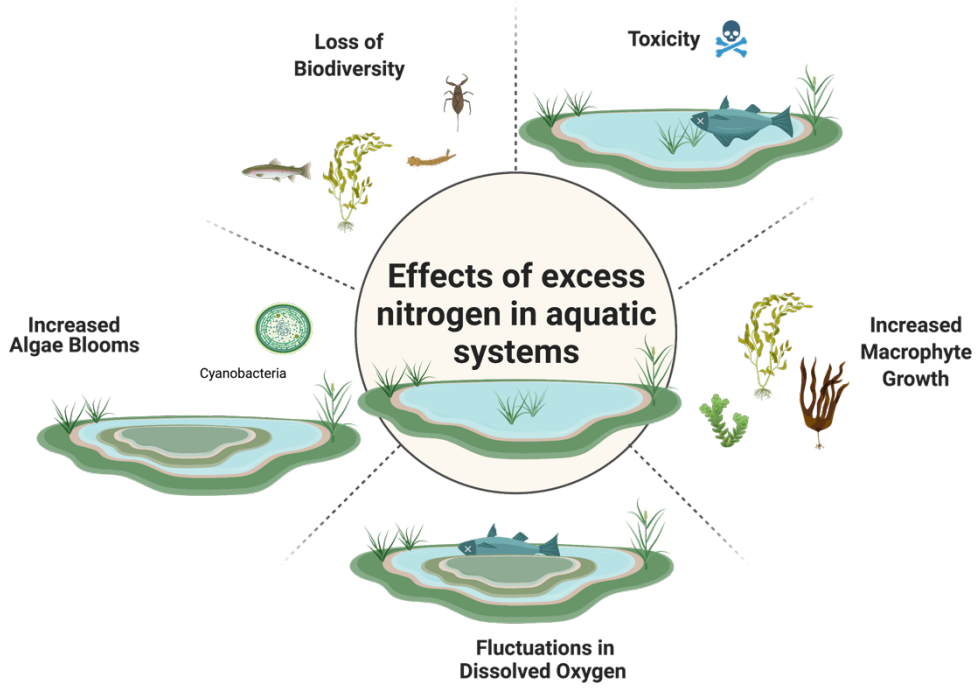

Figure 1.1 Potential effects of eutrophication.
Potential effects of excess nitrogen in aquatic systems include: habitat changes, resulting in a loss of biodiversity (plant and animal); increased likelihood of algal blooms; increased macrophyte growth; fluctuations in dissolved oxygen, potentially resulting in fish and invertebrate deaths; and direct toxicity to in-stream life (created using BioRender.com).

In addition to its toxicity to aquatic life and ecosystem degradation, there are negative human and animal health outcomes associated with high nitrate levels in drinking water (El-Wakf et al., 2009; World Health Organization, 2017; Ward et al., 2018; Sprong et al., 2020). Maximum nitrate-nitrogen (NO_3-N) levels in drinking water were originally established by the WHO (1993) at 11.3 mg NO_3-N/L to prevent acquired methemoglobinemia in infants, a physiological response to high levels of ingested NO_3-N resulting in hypoxia and potentially death (Ward et al., 2018). At that time the teratogenic and carcinogenic potentials of nitrate as a precursor to N-nitroso compounds were not fully appreciated (Ward et al., 2018). Evidence now suggests that human and animal exposure to N-nitroso compounds, potentially including those produced from ingesting drinking water with high NO_3-N levels, are linked to birth defects, pre-term labour, cancer, and/or other health issues (Stayner et al., 2017; Ward et al., 2018; Sprong et al., 2020; Sherris et al., 2021). These findings have led to researchers

questioning whether there may be a relationship between New Zealander's health and the increasing nitrate levels in their drinking water (Chambers et al., 2021; Richards et al., 2021).

1.1.4 Global Responsibility and Citizenship

Global exchange and trade enables some countries (e.g., China, Japan, Saudi Arabia (von Braun & Meinzen-Dick, 2009)) to outsource specific types of production, notably food and livestock farming, sparing their countries the direct public health and ecological degradation associated with those types of production (MacDonald et al., 2015; Oita et al., 2016; Vandergeten et al., 2016). Increasingly, governments, industry leaders, and policy makers are being urged to include accountability and sustainability measures, such as lifecycle analysis models and environmental footprints (e.g., TRACI and ReCiPe and/or eutrophication potential and carbon or water footprints), on resource use and products (Bare, 2002; Huang, von Lampe & van Tongeren, 2011; Bento & Klotz, 2014; Erickson & Lazarus, 2014; Konakli, Sudret & Faber, 2016; Orlova et al., 2016). Lifecycle analysis models are one way of measuring resource use and the environmental cost that products incur from beginning to end (Huang, von Lampe & van Tongeren, 2011; Erickson & Lazarus, 2014; Konakli, Sudret & Faber, 2016). By understanding how much of a resource is used from what is available and how, and in what form that resource returns to the system – with or without pollutants or as a different entity (e.g., fossil fuels to greenhouse gas) – potential limits can be set to ensure continued access and equitable accountability (Hoekstra, 2014). One lifecycle model of particular importance to freshwater ecosystems is the eutrophication potential which measures the impact food production has on nutrients in a system (Huijbregts & Seppälä, 2001; Romanelli et al., 2020).

When assessing the eutrophication potential of food production methods, red meat has the highest eutrophication potential followed by dairy products, chicken/eggs, and fish (Xue & Landis, 2010). Meat production's eutrophication potential is estimated to be 50 times that of grains and double that of fruit and vegetables when assessed by energy produced (g N equivalent/kcal) (Xue & Landis, 2010; Lassaletta et al., 2014). Not only does dairy hold the highest eutrophication potential but pastoral cattle's combined pollution footprint (e.g., microbial contaminants, sediment, water footprint, nitrogen footprint, and carbon footprint) is the highest of any livestock farming practice (McDowell & Wilcock, 2008; Yost, Diarra & Topp, 2011; Scholtz, Du Toit & Neser, 2014).

In addition to cattle farming having the highest pollution footprint of any food product, the method of rearing affects the amount and spread of the pollution (Scholtz, Du Toit & Neser,

2014; Monaghan et al., 2021). The diffuse nature of pollution associated with pastoral farming results in extensive environmental degradation, particularly to aquatic systems and forest habitats (Monaghan et al., 2021; Silva, Barioni & Moran, 2021). The amount of degradation is a direct result of size. Pastoral farming relies on applying artificial fertilizers to large areas of grassland for large livestock to graze and then spread waste over (Stats NZ, 2017; Chapman et al., 2020; Monaghan et al., 2021).

This need for grassland for grazing results in large scale deforestation (Pecl et al., 2017; Zu Ermgassen et al., 2020; Silva, Barioni & Moran, 2021). The detrimental effects of deforestation, nutrient pollution, and livestock waste on the functioning of adjacent freshwater systems (e.g., rivers, lakes, and groundwater) and their communities (e.g., fish, invertebrates, microorganisms, and plants) are a direct result of farm management (Pollans, 2016; Gluckman, 2017; Snelder, McDowell & Fraser, 2017; Parsons, Fisher & Crease, 2021). How this pertains to the degraded state of Aotearoa's rural waters should be clear. Yet responsibility for and the scope of freshwater contamination is often confounded by scientists, industry, and government agencies arguing over tenths of a mg/liter and myopic assessments of aquatic systems as disconnected units instead of a as single point in water's immense global lifecycle (Korenaga, Planavsky & Evans, 2017; Abbott et al., 2019a,b; Hansen, 2019; Silva, Barioni & Moran, 2021). Ultimately and distressingly, the financial gains associated with the polluting of water commons are generally privatised, sometimes subsidised, but rarely levied for remediation (Pollans, 2016; World Health Organization, 2017). More commonly, governments are made responsible for ameliorating the pollution *post hoc* to provide safe drinking water and clean waterways for their citizens to use (Pollans, 2016; World Health Organization, 2017).

1.2 Part Two - Microorganisms

1.2.1 Microorganisms in Aquatic Systems

Microorganisms are the driving force behind biogeochemical cycling on Earth (Madsen, 2011; Rousk & Bengtson, 2014; Anderson, 2018; Grossart et al., 2020). The microbial communities residing in freshwater ecosystems are, concurrently, juxtaposed with and influenced by adjacent terrestrial, groundwater, and atmospheric microbial communities (Hendricks, 1993; Zeglin, 2015; Battin et al., 2016). Riverine geomorphology facilitates the interaction of water with solutes, gasses, and solid materials supporting the diverse functionality of in-stream microbiota (Hendricks, 1993; Allan & Castillo, 2007). Moreover,

the same pressures (e.g., stochastic, dispersal, predation, commensalism) that influence community structure in macroorganisms are present in microbial communities (Groner & Novoplansky, 2003; Erken, Lutz & McDougald, 2013; Trøjelsgaard & Olesen, 2016; Albright & Martiny, 2017). In addition to the biotic and abiotic drivers of community structure, rapid generation cycling and complex genetic plasticity equip microbes with an adaptability and responsiveness to anthropogenic incursions that is unmatched in higher organisms (Logue, Findlay & Comte, 2015; Nuy et al., 2018; Sugden, 2020).

1.2.2 Pathogenic or Not

Microorganisms capable of causing disease, especially in humans, attract more attention and money for research, designing and improving treatments, and prophylaxis (e.g., vaccines, reducing vector contact, or preventative medications such as antimalarials) (Head et al., 2020; Prudêncio & Costa, 2020). Commonly, groups of microorganisms are ubiquitously and incorrectly referred to as 'pathogens' (Casadevall & Pirofski, 2014). The term 'pathogen' describes the relationship an infecting agent has with a particular host, identifying it as the causative agent of a disease process in that host species (Casadevall & Pirofski, 2002, 2014). Additionally, the term 'pathogen' is often used without a proper descriptive noun (e.g., human pathogen or plant pathogen), mistakenly implying it can cause disease in all organisms (untrue of any microbe) (Casadevall & Pirofski, 2002, 2014).

The virulence, or measurable loss of host fitness due to infection, of an infecting agent typically lies on a spectrum; it is a product of the unique interaction of the host's immune status (e.g., age, sex, stress levels, and genetic variation) and the infecting agent (Read, 1994; Weiss, 2002; Tahamtan et al., 2020). This explains symptom and clinical outcome variances across infected populations independent of treatment (Perry, 2012; Davidson et al., 2014; Tahamtan et al., 2020). Virulence factors, distinct characteristics – typically cellular structures or products (e.g., fimbriae and serum resistance) – imparting functional or physical properties that increase the ability of the organism to infect and damage the host, are an important aspect of pathogenesis (Johnson, 1991; Rahme et al., 1995). Virulence factors are genetic in origin and may be encoded on chromosomes or carried on mobile genetic elements (e.g., plasmids and/or phages) (Escobar-Páramo et al., 2004; Chen et al., 2005; Talukdar et al., 2013). In bacteria, mobile genetic elements may be shared across genera through horizontal gene transfer – via conjugation, transformation (acquisition of free DNA from the environment), transduction, and/or vesiduction (Figure 1.2) (Dutta & Pan, 2002; Soler & Forterre, 2020).

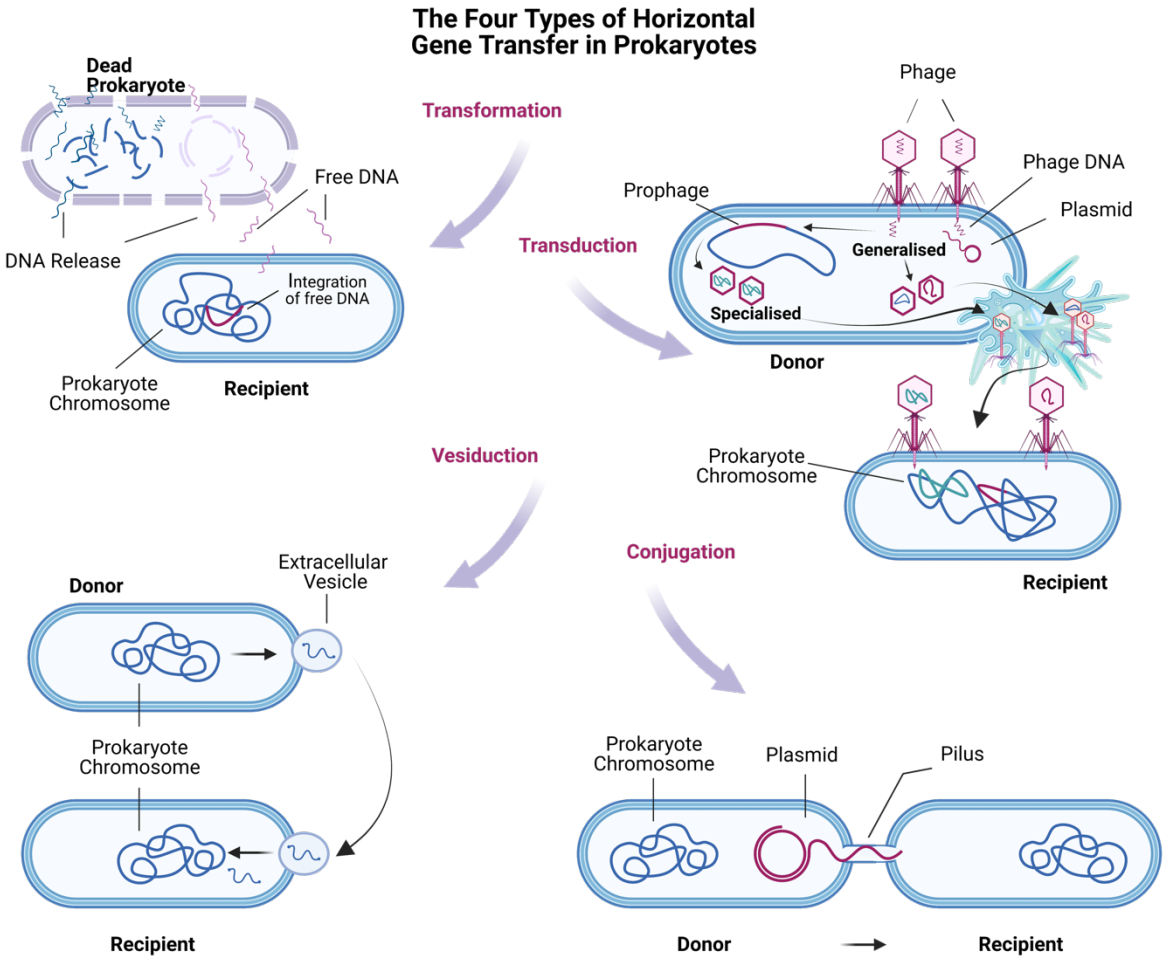

Figure 1.2 The four types of horizontal gene transfer.
The four types of horizontal gene transfer in prokaryotes: transformation, transduction, vesiduction, and conjugation (created using BioRender.com).

Shiga-toxin-producing *Escherichia coli* (STEC) are one type of bacterial human pathogen for which many of the genetic loci associated with virulence are well documented and include both prophage and plasmid encoding: Shiga-toxin production, stx_1 and stx_2; intimin attachment, *eae*; and haemolysin, *hlyA* and *ehxA* (Boerlin et al., 1999; Nakamura et al., 2020).
Attempts to explain the drivers of virulence through coevolution, or prudent exploitation – where host and infecting organisms evolve in tandem, ideally achieving a commensal relationship – leave a multitude of exceptions without explanation (van Valen, 1977; Ewald, 1983; Levin, 1989). Though avirulence (i.e., reduced virulence) may be selected in some instances, it is clearly not the outcome of every long-standing pathogen/host relationship;

human examples include tuberculosis and syphilis, which exhibit no reduction in virulence over time (Alizon et al., 2009; Shin et al., 2018). An alternative, tradeoff theory, correlates higher virulence with increased transmission, exchanging host damage for greater infectivity (Anderson & May, 1982; Frank, 1996). Both theories incorporate rigid assumptions about the genetic evolution of the invading organism; prudent exploitation assumes near-constant evolution, while trade-off theory assumes a constant state of equilibrium where the ideal exploitation-to-transmission ratio is always in effect, ignoring short-term evolution entirely (Stenseth & Smith, 1984; Frank, 1996; Galvani, 2003).

Ironically, both theories become problematic in instances where infection is occurring in novel hosts (spillover) and/or when more than one virulence genotype is competing for the same host - a 'tragedy of the commons' where host survival is the limiting resource (Hardin, 1968; Frank, 1996; Ekroth et al., 2021). If tradeoffs were the primary driver, genotypes associated with higher virulence would quickly consume the available resources and out compete prudent exploiters, burning through host populations quickly (Bremermann & Pickering, 1983; Frank, 1996). Additionally, reduced virulence may be advantageous where culling, restricted host availability, and/or high host resistance is present (Ebert & Bull, 2003; Geoghegan & Holmes, 2018; Ekroth et al., 2021; Wargo et al., 2021). Contemporary theories are increasingly turning to genomics to better understand where and how virulence is selected, but there is still much work to be done in the field (Geoghegan & Holmes, 2018; Najafi et al., 2018; Wang et al., 2018).

1.2.3 Where You Live Defines You

The environment in which a microorganism grows and multiplies without causing a disease process is referred to as its reservoir (e.g., bats are reservoir hosts to rabies-causing lyssaviruses (Rupprecht, Kuzmin & Meslin, 2017)) (Hubálek, 2003; Porta, 2014). Reservoirs may be biotic or abiotic; microorganisms living in or on abiotic substrates (e.g., soil or water) are sapronotic and form environmental reservoirs (e.g., cholera) (Webster, 2017). Human pathogens with an animal host, are classed as zoonotic (e.g., STEC - hosted by cattle (Browne et al., 2018)) (Webster, 2017). Human pathogens with zoonotic or sapronotic reservoirs often emerge and re-emerge in human populations unpredictably because they can complete their lifecycle without a human host (Woolhouse & Gowtage-Sequeria, 2005).

1.2.4 Zoonoses – Transmission and Multi-host Networks

Human pathogens and the interspecific transfer of disease-causing microorganisms became the public health 'wicked issue' of 2020 with the advent of the Covid-19 pandemic caused by the SARS-CoV-2 virus (Fauci, Lane & Redfield, 2020; Jefferies et al., 2020). Prior to Covid-19, the world had been through multiple zoonotic epidemics: the Black Death in 1350, Russian influenza in 1889, and Spanish influenza in 1918 (Smith, 1995; Perry & Fetherston, 1997). Then in 1976, the Ebola virus disease outbreak ran through 55 villages along the Ebola River from Yambuku, Democratic Republic of the Congo to Nzara, South Sudan, (World Health Organization, 1978). Over the decades following its discovery, the Ebola virus came and went with no predictable seasonality, the severity of the infections fluctuated, and it was absent for years at a time suggesting it likely had a wildlife reservoir (Chowell & Nishiura, 2014; Coltart et al., 2017; Kock et al., 2019).

In 2006, the World Health Organisation signalled that our understanding of the public health burden zoonotic diseases represent needed to improve and that zoonoses were important from economic and welfare perspectives (Schrag & Wiener, 1995; Wilcox & Gubler, 2005; Alexander et al., 2018). We now know that most emerging human diseases (~70%) are zoonotic and two thirds of all known zoonoses are hosted by more than one species (multi-host pathogens) (Cleaveland, Laurenson & Taylor, 2001; Fong, 2017; McMahon, Morand & Gray, 2018; Daszak et al., 2020). We also know that the phylogenetic relatedness of hosts, their interaction intensity, the health of the surrounding environment, and the amount of wildlife habitat destroyed are strong predictors of zoonotic disease emergence (Cleaveland, Laurenson & Taylor, 2001; Haydon et al., 2002; Daszak et al., 2020; Johnson et al., 2020; Wardeh, Sharkey & Baylis, 2020). However, we are still limited in our ability to predict novel zoonoses emergence (spillover events), as human systems are complex, there are multiple drivers involved, and there are many unidentified microorganisms with the capacity to be pathogenic to humans (Fitzgerald & Musser, 2001; Wilcox & Gubler, 2005; Jones et al., 2008).

More recently, the impacts to both national and global economies and supply chains resulting from zoonotic outbreaks have been felt across the world (Mackenbach et al., 2008a; Ahmed et al., 2020; O'Brien et al., 2020a; Rubino et al., 2020a). The economic impact of the 2014 Ebola epidemic in West Africa was estimated to be more than $50 billion ($2014 USD) (Huber, Finelli & Stevens, 2018) and the 2015-2017 Zika epidemic in South America and the Caribbean was likely more than $7 billion USD (UNDP & IFRC, 2017; Daszak et al., 2020). The economic impacts associated with Covid-19 are still evolving but are primarily related to measures aimed at reducing disease spread such as stay at home orders, compromised supply

chains, changes in demand (e.g., increased demand for some items - PPE, toilet paper, pipettes, and other testing related consumables - and decreased demand for others - vehicles and household appliances), and job losses (e.g., manufacturing plants, restaurants, tourist destinations) (Ceylan, Ozkan & Mulazimogullari, 2020; Inoue & Todo, 2020). Mental and physical health issues arising from the Covid-19 are widespread and dynamic, with current data suggesting women and minority groups are being disproportionately affected (Mackenbach et al., 2008b; Azcona et al., 2020; Garg et al., 2020; Fisher & Ryan, 2021). Reduced access to non-Covid-19 related healthcare services (Patt et al., 2020; Rubino et al., 2020b) in combination with isolation (Chiappini et al., 2020; Serlachius, Badawy & Thabrew, 2020), job losses (Crayne, 2020), and changes in routine (e.g., working/schooling from home) are all associated with poorer health outcomes (Bubb & Jones, 2020; Purwanto et al., 2020). The transfer of disease-causing organisms between species is also important from animal production and welfare perspectives, with the potential to impact wildlife conservation and agricultural management (Gortázar et al., 2007; Jones et al., 2013; Daszak et al., 2020; Morand, 2020). This is particularly true where spillover and spillback (the movement of a zoonotic pathogen back into a native population) may occur (Figure 1.3) (Nugent, 2011a). Wildlife welfare may be negatively affected as habitats are 'cleansed' with pesticides and detergents (Kaiser et al., 2015), ecosystems may be destroyed and/or poisoned to remove host reservoir species from areas populated by human and/or their livestock (Lainé & Morand, 2020), the illegal and/or indiscriminate culling of potential host species (McCulloch & Reiss, 2017a; Frutos & Devaux, 2020; Lu et al., 2021), and rise of associations (e.g., the National Association of Bush Meat Sellers in Nigeria after an Ebola virus outbreak) set on eradicating any potential host species (Kaiser et al., 2015; O'Brien et al., 2020b). Conservation efforts are impacted as visitors to zoological facilities and parks diminish reducing vital income streams, field research is put on hold and researchers recalled, invasive species eradication programs are halted, and/or indiscriminate culling of at risk/endangered species that may be hosts occur (Bates et al., 2021; Gibbons et al., 2021; Lu et al., 2021; Miller-Rushing et al., 2021). Agricultural production may be impacted by culling (e.g., minks during Covid-19 (Frutos & Devaux, 2020), and cattle with bovine tuberculosis (TB) (Barlow, 1994) or spongiform encephalopathy (mad cow disease) (Bhakdi & Bohl, 2003)), livestock import/export bans (Mtimet et al., 2021), and/or supply chain failures and/or sanctions (Udmale et al., 2020; Sodhi & Tang, 2021).

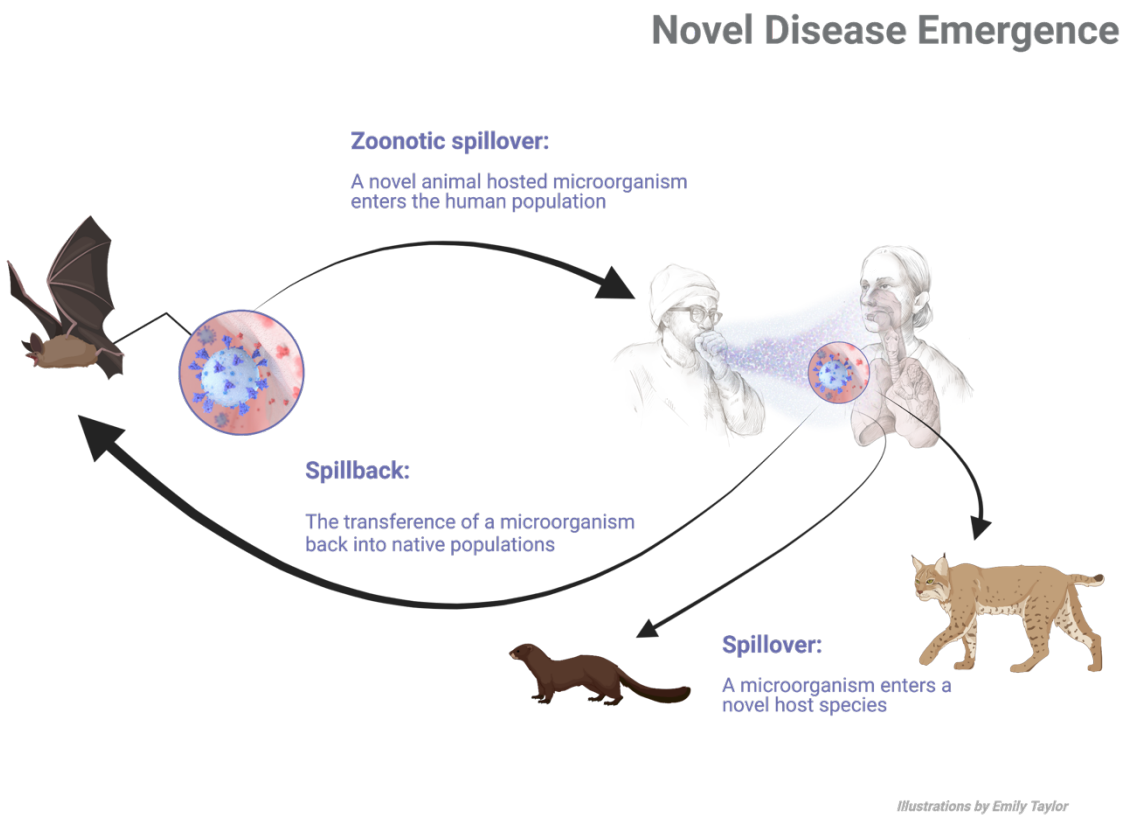

Figure 1.3 Pathways for disease transmission.
Novel disease emergence pathways: zoonotic spillover, spillover, and spillback (created using BioRender.com).

Zoonoses are just one type of multi-host pathogen (Cleaveland, Laurenson & Taylor, 2001). Multi-host pathogens are most shared between domesticated animal species, and humans, with the strength of interaction networks increasing multi-host disease formation (Figure 1.4) (Cleaveland, Laurenson & Taylor, 2001). For example, 90% of domestic carnivore and 77% of livestock pathogens have been found to form multi-host systems (Cleaveland, Laurenson & Taylor, 2001; Haydon et al., 2002). Taxa interact with these multi-host pathogens in a number of ways, as a: primary host, where the infectious organism reaches maturity and is capable of infecting others and there may be a disease process; reservoir host, where the infectious organism reaches maturity and is capable of infecting others and there is no disease process; b: secondary host or vector; the infectious organism does not mature and remains with the host for a short period (e.g., ingests and excretes); or c: initiates a disease process and is recovered from or kills the host (e.g., ingests and excretes) or c: initiates a disease process and is recovered from or kills the host (Russell, 1998; Martin, 2015). Owing to the ability of many multi-host pathogens to remain in the environment, particularly aquatic

environments, or use an arthropod vector (e.g., mosquito, flea, and/or tick), multi-host disease systems are more common between species with overlapping territory even if direct contact is limited (Cleaveland, Laurenson & Taylor, 2001; Lipp et al., 2001; Fong & Lipp, 2005; Karesh et al., 2012; Estrada-Peña et al., 2014; Malham et al., 2014; Silk et al., 2018).

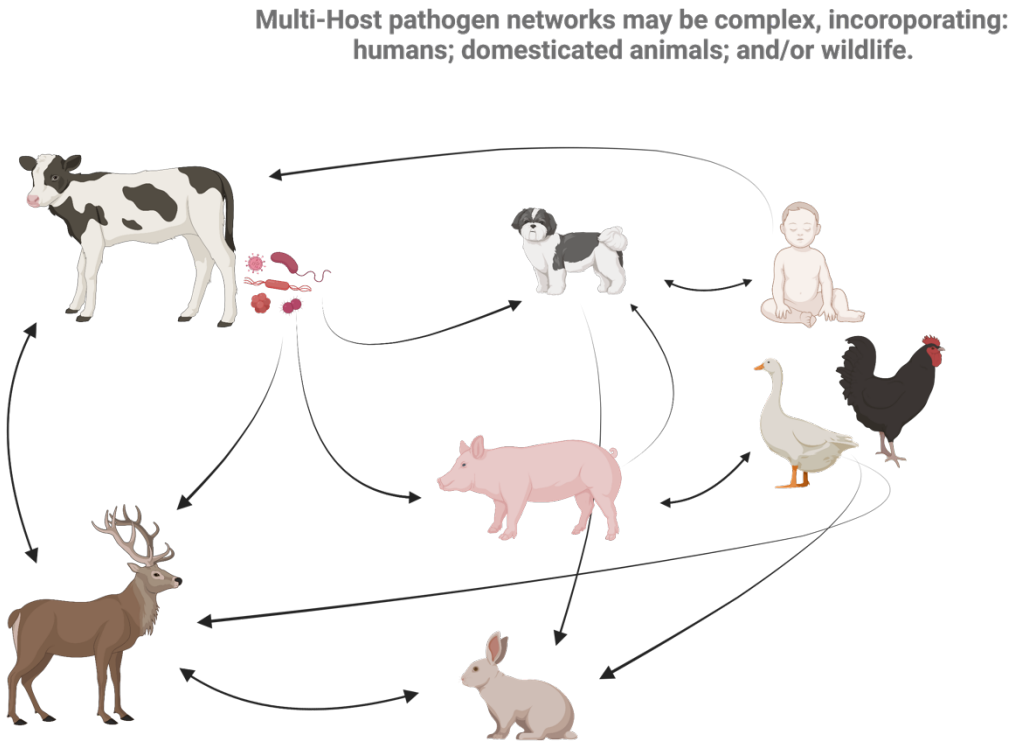

Figure 1.4 Multi-host pathogen networks.
Multi-host pathogen networks may include humans, domesticated animals, and/or wildlife. Transmission pathways may be stable or intermittent; unilateral, bilateral, or multimodal (created using BioRender.com).

1.2.5 Interfaces and Drivers of Novel Disease Emergence

Human land use change - deforestation, human settlement, and farming (both livestock and crops) - is the single most influential driver of novel human disease emergence (Plowright et al., 2016; Daszak et al., 2020; Mendoza et al., 2020; Swei et al., 2020; Wu, 2021). On average, five new human diseases emerge annually (Fong, 2017; Daszak et al., 2020; Plowright et al., 2021). Most novel human pathogens are uncharacterised microorganisms (e.g., virus, bacteria, protozoa), making them difficult to monitor in any meaningful or premeditated way (Daszak, Cunningham & Hyatt, 2000; Haydon et al., 2002; Daszak et al., 2020). Reciprocally, humans, their livestock, and pets harbour microorganisms that may

threaten wild biodiversity (e.g., bovine tuberculosis (McCulloch & Reiss, 2017b), bovine brucellosis (Cross et al., 2007)) (Morand, 2017; Gallina et al., 2020; Gryseels et al., 2020; Bouwmeester et al., 2021). Transmission of pathogenic organisms is often bi-directional, with multiple spillover and spillback events possible (Bengis, Kock & Fischer, 2002; Nugent, 2011b; Frutos & Devaux, 2020).

The potential for negative health outcomes, to both humans and animals, resulting from land use changes are mostly ignored in the planning stages of land development (Dulu, 2020; Plowright et al., 2021; Reaser et al., 2021). We know how terrestrial and aquatic ecosystem health degrade in response to certain human activities (e.g., intensive livestock farming (Menzi et al., 2010; Foote, Joy & Death, 2015) and urbanisation (Rahman & Vu, 2020)) and that disease emergence increases in response to ecosystem degradation (Häsler et al., 2012; Fong, 2017; Daszak et al., 2020); however, there is still a general disregard for the mechanisms behind disease emergence and lack of forethought towards prevention. The primary mechanisms of infection are well documented including: habitat loss (either total or fragmented), increasing wildlife/human/domestic animal interactions; a loss of biodiversity, removing customary hosts, driving spillover to new host species; and reduced ecosystem functioning, increasing interactions with pollutants urging pathogen evolution (e.g., heavy metal or herbicide pollutants driving antimicrobial resistance) (Schrag & Wiener, 1995; Daszak, Cunningham & Hyatt, 2000; Crump, Murdoch & Baker, 2001; Giller et al., 2004; Olson et al., 2015).

Spillover is not just a foreign issue. Although disease emergence is more common in tropical climates where research is poorly funded, the global movement of humans and animals leaves no country immune (Reed et al., 2003; Tatem, Rogers & Hay, 2006; Brockmann & Helbing, 2013). Ascribing pandemics only to foreign practices is xenophobic. Instead they should be attributed to risky practices (e.g. illegal wild animal trading, wet markets, and/or selling and eating exotic meats, intensive livestock farming, humans and domestic animals intruding into wildlife habitats, and/or the exotic pet trade – birds, sugar gliders, foxes, or reptiles) (Goławska et al., 2019; Gravesen, 2020; Onoma, 2020; Dhont, Piazza & Hodson, 2021; Moorhouse, D'Cruze & Macdonald, 2021). While trade in and consumption of wildlife, particularly mammals and birds that are well-known reservoirs for human pathogens, does increase the risk of spillover events, these practices and the risks associated with them vary significantly between countries and are not responsible for the majority of human zoonotic diseases (Aguirre et al., 2020; Daszak et al., 2020; Barnett & Fournié, 2021). Unfortunately, human population growth and climate change are likely to continue to reduce and move

wildlife habitats, increasing novel animal/human/pathogen interactions and accelerating novel disease emergence (Wessely et al., 2017; Baisero et al., 2020; Burden et al., 2020; Daszak et al., 2020).

1.2.6 Using *Escherichia coli* to Better Understand Rivers as Vectors

Antimicrobial resistant infectious diseases are emerging as one of the most pressing global public health issues (Hernando-Amado et al., 2019). Antimicrobial resistance (AMR) is a result of interactions between microbes and their environment, both biotic and abiotic; and in particular the use of antibiotics in livestock and humans is considered the predominant driver (Hernando-Amado et al., 2019). Enterobacteriaceae - including *Escherichia coli* (*E. coli*) which is commonly used as a proxy bacteria for disease risk - are recognised as an important group of bacteria that carry AMR (Ferri et al., 2017; Hay et al., 2018). Bacterial strains with AMR are linked to high mortality rates, recovery complications, and increased costs associated with treatment in clinical settings (Cosgrove, 2006; Pendleton, Gorman & Gilmore, 2013; Ferri et al., 2017; Hay et al., 2018). Until recently antimicrobial resistant bacteria were primarily of interest in hospital settings as they were only thought to be associated with antibiotic use as evidenced by the global push for increased antimicrobial stewardship (Amann, Neef & Kohl, 2019). However, AMR is increasingly being identified in the environment, wildlife, and domestic animals with other non-antibiotic factors promoting its emergence (e.g., heavy metal contamination (Chenia & Jacobs, 2017)) (Cosgrove, 2006; Bancroft, 2007; Wilharm et al., 2017; Singh, 2018; Wyres & Holt, 2018).

The production of extended spectrum β-lactamase (ESBL) enzymes commonly underpins drug inactivation or a reduction in sensitivity to β -lactam antibiotics and subsequent AMR emergence (Lewis et al., 2007). These enzymes provide resistance to β-lactam antibiotics, including first and third generation cephalosporins, carbapenems, and penicillins (Bonnet, 2004). Critically, many of the genes encoding β-lactamase production can be horizontally transferred via plasmids between bacterial genera (Carattoli, 2013). Globally, dairy farms, particularly their calves, have been identified as reservoirs of ESBLs (Ishii et al., 2005; Dahmen et al., 2013; Gonggrijp et al., 2016). In Aotearoa, where dairy farming is a major land use, there is limited research on the prevalence of AMR, including ESBLs, on farms, from livestock, or from adjacent aquatic systems (Burgess et al., 2021).

International studies are increasingly identifying $bla_{\text{CTX-M-1}}$ and $bla_{\text{CTX-M-9}}$ ESBLs from zoonotic Shiga-toxin-producing *E. coli* (STEC) strains hosted by cattle (Ishii et al., 2005; Browne et al., 2018; Elmonir et al., 2021; García-Meniño et al., 2021). The bovine intestine is

a known reservoir for AMR bacteria (Auffret et al., 2017) and STEC (Fukushima, Hoshina & Gomyoda, 1999; Irshad et al., 2016; Browne et al., 2018). Bacteria residing in the digestive tract are often impacted by antibiotic use and then, in pastoral farming systems, excreted onto land where they are potentially transported into waterways (Kirchner et al., 2013; Dwivedi, Mohanty & Lesikar, 2016). Shiga-toxin-producing *E. coli* are an important group of zoonoses because of their propensity to cause kidney disease, neurological damage, and haemorrhagic colitis particularly in young children and the elderly (Oakes et al., 2006; Tarr, 2009). As a group, STECs are the fourth most reported zoonotic disease in the EU (European Centre for Disease Prevention and Control, 2018) and USA (Centres for Disease Control and Prevention, 2018) and increasing in prevalence in Aotearoa (Public Health Surveillance: Environment Group ESR, 2019). However, multi-drug resistant and ESBL producing STEC strains are an underappreciated emerging public health risk around the world.

Both AMR and STEC have been responsible for human outbreaks related to contact with recreational waters as they are transmitted via the oral-faecal route (Ahmed, Gyawali & Toze, 2015; Swaggerty et al., 2018). In the United States as many as 10% of human STEC O157 outbreaks may be waterborne (Rangel et al., 2005). Additionally, STEC have demonstrated the ability to invade the tissues of terrestrial produce (e.g., lettuces and sprouts (Merget et al., 2019; Wang, J Deering & Kim, 2021)) and there is the potential that aquatic vegetation, such as water cress, could be similarly infected. Wildlife (e.g., foxes, deer, and boar (Asakura et al., 1998; Díaz-Sánchez et al., 2012; Mora et al., 2012)), including birds and insects (Nielsen et al., 2004; Gibbs et al., 2005; Brandl, 2006; Foster et al., 2006) can transport STEC from one habitat to another (e.g., physically move on skin/fur/feathers/feet or by ingesting and excreting), including into waterways (Probert, Miller & Ledin, 2017).

The two strains of STEC responsible for most human disease globally are O157 and O26 (Centers for Disease Control and Prevention, 2018; European Centre for Disease Prevention and Control, 2018; Public Health Surveillance: Environment Group ESR, 2019; Joseph et al., 2020). Ruminants are the primary hosts for STECs (Aktan et al., 2007; La Ragione et al., 2009) and direct contact with ruminants, their waste, environment, and food products, including produce farmed using animal manure and/or contaminated water, are the most common risk factors for human outbreaks (Henderson, 2008). However, there is a growing list of wildlife spillover hosts (e.g., mammals, birds, amphibians, fish, and invertebrates (Espinosa et al., 2018)) resulting from environmental contamination by domesticated ruminants (Henderson, 2008). Although STECs are not the most common or economically

impactful zoonoses, they offer excellent model bacterial organisms in catchments impacted by livestock farming where *E. coli* is already being monitored for water quality.

1.3 Part Three - Approaches

1.3.1 Disease Research - A History

Historically, successful human pathogen spillover events have been studied and communicated in silos (i.e., isolation) (Wolfe, Dunavan & Diamond, 2007; Evans & Leighton, 2014; Johnson, Hansen & Bi, 2018). Ecologists studied host population dynamics and distribution, host-pathogen evolution, and the relationship between environmental health and disease outbreaks (Schrag & Wiener, 1995; Morse et al., 2012; Rabinowitz & Conti, 2013; Estrada-Peña et al., 2014; Alexander et al., 2018; Carver & Lunn, 2020). Epidemiologists focused on the animal human interfaces and identifying drivers, modes, and frequency of disease spread (Van Kerkhove et al., 2011; Chowell & Nishiura, 2014; Kock et al., 2019; Peel et al., 2019; Nikolay et al., 2020). Immunologists and microbiologists investigated the pathogenesis of disease-causing agents, intraspecific variation, phylogenies, and the physiological characteristics of various hosts and disease-causing agents (Hoiby, Doring & Schiotz, 1986; Wakelin, 1996; Bean et al., 2013; Preziusoa et al., 2014; Kash & Taubenberger, 2015; Mandl et al., 2015; Plowright et al., 2016). The lone interdisciplinary cyborgs, technology heavy bioinformaticians, used computer scripts and genomic sequences to further illuminate host and pathogen biological data through codes and binaries (Cortes et al., 2018; Murillo et al., 2020). Crucial research, isolated by cognitive, lingual, and professional space, restricting its reach to the sounding box of preferred disciplinary journals and is written in subtly discriminatory scientific ‘dialects’ (Mayer, 1968; Fry, 2001; March, 2005; Barzilai-Nahon, 2009; Bimpitsos & Petridou, 2012; Reeve & Partridge, 2017).

1.3.2 One Health

While predicting outbreaks is a complex issue, a multi-disciplinary research approach is essential (Bidaisee & Macpherson, 2014; Cleaveland, Borner & Gislason, 2014). The overarching goal, to reduce the spread of infectious diseases, has resulted in many inter-, multi-, and trans-disciplinary collaborations with varying degrees of participation and engagement (Campbell, 2005; Horton et al., 2014; Scholz & Steiner, 2015; Harrison et al., 2020). One of the more successful approaches - One Health - maintains as its core belief that human, animal, and environmental health are interconnected (Rabinowitz et al., 2013; Evans & Leighton, 2014). However, through the hierarchy of words in its simple definition,

> "*One Health is a collaborative, multisectoral, and transdisciplinary approach—working at the local, regional, national, and global levels—with the goal of achieving optimal health outcomes recognizing the interconnection between people, animals, plants, and their shared environment.*"
>
> (Center for Disease Control and Prevention, 2021)

it is clear whose health is viewed as most important and how we as a scientific community rank the value of the health of our environment and, in effect, those who study it (Johnson, Hansen & Bi, 2018; Lainé & Morand, 2020). If we look more closely, we find One Health was formed around the idea that human and animal health and their shared environment are considered important and that both humans and animals have a higher burden of infectious disease when that shared environment is degraded (Rulli et al., 2017; Daszak et al., 2020). The most recent One Health definition aims to increase the importance of the environment, by including ecosystems and plants in its wording (One Health High-Level Expert Panel (OHHLEP), 2022). However, while being a significant improvement, this definition still places greater emphasis on the impacted species, not the driver of adverse health – environmental health.

> *"One Health is an integrated, unifying approach that aims to sustainably balance and optimize the health of people, animals and ecosystems. It recognizes the health of humans, domestic and wild animals, plants, and the wider environment (including ecosystems) are closely linked and inter-dependent."*
>
> *(One Health High-Level Expert Panel (OHHLEP), 2022)*

I would suggest that the definition, our research priorities, and our value system are backwards; that the environment's health is the most important factor for all life residing within it, and that it is the spaces in between, the edges and overlaps, not the defined boxes, that are most important in health research (Fong, 2017; Daszak et al., 2020).

1.3.3 The Role of the Environment

That an unhealthy environment results in poorer health outcomes for humans and animals is well documented (Daszak, Cunningham & Hyatt, 2000; Hubálek, 2003; Jones et al., 2008; Daszak et al., 2020), but there is little mention of environmental or ecosystem health being intrinsically important or of how either is defined in the One Health approach (Johnson & Degeling, 2020). This lack of clarity under the One Health umbrella may reflect the base membership of medical professionals (both human and animal) around the world; with a low proportion of membership comprised of environmental scientists and/or ecologists (Zinsstag

et al., 2011; Rabinowitz et al., 2013; Bidaisee & Macpherson, 2014; Cleaveland, Borner & Gislason, 2014). This reduced engagement amongst environmental experts may be related to One Health's origins in the medical community: a lack of exposure for those who aren't medical professionals, a perceived focus on human outcomes and not environmental ones, or that environmental experts may be viewed as less valued members (Destoumieux-Garzón et al., 2018; Johnson, Hansen & Bi, 2018; Reaser et al., 2021). Whatever the reason, ensuring the engagement of ecologists and environmental scientists should be prioritised in any future One Health initiative to better understand the ecological drivers of pathogen transmission and in implementing effective ecological remediation and prophylactic conservation (Reaser et al., 2021). We must recognise that zoonotic spillover events are increasing in frequency around the world because we have underprioritised the role of the environment for too long (Daszak, Cunningham & Hyatt, 2000; Olson et al., 2015; Daszak et al., 2020).

To begin that process, basic universal defined measures of environmental health need to be incorporated under the One Health umbrella and these measures must include local indigenous knowledge to ensure engagement at all levels (Montenegro & Stephens, 2006; Thomson et al., 2012; Harmsworth & Awatere, 2013; Mantyka-Pringle et al., 2017; Isaac et al., 2018). One can argue that every environment is different, and each will have special needs; however, we can certainly do better than what is currently in place, which is nothing. By defining environmental health in measurable scientific and historic terms, we enhance global accountability while respecting and empowering indigenous knowledge (Harmsworth & Awatere, 2013; Mooney & Cullen, 2019; Gonzales Tovar et al., 2021). Only after agreeing on terms can we identify those environments most in need and prioritise, as a global community, how best to support those regions. By continuing to focus on identifying potential human pathogens from animal hosts and/or the environment, we are treating the consequences not the causes of novel disease emergence; functioning in the same manner as the ambulance at the bottom of a cliff, when a fence is what we need.

1.3.4 Current Measures of Riverine Ecosystem Health

There are numerous ways to measure the health of river ecosystems, including: abiotic water quality through water clarity, turbidity, temperature, pH, dissolved oxygen, and nutrient levels (Dufour, 1984) and the use of community indices, macroinvertebrate, fish, and microbial (Stark, 1998; Joy & Death, 2004; Niu et al., 2018); microbiological cultures of indicator organisms (e.g., *E. coli* and/or faecal coliforms) (Odonkor & Ampofo, 2013); chlorophyl metrics (Harris & Dash, 2010; Mishra & Mishra, 2012); and habitat quality

assessments (Christensen et al., 1996; Maddock, 1999). However, no single measure or sampling method provides a holistic framework with which to assess the health of our freshwater systems and those reliant on them (Boulton, 1999; Maddock, 1999; Vollmer, Regan & Andelman, 2016).

1.3.5 Microbiological Monitoring in Waterways - Faecal Indicator Bacteria

Globally and nationally, the risk of contracting a human pathogen capable of causing gastrointestinal disease from drinking water and recreational waterways is assessed by culturing and counting faecal indicator bacteria such as *E. coli* or faecal coliforms (Havelaar & Melse, 2003; Hrudey, Hrudey & Pollard, 2006; Ministry of Health, 2017; Porter et al., 2017). Despite these protocols waterborne disease outbreaks continue to occur (Baldursson & Karanis, 2011; Probert, Miller & Ledin, 2017; Gilpin et al., 2020). In Aotearoa, this may be a result of the extended sample-to-result time (Hamilton, Kim & Thackston, 2005), the infrequency of sampling (commonly one to two week intervals for recreational waterways and limited to 'swimming months') (Gluckman, 2017), the delivery of unsanitised drinking water (Ministry of Health, 2018), and/or the lack of standardised culturing/counting/reporting methods practiced nationally (Larned et al., 2016). Aotearoa's complacency and 'she'll be right' attitude regarding the high levels of faecal contamination in its aquatic systems have resulted in human gastrointestinal disease outbreaks associated with: drinking water, the largest recorded campylobacteriosis outbreak to be specific (New Zealand Government, 2017); recreational water (King, Lake & Campbell, 2011); and the commercial sale of contaminated farmed shellfish (Simmons et al., 2001; Wall et al., 2011). Clearly, there is a problem that current monitoring is not alleviating.

1.3.6 Challenges in Current Monitoring Methodology

Current water monitoring techniques are labor intensive (both to collect and process) and rely on a minimum level of expertise to process and interpret (Haase et al., 2010; Pule, Yahya & Chuma, 2017). Assessing a single reach often entails numerous replicates of multiple sample types with varied, bulky storage and transportation requirements and specific processing times which can limit where sampling is able to be carried out (Stainton, Capel & Armstrong, 1977; Gregor & Maršálek, 2004; Schwoerbel, 2011).

The various sample types are rarely processed in a single laboratory increasing the potential for mistakes, contamination, degradation, and/or loss. Additionally, this type of monitoring encourages specialisation based on methodology (e.g., macroinvertebrate identification,

microbiology, environmental chemistry, and plant science) creating pseudo-silos within aquatic ecology, incorrect application of methods, and a fractured understanding of the system as a whole (Morse et al., 2006).

1.3.7 Environmental DNA as an Alternative Method

Advances in molecular technology may offer an alternative to many or all the current biotic measures used for environmental assessments if developed and applied appropriately (Doi et al., 2017; Stewart, 2019; Pansu et al., 2021). Molecular methods have the potential to assist with assessing river connectivity, monitoring conservation and restoration projects, bio-surveillance for invasive species, and monitoring species re-introduction (Hughes, Schmidt & Finn, 2009; Woods et al., 2010; Garlapati et al., 2019).

The use of environmental DNA (eDNA), intra- and extracellular DNA collected from the environment, to identify organisms is an increasingly popular alternative to traditional survey methods, particularly in aquatic systems (Deiner & Altermatt, 2014; Maechler et al., 2014; Balasingham, Walter & Heath, 2017). Rivers are ideal habitats in which to employ eDNA techniques due to their connectivity, rapid mixing, and unidirectional flow supporting effective passive collection (Wacker et al., 2019). Additionally, the different substrates (e.g., water and sediment) may offer temporally distinct survey perspectives. The mobile nature and speed of DNA degradation in the water column, a result of microbial processing and abiotic conditions (e.g., UV light, pH, and temperature (Strickler, Fremier & Goldberg, 2015; Troth et al., 2021)), typically denotes recent presence (Nielsen et al., 2007). While sediment samples may provide a more stable and protected environment preserving eDNA for longer and/or in higher concentrations (Nielsen et al., 2007; Boehm Jr et al., 2009; Turner, Uy & Everhart, 2015). Riverine and flood plain core samples using ancient eDNA have informed many important planetological findings (Anderson-Carpenter et al., 2011; Lejzerowicz et al., 2013; del Carmen Gomez Cabrera et al., 2019).

1.3.8 Advantages of eDNA

There are considerable advantages to animal welfare in developing non-invasive survey methods using eDNA. Replacing or at least reducing the need to handle and/or harvest living biological entities (e.g., macroinvertebrates, fishes, periphyton, and bacteria) is especially important when studying lifecycle events (e.g., spawning (Tillotson et al., 2018; Thalinger et al., 2019)), cryptic, endangered, protected, or hard to access/culture species (Lor et al., 2020; Mauvisseau et al., 2020; Plough et al., 2021). Use of eDNA is proving useful when

identifying bacteria that take weeks to grow (e.g., *Leptospira* sp. (Picardeau, 2013)), require media and growth conditions (e.g., carbon dioxide incubation (Bolton & Coates, 1983)) that are inaccessible to all laboratories or are viable but unable to grow in a lab environment (e.g., viable but not culturable STECs (Ding et al., 2017)).

Additionally, eDNA is an efficient methodology - in cost, processing time, and analysis. It can be used study any organisms and/or stored to be (re-)analysed in the future when new techniques and/or species are identified making it an attractive multi-disciplinary research tool. This, in combination with the array of molecular techniques available (from functional studies to broad community surveys to targeting genetic loci, such as antibiotic resistance or toxin-producing genes, chosen for their relevance to plant, animal, and/or human health (Lyautey et al., 2003; Song et al., 2008; Jerde et al., 2011; Maechler et al., 2014; Olds et al., 2016; Shaw, Weyrich & Cooper, 2016; Simon et al., 2019)) makes eDNA an exciting, fast paced, and constantly evolving field of study. However, there is much work needed around standardisation of protocols to ensure reproducible results and to prevent the misuse and misinterpretation of data (Cristescu & Hebert, 2018; Loeza-Quintana et al., 2020; Burian et al., 2021; Trujillo-González et al., 2021).

1.3.9 Molecular Analysis

Modern genomics, the study of genetic material to better understand its function and structure, has its roots in methods developed decades ago (Olsen et al., 1986; Hardison, 2003). The advent of Polymerase Chain Reaction (PCR) (Schochetman, Ou & Jones, 1988), single fragment DNA sequencing (Sanger) and then Next Generation Sequencing (NGS) methods, able to target individual gene loci and sequence millions of DNA fragments simultaneously, provided a quick, accurate method for producing large scale genetic data (Gill et al., 2006; Sogin et al., 2006; Thorp, Thoms & Delong, 2006; Nakamura et al., 2008; Qin et al., 2010; Prescott, 2017). Pace (1985) pioneered analysis techniques using eDNA which, in conjunction with NGS, has proven to be rigorous and precise, capable of revealing the biological diversity contained within microbiomes, communities of microorganisms within a shared defined ecosystem (Lederberg & McCray, 2001; Murienne et al., 2019). Today, PCR is a method commonly used to target specific genetic elements, or loci. A PCR reaction anneals molecular primers, specific to targeted loci, onto complementary DNA fragments present in a sample and then amplifies (i.e., makes millions of copies) those loci through cycled heating, producing millions of replicates of the targeted DNA (Schochetman, Ou & Jones, 1988). There are a number of PCR methodology variants in use (e.g., Loop-

mediated isothermal AMPlification (LAMP) (Wong et al., 2018) and real-time PCR (Mackay, 2004)) which produce comparable results.

Early microbiome research using genomics focused on techniques that utilised a PCR step to amplify targeted areas of the 16S ribosomal RNA gene's variable regions (16S) producing short DNA sequence lengths (i.e., reads) with which taxa could be differentiated (Pace, 1985; Acinas, Rodríguez-Valera & Pedrós-Alió, 1997; Fuhrman & Campbell, 1998). However, because 16S relies on a single gene, it is limited in its resolution power by the genetic similarity between bacterial species and the prevalence of horizontal gene transfer (Rosselló-Mora & Amann, 2001).

1.3.10 Choice of Techniques

One alternative to 16S targeted sequencing is whole genome shotgun metagenomics. Whole genome shotgun metagenomics reduce PCR amplification bias (i.e., they do not use primers to amplify any targeted loci but instead, as the name suggests, target a wide range of amplicons/sequences/reads across the genome) and provide a robust method for assessing microbial community composition (Figure 1.5) (Poretsky et al., 2014; Tessler et al., 2017; Johnson et al., 2019). Although both techniques have strengths, it is now widely agreed that 16S sequencing is less sensitive and less accurate than whole genome shotgun approaches (Jovel et al., 2016; Hillmann et al., 2018; Khachatryan et al., 2020). Whole genome shotgun metagenomics can provide more than just taxa identification. It can be used for whole genome assembly when there are enough reads to cover of the entire genome (i.e., coverage), overlap sufficiently (i.e., are contiguous), and there are ample copies (i.e., acceptable read depth) (Forouzan et al., 2018; Liang & Sakakibara, 2021). Whole genome shotgun metagenomics can also provide high resolution data useful in AMR surveillance (Oniciuc et al., 2018), functional group differentiation (Kalyuzhnaya et al., 2008), and strain/variant identification (Zhang et al., 2015; Geoghegan et al., 2020). Because of this flexibility in data use, I chose to use whole genome shotgun metagenomics for the microbial community and surveys of notifiable diseases I carried out on the Waiotahe river.

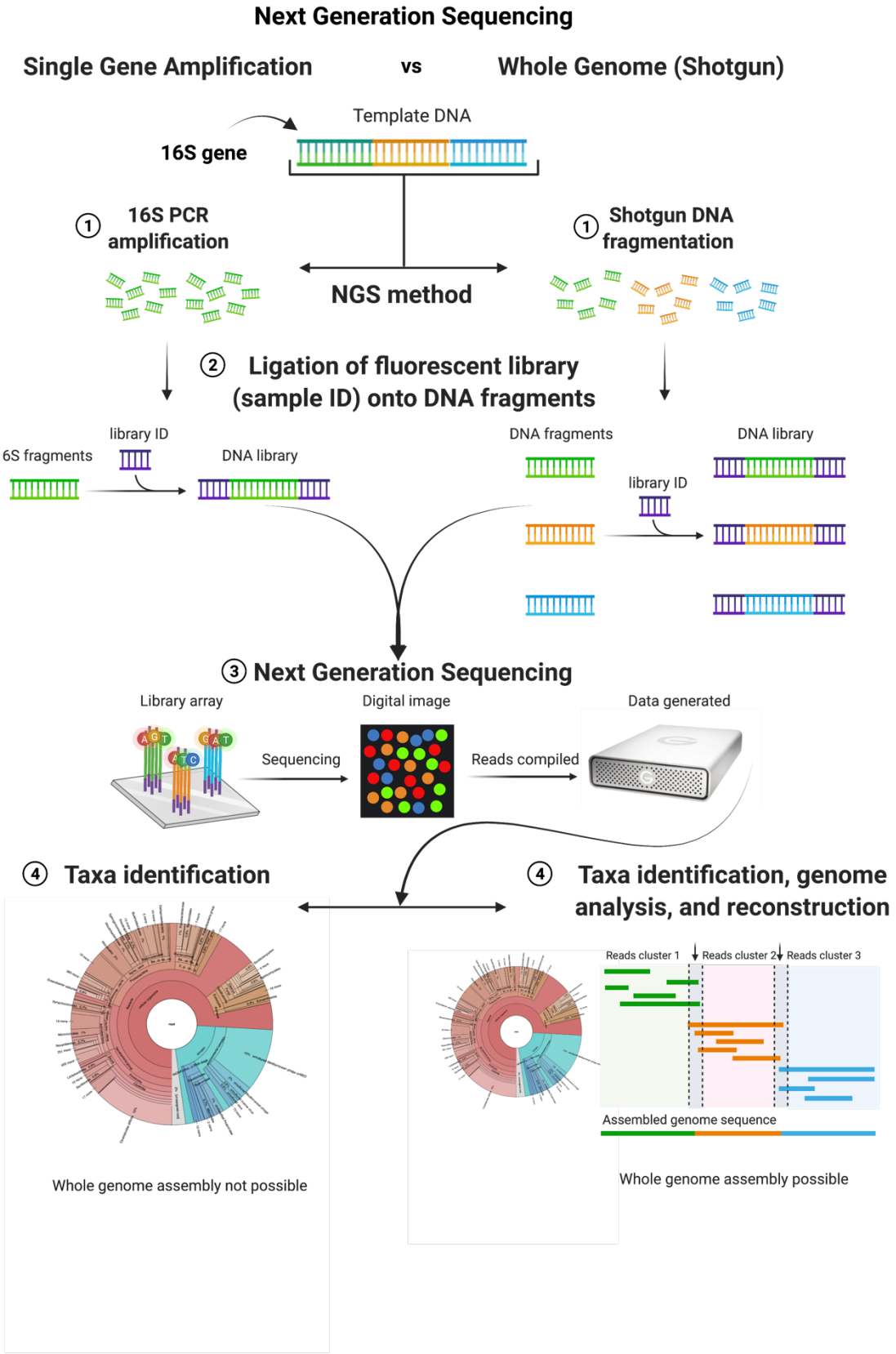

Figure 1.5 Next generation methods for analysing DNA.

A comparison of next generation methods for analysing DNA and the data types produced from 16S amplicon sequencing and whole genome shotgun metagenomics (created using BioRender.com).

1.3.11 Limitations

The primary limitations associated with whole genome shotgun metagenomics when compared to 16S, are the inability to target specific loci and reduced identification of cryptic species, because there is no targeted amplification step to increase DNA concentrations that may result in concentrations remaining below detection thresholds (Shah et al., 2011). Reduced identification of cryptic species, while a limitation, should not be seen as a drawback when assessing waterways. The heightened sensitivity of some molecular testing methods (e.g., some LAMP assays able to detect 100 ag of targeted DNA in mixed DNA samples (Winkworth et al., 2020)) that are advantageous in a clinical setting, when applied to environmental monitoring could lead to overestimating the abundance and therefore the risk associated with targeted organisms.

Overall, resolution of taxa using whole genome shotgun metagenomic approaches are robust; however, identification at the species/strain level in mixed samples is less accurate than from isolated organisms (Tringe & Rubin, 2005; Shah et al., 2011). Whole genome shotgun with Illumina produces short reads, ~300 bp in total length. Due to the length of the reads, there is the potential for acquired DNA (e.g., prophage insertions and/or plasmids acquired through some form of gene transfer (Figure 1.2)) to identify back to an originating organism. While longer reads may offer slightly better resolution at lower taxonomic levels, the cost is currently prohibitive (Tedersoo et al., 2021). However, this is just part of the general excitement of working with microscopic genetic hoarders that enjoy mocking the arbitrary and unnecessarily overthought ‘what is the source?’ question.

1.3.12 Challenges Assessing Aquatic Microbial Communities

Aquatic systems are complex, containing allochthonous (from external sources) and autochthonous (locally derived) microorganisms (Deiner et al., 2016; Ushio et al., 2017). Despite large amounts of high-quality genomic data, we are limited in our understanding of freshwater microbial communities and restricted in our ability to accurately predict community dynamics (Grossart et al., 2020; Hilderbrand et al., 2020). This is due, in equal parts, to the size of the communities, the high proportion of unidentified organisms, our poor understanding the range of potential interactions, and the organism’s inherent functional

mutability (Cotner et al., 2010; Fuhrman, Cram & Needham, 2015; Rodriguez-R et al., 2020). The lack of any routine, standardised approach does not help.

The most common solution for simplifying these complex communities has been to identify core microbiomes, the microbiota present in every sample from a defined ecosystem (Shade & Handelsman, 2012), at higher taxonomic levels (e.g., phylum, class, and genus) (Zorz et al., 2019). A core biome is the least common denominator between samples, providing variation between samples as a unit with which to measure community change (Urban et al., 2021). However, as in all community approaches, it presents some challenges in application, such as: where does the core biome begin and end when the habitat is extensive, but the organisms are microscopic; at what taxonomic level do we assess the core biome when there can be hundreds to thousands of taxa; how do we define abundance and how important are the rare taxa; and how do we account for all taxa, when read numbers bias our analysis?

1.3.13 Research Goals

In this research I used molecular and microbiological methods (e.g., cultures, targeted gene amplification, and whole shotgun metagenomics) to gain a better understanding of how time, space, water chemistry and quality, and the environment related to aquatic microbial communities and their function (e.g., archaea, bacteria, and microbial eukaryotes). To understand the riverscape holistically, I sampled and compared the microbial communities in the water column with those in the benthic substrates. This was important as most freshwater sampling protocols are restricted to water column samples alone. While human pathogenic and zoonotic bacteria were a focus of this research, the scope was limited to understanding the relationship of STEC and prevalence of AMR loci to water chemistry, water and benthic substrate *E. coli* concentrations, and/or land use. The overarching objective was to understand aquatic microbial communities as a whole, the factors, interdependencies, and current aquatic monitoring techniques that affected and/or reflected their structure.

The first step was to determine whether *E. coli* concentrations, the primary water quality metric and a proxy for faecal contamination in fresh and estuarine waters, was related to the presence of genes associated with human pathogenic bacteria in rivers and/or benthic substrates. This also allowed me to discern whether aquatic samples could be tested quickly and inexpensively for specific loci associated with human pathogenicity (e.g., STEC virulence or AMR), with the goal of reducing the current reliance on culturing proxies. Proxies are generally used when it is too time consuming, expensive, and/or difficult to identify the target organism(s) directly (Leightner & Inoue, 2007; Odonkor & Ampofo,

2013). However, there are several issues associated with the use of proxies; primarily oversimplifying relationships, erroneously equating the proxy with what it is a proxy for, misidentifying the proxy, and potentially identifying drivers for the proxy, not the target organism (Eigenbrod et al., 2010; Daly, Baetens & De Baets, 2018). Suffice to say, reducing the use of proxies and direct monitoring should be prioritised wherever possible.

The next step was to determine whether nitrate pollution, a significant concern for waters in Aotearoa and globally (e.g., ground, fresh, marine, and estuarine), affected enteropathogenic and/or ubiquitous environmental *E. coli* persistence. Nitrogen is a significant source of nutrition for many microorganisms including *E. coli*. Therefore, understanding how chronic environmental enrichment affects the distribution and longevity of virulent *E. coli* strains outside of a host is essential to estimating risk and protecting human and animal health.

Finally, I used whole genome shotgun metagenomics to investigate the relationships between microbial communities and their environment and with each other. I performed comprehensive seasonal surveys of the freshwater and estuarine microbial communities (e.g., archaea, bacteria, and microbial eukaryotes) along the Waiotahe river and Te Ahiaua, in 2018. I analysed archaea community structure individually and archaea, bacteria, and microbial eukaryotes together to investigate the greater microbial community as a whole. Where possible, I explored taxa function and morphology to complement basic community structuring mechanisms.

To counteract the reduced species/strain/virulence gene resolution associated with whole genome shotgun metagenomics, I augmented it with polymerase chain reaction (PCR) (Anklam et al., 2012; Lalzampuia et al., 2013). Adding this to the genomic data provided side-by-side validation for the whole genome shotgun metagenomics results as well as a stand-alone technique for assessing aquatic eDNA samples.

Preface to Chapter 2

"What's natural is the microbe. All the rest — health, integrity, purity (if you like) — is a product of the human will, of a vigilance that must never falter. The good man, the man who infects hardly anyone, is the man who has the fewest lapses of attention."

The Plague – Albert Camus

2 Overview of *Escherichia coli*, bacterial transportation into waterways, and persistence factors

2.1 Introduction

Ensuring clean water and sanitation for all humans by 2030 is one of the United Nation's 17 Sustainable Development Goals (United Nations, 2015a, 2020). Each of the Sustainable Development Goals is focused on improving human outcomes, without a single goal prioritising resources for sole the benefit of any non-human species (United Nations, 2015b,a). With just eight years left, access to improved water sources and sanitation has improved the least of the 17 goals (Tortajada & Biswas, 2018). Errors in initial reporting and in qualifying water safety have resulted in a compounding overestimation of the number of people with access to safe drinking water by approximately 1.8 billion people as of 2017 (United Nations, 2015b; Tortajada & Biswas, 2018).

Although poor water sanitation disproportionately affects developing countries, it is not restricted to them (United Nations, 2015b). Countries with a high proportion of their land used for livestock farming, similar to Aotearoa, report that adjacent waterways contain substantial levels of faecal contamination at both recreational and drinking water abstraction sites (Thornton & Gerber, 2010; Ministry of Health, 2017; Porter et al., 2017). In regions where agriculture is not a significant land use, the majority of freshwater faecal contamination may originate from untreated or overflowing human wastewater and/or wildlife (Borel, 2019; Honda et al., 2020; Korajkic et al., 2020; Américo-Pinheiro et al., 2021).

Human health risks related to faecal contamination of water is not restricted to drinking water. Health risks are also associated with participating in recreational use of waterways (McBride et al., 2002; Gluckman, 2017) and/or indirect ingestion by harvesting food from or irrigated with contaminated water (Doré, Henshilwood & Lees, 2000; Rose, 2001; Rose et al., 2001; Perkins et al., 2016). Additionally, failures in or fragmented application of sanitation treatments for drinking water drawn from contaminated water sources leave openings for outbreaks (Viñas, Malm & Pettersson, 2019; Phiri et al., 2020). With over 100 known human pathogens including *Leptospira*, *Clostridium*, *Mycobacterium*, and *Salmonella* genera able to survive in aquatic environments (Rose, 2001; Anderson, Whitlock & Harwood, 2005; Byappanahalli & Ishii, 2011) streamlining monitoring processes and implementing remediation of water sources, both recreational and drinking, are necessary.

In Aotearoa, outbreaks of waterborne diseases tripled during the 2004-13 period (Table 2.1) (Ministry of Health, 2017). The number of people affected by a single drinking water outbreak peaked in 2016, when the highly publicised campylobacteriosis outbreak, which affected more than 6,000 people in Havelock North, Aotearoa, occurred (New Zealand Government, 2017; Gilpin et al., 2020). Campylobacteriosis is typically caused by a livestock hosted human pathogen and is the most common bacterial cause of human enteric disease globally (World Health Organization, 2020a). The Government Inquiry into Havelock North Drinking Water (New Zealand Government, 2017) found multiple bore breaches, leading to surface water (contaminated by sheep faeces) likely entered the drinking water supply. It blamed a lack of action, accompanying the detection of elevated *Escherichia coli* (*E. coli*), a faecal indicator bacteria, levels in the water, to be at least partially responsible for the high number of residents affected (Clark, 1998; New Zealand Government, 2017).

Table 2.1 Number of disease outbreaks and associated cases recorded for enteric disease and waterborne enteric disease, 2004-2013. Adjusted from Ministry of Health (2017).

	Waterborne enteric outbreaks		**All enteric outbreaks**	
Year	**Outbreaks**	**Cases**	**Outbreaks**	**Cases**
2004	24	118	363	4,623
2005	27	184	342	2,365
2006	18	284	483	6,171
2007	15	205	479	7,866
2008	26	159	429	6,311
2009	24	87	587	10,217
2010	56	235	571	6,153
2011	45	141	545	6,715
2012	51	379	659	9,489

	Waterborne enteric outbreaks		All enteric outbreaks	
Year	Outbreaks	Cases	Outbreaks	Cases
2013	62	227	616	6,950

2.2 *Escherichia coli*

Escherichia coli are Gram-negative bacteria that are facultatively anaerobic (capable of reproduction and growth with or without oxygen) and shed in faeces (Blount, 2015). They are one of the most diverse bacterial species with thousands of strains described (Guo et al., 2012). The species' genetic diversity is so high that only ~20% of the base genome is shared within the species and in some instances, other bacteria are genetically more similar to strains of *E. coli* than other *E. coli* (Lukjancenko, Wassenaar & Ussery, 2010; Guo et al., 2012). They are metabolically complex and genetically mutable, able to adapt to various energy sources and habitat conditions (Reisner et al., 2006; Clermont et al., 2011). This is partially due to horizontal gene sharing among bacteria, commonly in the form of plasmids, across genera (Albert et al., 1992; Rousset et al., 2021). Importantly, ubiquitous (i.e., unidentified strains) *E. coli* are commonly used as a proxy to assess the extent of faecal contamination present in aquatic systems (Odonkor & Ampofo, 2013).

2.3 Microbes Deposited to Land

Sources of faecal matter are either point (wastewater treatment plant, discharge pipes, irrigation systems, and/or drainage ditches) or non-point (grazed pastures, wildlife, runoff, and/or failed septic systems) in origin (Jamieson et al., 2004). Non-point sources are more common in pastoral catchments and contamination of waterways by these sources often incur higher costs associated with identification, due to the extensive list of potential species implicated (Jamieson et al., 2004), and decreased remediation success (Harwood et al., 2014). Though livestock are the primary source of faecal microbes in water systems adjacent to land used for livestock farming (Jamieson et al., 2004; Yost, Diarra & Topp, 2011), lesser non-point sources include wildlife and birds (Lévesque et al., 2000) and onsite wastewater treatment systems (Dwivedi, Mohanty & Lesikar, 2016). The microbial loading potential from livestock is considerable, with one cow/pig/sheep each depositing twice the faecal

coliforms (e.g., 7 $\log_{10}$ CFU/g) per day of a single human (Jamieson et al., 2003). Additionally, irrigation with effluents has been linked to increased faecal bacteria survival in soils that is likely a result of increased soil moisture (Reddy, Khaleel & Overcash, 1981; Close et al., 2010).

Livestock density, effluent irrigation rate and regimen, and grazing management govern the number of microbes deposited to pastoral land (Reddy, Khaleel & Overcash, 1981; Porter et al., 2017). Microbial load combined with environmental conditions (e.g., other microorganisms present in the soil, nutrients available, sunlight, and soil pH (Reddy, Khaleel & Overcash, 1981; Xing et al., 2019; Petersen & Hubbart, 2020)) determine the land area, time, and distance from water necessary for adequate physical filtration and remediation of faecal microbes to ensure they are not available for transportation into waterways (Reddy, Khaleel & Overcash, 1981).

2.4 Transportation

Faecal microbes draining from pastoral land is the preeminent source of human pathogens entering waterways in rural areas (Graczyk et al., 2000; McLeod et al., 2003; USEPA, 2016). Microbes move from land into waterways through surface (Reddy, Khaleel & Overcash, 1981; Anderson, Whitlock & Harwood, 2005; Byappanahalli & Ishii, 2011) or subsurface transportation (Jamieson et al., 2003). Both methods depend on the concentration of microbes deposited (Edwards et al., 1997; Graczyk et al., 2000), surface and soil type (McLeod et al., 2008), distance to travel (McLeod et al., 2003), and precipitation volume (Lipp et al., 2001; Malham et al., 2014). Physical filtration and die-off are considered the primary mechanisms limiting bacterial mobility in soils (Figure 2.1) (Gerba & Bitton, 1984).

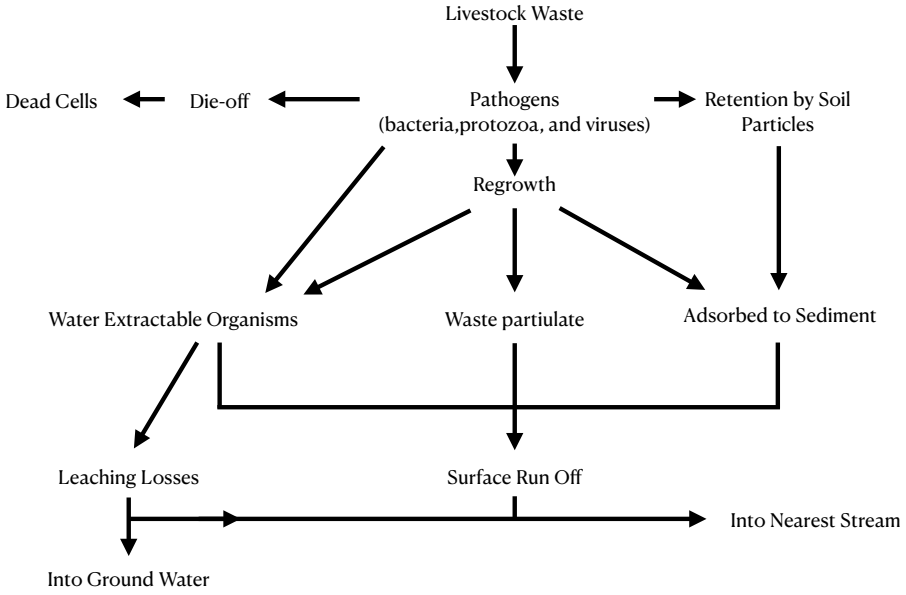

Figure 2.1 Pathogen transformation and transportation.
Pathogen transformations and transport from land areas receiving livestock wastes. Modified from Reddy, Khaleel & Overcash (1981).

2.5 Adsorption and the Importance of Soil Type

Movement of soils containing bacteria into waterways is the primary method of surface transportation (Desmarais, Solo-Gabriele & Palmer, 2002; Oliver et al., 2005; Droppo et al., 2010). Bacterial adsorption to soil particles is classified into two types, weak (utilising van der Waals forces) and strong (cellular appendages or polymers cementing bacteria to soil particles) (Palmateer et al., 1993). Soils with larger particle size and/or high clay content encourage bacterial adsorption (McLeod et al., 2003). Bacteria that use cellular appendages to attach to soil particles are more likely to regrow and survive for longer due to the availability of nutrients and soluble organic matter (Gannon, Manilal & Alexander, 1991; Marino & Gannon, 1991; Auer & Niehaus, 1993; Davies et al., 1995). *E. coli* grown in liquid media and/or sourced from aquatic systems commonly form biofilms and/or attach to sediment particles, likely due to the presence of fimbriae (Schillinger & Gannon, 1985; Jeng, England & Bradford, 2005).

Adsorption increases bacterial survival when: sheltering them from parasitism or predation (Teunis et al., 1997; Pachepsky & Shelton, 2011); soil particle size decreases (Howell, Coyne & Cornelius, 1996); temperatures stabilise; pugging or compaction by livestock occurs (McLeod et al., 2008); and soluble nutrients are increased (Garzio-Hadzick et al., 2010). In

saturated soils, bacterial transportation is influenced by low water storage ability (Mubiru, Coyne & Grove, 2000; Sojka & Entry, 2000) and decreased interaction time with soil particles, leading to increased pathogen movement into waterbodies (Chandler, Farran & Craven, 1981; Zhang et al., 1995).

Erosion, livestock disturbance, and runoff are all mechanisms of surface transportation for microbially contaminated soils to enter waterways (Muirhead et al., 2004; Davies-Colley et al., 2004; Byappanahalli & Ishii, 2011). Those soils, once in streams, become aquatic sediments, where microbial contaminants are retained and may form environmental reservoirs (Byappanahalli & Ishii, 2011). McDonald et al. (1982) identified the two environmental faecal bacterial reservoirs in pastoral catchments capable of colonising and/or re-colonising livestock, pastoral and in-stream. Movement of bacteria from pasture into in-stream reservoirs was dependent on land slope and hydrological processes, while movement within the channel system was related to sediment mobility (McDonald, Kay & Jenkins, 1982).

In-stream sediment mobility is closely associated with physical disturbances (e.g., precipitation, in-stream engineering, or stock movement) resulting in the displacement of sediments downstream by re-suspending them in the water column (Muirhead et al., 2004). Studies on water column *E. coli* concentrations have found increases, by one to two orders of magnitude, when sediments are resuspended (McDonald, Kay & Jenkins, 1982; Wilkinson et al., 1995; Muirhead et al., 2004) and that water clarity is closely linked to faecal contamination measures (Davies-Colley, Valois & Milne, 2018).

2.6 Advective transportation

Advective transportation, or movement by water flow, is the most common method of moving non-particle associated microbes from land into water (McLeod et al., 2003; Jamieson et al., 2004). This mechanism pushes microbes in the subsurface layer through soil pores, or if the soil is highly compacted or the water table is high, over land as runoff (McLeod et al., 2008). Advective transport moves water from areas of low to high concentrations, resulting in slower movement in soils where the water table is high. Although subsurface transportation is slowed by a high water table, there is an increased risk in over-land flow of bacteria, through runoff (Dwivedi, Mohanty & Lesikar, 2016).

Some soils are less suited to retaining bacteria, and some bacteria are less suited to retention, whether it's due to soil type, water-saturated soils, microbial saturation, or an inability of the bacteria to adsorb to soil, leading to microbial leaching between soil pores (McLeod et al.,

2003). Collins and Rutherford (2004) traced over-land shed of bacteria into waterways and its relationship to heavy precipitation events in hill country bordering on wetlands (where surface and subsurface flows converge). Their study found the number of *E. coli* reaching waterways was impacted by the velocity and volume of precipitation and that numbers dropped significantly as the time between precipitation events and livestock grazing increased (Davies-Colley et al., 2004; Blount, 2015). Interestingly, bacterial loading to the waterway also increased with faeces solubility, further supporting the theory that in certain *E. coli* strains are better able to survive in liquid habitats (Schillinger & Gannon, 1985; Collins & Rutherford, 2004). Ultimately, over-land advective transportation (e.g., bypass flow) is the primary input of faecal bacteria into waterways (Hunter, McDonald & Beven, 1992; Phiri et al., 2020; Boithias et al., 2021).

2.7 Bypass flow

Land vulnerability to bypass flow is categorised by soil classification, structure, and slope (McLeod et al., 2008). Soils exhibiting a high rate of bypass flow are strongly structured, heavily compacted (often from pugging by livestock or vehicles), and likely to show pooling (Young et al., 2005). Precipitation increases bypass flow by decreasing the time bacteria have to adsorb to soil, disturbing bacterial reservoirs (on land), and increasing water flow into channels (Graczyk et al., 2000). Irrigation with effluents magnifies the effects of bypass flow through microbial saturation, increased mobilisation of sediment, and increased soil saturation (Curriero et al., 2001; Lipp et al., 2001; Malham et al., 2014).

In addition to increasing microbial pollution entering surface water, irrigation-induced bypass flow is the primary driver of groundwater contamination (Davies-Colley et al., 2004). Close et al. (2010) documented *Campylobacter* sp. and *E. coli*, in Canterbury wells, as deep as 11 m after grazing coincided with border-strip irrigation and heavy precipitation. Peak concentrations of bacteria in the groundwater occurred 20-30 days after the event, demonstrating how long the delay between a bypass flow event and bacteria reaching the aquifer can be. They calculated the probability of a human outbreak associated with the contaminated wells at 60-75% during the irrigation season (Close et al., 2010).

2.8 Die-off and Regrowth

The two factors that influence microbial survival on land, and thus their availability to move into aquatic systems, are die-off and regrowth. Reddy et al. (1981) calculated first-order die-off rates for *E. coli* and found for every 10°C rise, between 5°C and 35°C, die-off almost

doubled. They also found that survival time of *E. coli* was shorter in soils with a pH range outside 5.8 to 8.4, and decreased linearly with soil moisture (Reddy, Khaleel & Overcash, 1981). Reduced microbial survival is also related to UV light exposure, predation, parasitism, competition, and low nutrient availability (Klein & Casida Jr, 1967). Microbial retention (i.e., increased survival) is positively correlated with the clay content of soils as it's interstitial spaces may protect against predation and promote adsorption (Reddy, Khaleel & Overcash, 1981).

Regrowth of *E. coli* in soils is known to occur; however, growth rates in natural habitats have not been as well studied as die-off rates (Reddy, Khaleel & Overcash, 1981; Gerba & Bitton, 1984; van Elsas et al., 2011). There are a number of reasons for this including: some strains do not grow on culture media/in laboratories, a lack of growth does not equate to non-viable, absent, or deceased bacteria (Semenov et al., 2008); bacteria emigrate, soil is an open system subject to multiple transportation mechanisms (Mankin et al., 2007; Lucy et al., 2008); and fluctuating environmental conditions and resource availability which may result in die-off rates exceeding growth rates or an inability to quantify regrowth (van Elsas et al., 2011). Although the biotic and abiotic factors associated with *E. coli* persistence on land are well documented, complex, and inconstant habitat conditions severely restrict our ability to model terrestrial survival of *E. coli* (Semenov et al., 2008). However, some of the abiotic factors that limit terrestrial modelling are more stable in aquatic habitats (e.g., moisture, temperature, carbon availability). One example of this is the survival of human pathogenic, Shiga toxin-producing *E. coli* in aquatic systems, which has been documented from months to almost a year (Warburton et al., 1998; Berthe et al., 2013, see chapter 4). Further investigation into the persistence and growth of enteropathogenic and environmental strains of *E. coli* and its relationship to other human pathogens in aquatic systems are needed to better understand its usefulness as a faecal indicator bacterium.

2.9 Suitability of *E. coli* as a Proxy for Faecal Contamination in Waterways

The most common method used to estimate faecal contamination in fresh-, brackish-, and salt-water is by monitoring ubiquitous strains of *E. coli* as faecal indicator bacteria (United States Environmental Protection Agency, 1985; Sayre, 1988; Carney, 1991; Ministry for the Environment, 2020). Although better than faecal coliforms at indicating contamination (Odonkor & Ampofo, 2013), there are numerous issues with the use of *E. coli* as a proxy. *E. coli* are complex organisms. Commensal strains reside in animal gastrointestinal tracts without incident, while other strains are major causes of diarrhoeal disease, urinary tract

infections, and sepsis in affected species (Shehata & Marr, 1971; Blount, 2015). *E. coli* presence and persistence in the environment is more complicated, and longer, than originally assumed (Anderson, Whitlock & Harwood, 2005; Odonkor & Ampofo, 2013; Korajkic et al., 2019) and human pathogenic strains continue to emerge from the environment (Hiruta, Murase & Okamura, 2001; Boss, Overesch & Baumgartner, 2016; Centers for Disease Control and Prevention, 2018; European Center for Disease Prevention and Control, 2018; Public Health Surveillance: Environment Group ESR, 2019).

Due to a highly resilient persister phenotype (a stress response resulting in metabolic slowing or inactivity and increased antibiotic resistance), *E. coli* is well equipped for survival and growth *ex vivo* (Madigan, Martinko & Parker, 1997; Zhang, 2014). Additionally, *E. coli* presence in the gastrointestinal tract is not restricted to mammals; invertebrates (Frick et al., 2018), fish (del Rio-Rodriguez, Inglis & Millar, 1997), birds (Glünder, 2002; Ewers et al., 2009; Markland et al., 2015), amphibians and reptiles (Gopee, Adesiyun & Caesar, 2000; Gordon & Cowling, 2003) are all able to host and transport it.

Non-mammalian hosted bacteria can be associated with a reduced risk of disease formation in humans and lesser associations with other human pathogens (Bloomfield et al., 2020); however, these hosts play important roles in human systems and may be important in *E. coli* pathogenesis (Hilbi et al., 2007). *E. coli* hosted by mammals closely related to humans and those humans interact with regularly, such as livestock, are more likely to be pathogenic to humans (e.g., Shiga toxin-producing and Enteropathogenic *E. coli*) (Soller et al., 2010; Devane et al., 2018, 2020). Of particular interest are *E. coli* producing extended-spectrum β-lactamase enzymes (rendering them resistant to multiple antibiotics) and toxin-producing strains (e.g., Shiga toxin-producing *E. coli*) (Melzer & Petersen, 2007; World Health Organization, 2020b).

Other flaws in the use of *E. coli* as a proxy for human pathogens in waterways include: a lack of correlation with viral waterborne human pathogens (Doré, Henshilwood & Lees, 2000; Sharp et al., 2021); other bacteria, such as *Enterobacter cloacae*, produce the β-glucuronidase enzyme that is used in water sampling methods to identify *E. coli* (Pearez, Berrocal & Berrocal, 1986b) resulting in misidentification; no further routine testing of the ubiquitous *E. coli* is performed to identify whether it is a human pathogenic strain or where it came from (Manges et al., 2019; Carver & Lunn, 2020); waterborne human pathogens that are not related to faecal contamination (e.g., *Vibrio* sp. and *Legionella* sp.) (Ericksen & Dufour, 2018); not all viable cells are culturable leading to a potential underestimation of concentrations (Ding et al., 2017); and because the source of the *E. coli* is unknown, the

relationship of that source to other human pathogens is unknown (Frick et al., 2018; Lekshmi, Oishi Das & Nayak, 2018; Wang, Deering & Kim, 2020).

Waterborne transmission of human pathogens is particularly high in estuaries/rivers subject to combined sewage overflows (Rodríguez et al., 2012; Anne-Sophie et al., 2015; Jalliffier-Verne et al., 2016), catchments with a high percent of pastoral land (USEPA, 2016; Phiri et al., 2020; Endris et al., 2022), and is often preceded by periods of increased precipitation resulting in runoff or re-suspension of sediment in the water column (Curriero et al., 2001; Lipp et al., 2001). While some bovine strains of *E. coli* have increased survival time in estuarine environments (Lipp et al., 2001; Malham et al., 2014); other *E. coli* may be a poor indicator of faecal contamination in brackish or marine habitats, as it is sensitive to osmotic stress (having a negative correlation with salinity) (Kirschner et al., 2004). However, the use of *Enterococci* sp. in marine environments, as an alternative to *E. coli*, is concerning as this method has never been fully validated in Aotearoa.

Coastal and estuarine environments as the terminal point for rivers and the sediments they carry can be highly contaminated by human and animal faeces (Boss, Overesch & Baumgartner, 2016). Although *E. coli* is often correlated with swimming-associated gastroenteritis, it is also used to measure the safety of shellfish and other aquatic sourced foods for consumption with little evidence supporting that application (Walker et al., 2018; Sharp et al., 2021). Additionally, information on viral associations in shellfish (e.g., norovirus, commonly linked to shellfish gastroenteritis) to *E. coli* levels in estuarine waters and the shellfish's flesh is currently lacking (Doré, Henshilwood & Lees, 2000; Sharp et al., 2021).

2.10 Conclusion

Microbial contamination of surface and groundwater is a serious public health concern that is overseen by politicians. Faecal contamination and the subsequent microbial pollution of aquatic systems is closely related to land use, with non-point sources (e.g., livestock) the principal contributor in pastoral catchments and point sources (e.g., human wastewater) in urban settings (Ministry of Health, 2017; Porter et al., 2017; Américo-Pinheiro et al., 2021). Microbial transportation, from land into aquatic systems, occurs by adsorption to particles (Droppo et al., 2010), advective flow (McLeod et al., 2003), and/or bypass flow (McLeod et al., 2008). It is closely tied to the movement of both water and sediment particles, above and through the soil profile, with precipitation, soil saturation, and/or irrigation associated with the speed of mobilisation (Curriero et al., 2001; Jamieson et al., 2004; McLeod et al., 2008).

The quantity of faecal microbes available for transportation is determined by to land loading, growth, and die-off rates (Reddy, Khaleel & Overcash, 1981; Jamieson et al., 2004). Microbial growth and die-off rates are affected by multiple environmental factors, such as UV light, temperature, nutrients, and moisture, as well as community interactions (e.g., competition and predation) (Klein & Casida Jr, 1967; Reddy, Khaleel & Overcash, 1981; van Elsas et al., 2011). Soil type, distance from deposition to the aquatic system, and land slope are accessory determinants to microbial mobilisation and explain why delayed detection of microbial pollution may occur (Reddy, Khaleel & Overcash, 1981; Close et al., 2010).
Due to our expanding knowledge of *E. coli* – its sources and relationships to human pathogens – the current method of monitoring recreational and drinking water using ubiquitous *E. coli* from the water column may need to change. *E. coli*'s use as a faecal indicator, while inexpensive and globally practiced, may no longer be the best measure of microbiological water safety (Odonkor & Ampofo, 2013). Limiting factors to its use include a poor relationship to viral human pathogens (Doré, Henshilwood & Lees, 2000; Sharp et al., 2021), environmental and heterothermic reservoirs (del Rio-Rodriguez, Inglis & Millar, 1997; Gordon & Cowling, 2003; Zhang, 2014; Frick et al., 2018), the potential for misidentification (Pearez, Berrocal & Berrocal, 1986a), and the presence of waterborne human pathogens that are unrelated to faecal contamination (Ericksen & Dufour, 2018). Additionally, current methods do not account for the entire aquatic environment as they typically ignore microbial reservoirs in benthic substrates (Ministry for the Environment, 2003, 2020).

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STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the candidate and the candidate's Primary Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of candidate:	Meredith T. Davis
Name/title of Primary Supervisor:	Prof. Russell Death
Name of Research Output and full reference:	
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In which Chapter is the Manuscript /Published work:	Ch 3
Please indicate:	
• The percentage of the manuscript/Published Work that was contributed by the candidate:	95%
and	
• Describe the contribution that the candidate has made to the Manuscript/Published Work:	
MT Davis: designed the study with input supervisors, performed 100% of the lab work, drafted and submitted the manuscript, created all the figures tables.	
For manuscripts intended for publication please indicate target journal:	
Candidate's Signature:	Meredith Davis Digitally signed by Meredith Davis Date: 2022.08.23 20:38:12 +12'00'
Date:	
Primary Supervisor's Signature:	Russell Death Digitally signed by Russell Death Date: 2022.08.24 14:17:45 +12'00'
Date:	24/8/2022

(This form should appear at the end of each thesis chapter/section/appendix submitted as a manuscript/ publication or collected as an appendix at the end of the thesis)

Preface to Chapter 3

"That terrible darkness where the water rushed and roared and bellowed as it bore its cargo of storm debris toward the sea."

IT – Stephen King

3 Detecting genes associated with antimicrobial resistance and pathogen virulence in three of Aotearoa's rivers

3.1 Abstract

The emergence of clinically significant antimicrobial resistance (AMR) in bacteria is frequently attributed to the use of antimicrobials in humans and livestock and is often found concurrently with human and animal pathogens. However, the incidence and natural drivers of antimicrobial resistance and pathogen virulence in the environment, including waterways and ground water, are poorly understood. Freshwater monitoring for microbial pollution relies on culturing bacterial species indicative of faecal pollution, but detection of genes linked to antimicrobial resistance and/or those linked to virulence is a potentially superior alternative. We collected water and sediment samples in the autumn and spring from three rivers in Canterbury, Aotearoa; sites were above and below reaches draining intensive dairy farming. Samples were tested for loci associated with the AMR-related group 1 CTX-M enzyme production (*bla*$_{\text{CTX-M}}$) and Shiga toxin-producing *Escherichia coli* (STEC). The *bla*$_{\text{CTX-M}}$ locus was only detected during spring and was more prevalent downstream of intensive dairy farms., Loci associated with STEC were detected in both the autumn and spring, again predominantly downstream of intensive dairying. This cross-sectional study suggests that targeted testing of environmental DNA is a useful tool for monitoring waterways. Further studies are now needed to extend our observations across seasons and to examine the relationship between the presence of these genetic elements and the incidence of disease in humans.

3.2 Introduction

Waterborne diseases are a significant threat to public health and the majority of them are zoonotic (e.g., hosted by animals) (Cleaveland, Laurenson & Taylor, 2001; World Health Organization, 2011). There is a growing body of evidence that intensive livestock farming degrades freshwater ecosystems and has the potential to create stores of zoonotic bacteria in rivers (Monaghan, de Klein & Muirhead, 2008; Daszak et al., 2020; Phiri et al., 2020). Environmental degradation is a common precursor to infectious disease emergence (Pimentel et al., 2007; Daszak et al., 2020). Therefore, understanding the role of catchment land use and recreation in waterways in the spread of waterborne zoonoses has become even more critical. Antimicrobial resistant infectious diseases are emerging as one of the most pressing global public health issues (Hernando-Amado et al., 2019). Antimicrobial resistance (AMR) is a

result of interactions between microbes and their environment, both biotic and abiotic; the predominant driver is considered the use of antibiotics in livestock and humans (Hernando-Amado et al., 2019). Patterns of AMR emergence are non-random; however, our ability to predict emergence is limited because it involves interactions between multiple drivers and non-linear patterns associated with human systems (Daszak, Cunningham & Hyatt, 2000; Jones et al., 2008). These systems are best investigated using a One Health approach, which considers human, animal, and environmental health as interconnected, providing a useful framework for addressing the emergence and spread of both AMR and zoonotic diseases (Rubin et al., 2013; Harrison et al., 2020).

Enterobacteriaceae -including *Escherichia coli*- are recognised as an important group of bacteria that carry AMR in clinical settings (Pendleton, Gorman & Gilmore, 2013). Until recently AMR bacteria were primarily of interest in hospital settings (Cosgrove, 2006; Bancroft, 2007), but they have increasingly been identified in the environment (Wyres & Holt, 2018), wildlife (Wilharm et al., 2017), and domestic animals(Singh, 2018). The bovine rumen is also a known reservoir for AMR bacteria (Auffret et al., 2017). Bacteria residing in the digestive tract are often impacted by antibiotic use and then, in pastoral farming systems, excreted onto land where they are potentially transported into waterways (Kirchner et al., 2013; Dwivedi, Mohanty & Lesikar, 2016).

The extended spectrum β-lactamases (ESBL) enzymes provide resistance to β-lactam antibiotics, including first and third generation cephalosporins, carbapenems and penicillins (Bonnet, 2004). The production of ESBLs is an emerging and spreading AMR (Lewis et al., 2007). Critically, many of the genes encoding β-lactamase production can be horizontally transferred via plasmids (Carattoli, 2013). Some ESBLs, specifically the group 1 CTX-M β-lactamases, are recurrently co-morbid with zoonotic pathogens (e.g., Shiga toxin-producing *Escherichia coli* – STEC – (Ishii et al., 2005; Valat et al., 2012), a group of human pathogens commonly hosted by cows (Oporto et al., 2019)). STEC are an important group of zoonoses by themselves; the fourth most reported zoonotic disease in the EU (European Center for Disease Prevention and Control, 2018) and USA (Centers for Disease Control and Prevention, 2018) and increasing in prevalence in Aotearoa (Public Health Surveillance: Environment Group ESR, 2019). STEC have been found in recreational waters and are transmitted via the oral-faecal route (Ahmed, Gyawali & Toze, 2015; Swaggerty et al., 2018).

Molecular characterisation is routinely used to classify cultured bacterial isolates, as culturing alone is unable to identify all the genetic components imparting AMR or virulence (Ram et al., 2009). Pre-isolation culturing commonly uses an enrichment step prior to molecular

characterisation which has been found to affect plasmid retention (reportedly up to a 95% loss) and may select against the strains being sought (Hill & Carlisle, 1981; Sowers, Wells & Strockbine, 1996; Wein et al., 2019). However, an alternative is to test environmental DNA (eDNA) directly. This offers a rapid, inexpensive survey of specific genetic elements chosen for their relevance to environmental, animal, or human health, such as loci associated with AMR and/or virulence. Although, this approach does not yield individual pathogenic/AMR colony isolates, it does provide an indication of whether the relevant genetic elements are present and whether further investigation is warranted. Testing samples for genetic loci associated with human pathogens is commonly employed in screening food and has been used in waterways internationally; however, this is a novel variation of those methods and new in Aotearoa (Haberecht et al., 2019; Fraiture et al., 2020; Koutsoumanis et al., 2020).
In this case study we tested eDNA collected from sites on three Canterbury, Aotearoa rivers during autumn (May) and spring (September), which coincides with seasonal peaks in human STEC cases (Public Health Surveillance: Environment Group ESR, 2019). We evaluated both benthic sediments and water column samples for seven genes; one, a general indicator of *E. coli* (*uidA*), with the remaining six frequently associated with group 1 CTX-M β-lactamase production (*bla*$_{\text{CTX-M}}$), human-pathogenic STEC virulence (*stx*$_1$, *stx*$_2$ and *eae*), and STEC serotype (O157 *rfbE* and O26 *wzy*).

3.3 Materials and Methods

3.3.1 Sample Collection

Two substrates, water and sediment, were sampled, collected from the Ashley, Rangitata, and Selwyn rivers once in austral autumn and spring, 2018. Collections were made at two sites along each river, these sites were 10-15 km apart, with one above and the other below reaches draining intensive dairy farms (Figure 3.1). At each site, water and benthic sediment samples were collected in separate sterile containers. Three 1 L water and three 25 g sediment samples were collected at each site. Samples were packed on ice, transported to the laboratory, and processed within 24 hours of collection.

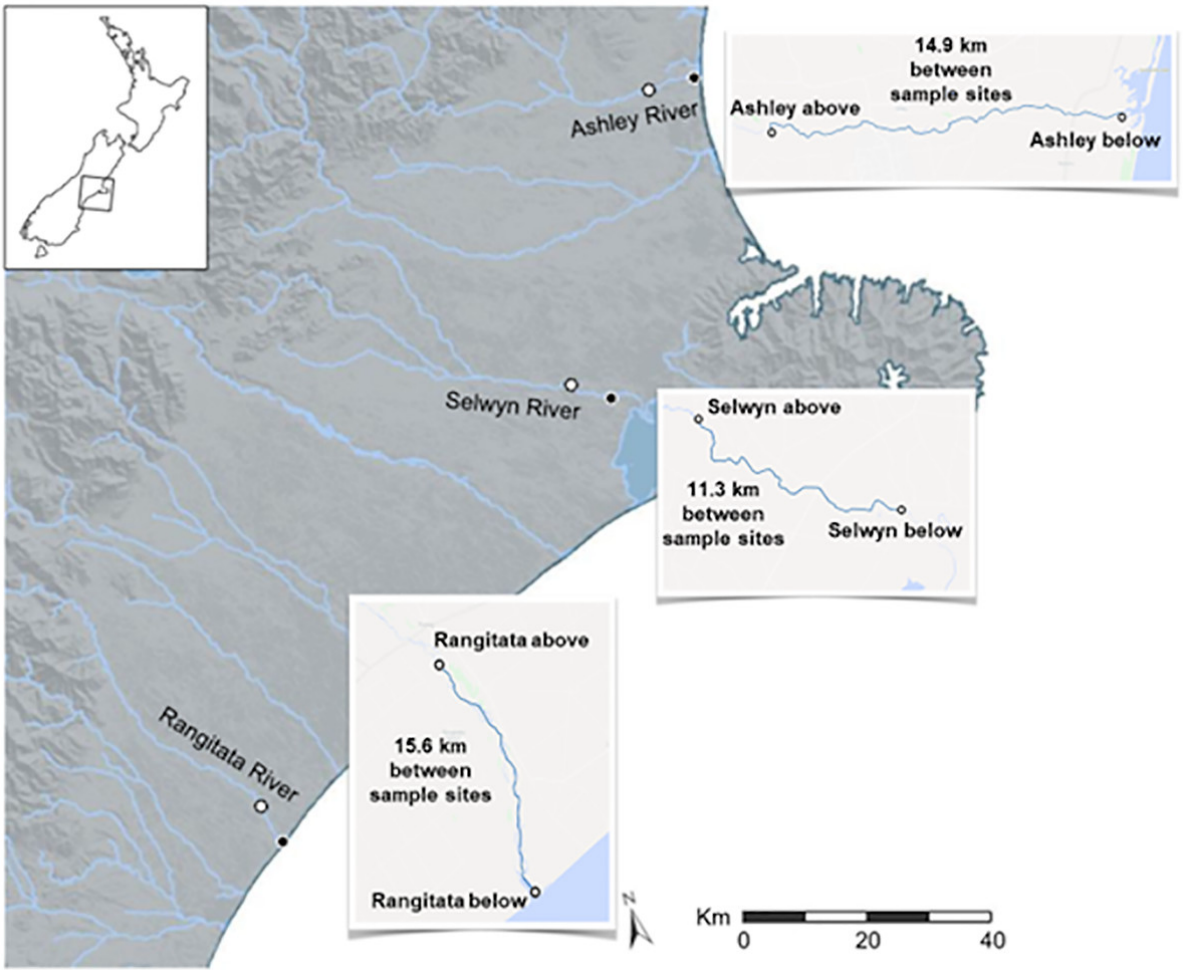

Figure 3.1 Map of central and southern Canterbury rivers.

Map of central and southern Canterbury, Aotearoa, with the Ashley, Rangitata, and Selwyn rivers labelled. Locations at which water and sediment were collected during May and September 2018 are marked with circles; for each river, the white centered dot indicates the 'above intensive dairy' site, and the black centered dot the 'below intensive dairy' site.

3.3.2 Sample Processing for Bacterial Culturing

Water column sample aliquots were diluted 1:10, 1:5, and 1:2.5 with sterile MilliQ H_2O to a final volume of 50 ml. At sites where the level of suspended sediments was high, an additional 1:50 dilution was also prepared to ensure ease of enumeration. For each sample we prepared three technical replicates at each dilution (i.e., 9-12 dilutions per sample).

For sediment samples, 2 g of wet sediment was first transferred to a 5 ml microtube, 3 ml MilliQ H_2O added, and the mixture then agitated vigorously for 30 seconds. Aliquots of the resulting supernatant were diluted 1:500, 1:200, 1:100, and 1:50 with MilliQ H_2O to a final

volume of 50 ml. For each sample we prepared three technical replicates at each dilution (i.e., 12 dilutions per sample).

For each dilution the total 50 ml volume was vacuum filtered through a single sterile 0.45 µm cellulose ester membrane filter (Merck KGaA, Darmstadt, Germany).

3.3.3 Bacterial Culturing

Bacterial culturing followed United States Environmental Protection Agency method 1603 (EPA, 2015). Each filter was placed onto a Difco Modified mTEC Agar (VWR, Radnor, PA) plate, incubated at 37.5°C for two hours, and then incubated at 45°C for 18-20 hours. Following incubation, colonies indicative of *E. coli* (red/magenta colonies) were counted.

3.3.4 Sample Processing for Molecular Testing

Three 500 ml aliquots of water from each site were vacuum filtered through separate 0.45 µm cellulose ester membrane filters. Environmental DNA was extracted from half of each filter using the NucleoSpin® soil kit (Machery-Nagel GmbH and Co. KG, Düren, Germany) following the manufacturer's instructions. For each sediment sample eDNA was extracted from three 0.5 g aliquots of wet sediment, using the NucleoSpin® soil kit.

3.3.5 Molecular Testing for Target Genes

We evaluated the presence of antimicrobial resistance using a polymerase chain reaction (PCR) targeting the *bla* gene associated with group 1 CTX-M β-lactamases ($bla_{\text{CTX-M}}$) using the primers of Lalzampuia et al. (2013). Molecular characterisation of STEC focused on genes associated with virulence. Specifically, we targeted genes associated with serogroup specific antigen biosynthesis, *rfbE* for O157 and *wzy* for O26, the stx_1 and stx_2 toxin genes, and the intimate attachment and effacing gene, *eae*, using the primers reported by Anklam et al. (2012). The detection limits of the STEC assays have been reported at 10^3 CFU/ml (Anklam et al., 2012) with a specificity to sensitivity ratio at 95%:92% for O157 strains 92%:91% for O26 strains (*eae*, stx_1, and stx_2 genes inclusive) (Browne et al., 2018). As an amplification control, we targeted the β-glucuronidase gene, *uidA*, which is present in most *E. coli* (Table 3.1) (Anklam et al., 2012).

Table 3.1 Details of oligonucleotide primers used in this study.

Gene	Primer sequences	Product size	References
bla_{CTX-M}	Forward: 5′ CCCATGGTTAAAAAACACTGC-3′ Reverse: 5′ CAGCGCTTTTGCCGTCTAAG-3′	950 bp	(Lalzampuia et al., 2013)
uidA	Forward: 5′ AGTGTGATATCTACCCGCTT-3′ Reverse: 5′ AGAACGGTTTGTGGTTAATCAG-3′	84 bp	(Anklam et al., 2012)
stx_1	Forward: 5′ GGATAATTTGTTTGCAGTTGATGTC-3′ Reverse: 5′ CAAATCCTGTCACATATAAATTATTTCGT-3′	107 bp	(Anklam et al., 2012)
stx_2	Forward: 5′ GGGCAGTTATTTTGCTGTGGA-3′ Reverse: 5′ GAAAGTATTTGTTGCCGTATTAACGA-3′	131 bp	(Anklam et al., 2012)
eae	Forward: 5′ CATTGATCAGGATTTTTCTGGTGATA-3′ Reverse: 5′ CTCATGCGGAAATAGCCGTTA-3′	102 bp	(Anklam et al., 2012)
wzy O26	Forward: 5′ AGCGTATGTTGATATATTTAATGTC-3′ Reverse: 5′ AATGTGGTCCCAAGGAATAAA-3′	141 bp	(Anklam et al., 2012)
rfbE O157	Forward: 5′ ATGCTGCCCACAAAAATAATGTAAA -3′ Reverse: 5′ CATAATCGGTTGGTGTGCTAA-3′	86 bp	(Anklam et al., 2012)

Amplification reactions were performed in 20 µl reaction volumes containing 0.5 × iQ PerfeCTa® qPCR ToughMix™, ROX™ (QIAGEN, Düsseldorf, Germany), 1 pM of each primer, and 2.5 µl of DNA template. Thermocycling was performed in a T1 thermocycler (Biometra GmbH, Göttingen, Germany) using standard cycling conditions including an initial denaturation at 94°C for 3 mins, followed by 35 cycles of 94°C for 30 s, 60°C for 30 s and 72°C for 1 min, with a final extension at 72°C for 5 mins. Amplification products were visualised using SYBR Safe (ThermoFisher Scientific, Waltham, MA, USA) following electrophoresis in 2% Tris-acetate-ethylenediamine tetraacetic acid agarose gels.

3.4 Results

3.4.1 Bacterial Culturing

With one exception, (the spring sampling at the ‘above intensive dairy’ site on the Selwyn River), colonies indicative of *E. coli* were consistently higher from sediment than water column samples (Table 3.2). Moreover, counts were also higher for all but one sample taken at the sites ‘below intensive dairy’ sites. Only the spring sediment sample from the site ‘above intensive dairy’ on the Rangitata River had a more *E. coli* than the corresponding sample from the site ‘below intensive dairy’.

3.4.2 Molecular Testing for Target Genes

The *uidA* locus was successfully amplified from every sample (Table 3.3). In contrast, detection of the gene associated with antibiotic resistance (*bla*$_{CTX-M}$) and the three gene loci associated with pathogenic STEC (*stx*$_1$, *stx*$_2$ and *eae*) varied with location, time and substrate. The antibiotic resistance gene was detected in both substrates, with equal frequency, in samples from the Selwyn and Rangitata rivers. The *stx*$_1$, *stx*$_2$ and *eae* genes were more frequently detected in water samples in the autumn but in sediment samples in the spring. In both the autumn and spring, serotype genes (O26 *wzy* and O157 *rfbE*) were amplified from the same sample as one or more toxin genes (*stx*$_1$ or *stx*$_2$) and the effacement gene (*eae*). The O26 marker was more frequently recovered from water, while that for O157 was more common in sediment.

In autumn, the genes associated with pathogenic STEC O157 (i.e., *stx*$_1$, *stx*$_2$, *eae* and *rfbE*) were present in 25% of the samples. All three virulence and both serogroup genes were detected in the autumn, both substrates from the Ashley and the water from the Rangitata had both serotype markers in the 'below intensive dairy' samples. Additionally, the *stx*$_1$, *eae*, and *rfbE* genes were present in water from the site 'below intensive dairy' on the Ashley River in spring. The *bla*$_{CTX-M}$ gene associated with AMR was not detected in any autumn samples.

In spring the *bla*$_{CTX-M}$ gene was detected in both substrates on the Rangitata and Selwyn rivers and was more frequent 'below intensive dairy'. However, it was also detected in one sediment sample 'above intensive dairy' on the Rangitata. The *stx*$_1$, *stx*$_2$ and *eae* STEC virulence genes were detected in all three rivers. The three STEC virulence genes were detected in sediment samples from five of the six sites but only from 'below intensive dairy' in the water samples; the *eae* gene alone, was only detected in water 'above intensive dairy' from the Rangitata (Figure 3.2). In spring samples, the gene associated with the O157 serogroup (*rfbE*) was detected four times more often than that associated with the O26 serogroup (*wzy*). In three samples, *stx*$_1$, *stx*$_2$ and *eae* were all detected (Figure 3.2); in one of these, the gene associated with the O157 serogroup was present, but the genes associated with O157 and O26 were not detected in the remaining two. The *stx*$_1$ and *eae* genes were detected in two water samples from 'below intensive dairy' whereas *stx*$_2$ and *eae* were detected in three sediment samples, two from 'below' and one 'above intensive dairy'.

Table 3.2 Presumptive *Escherichia coli* concentrations, in CFU/100 ml, for water and sediment samples collected from the Selwyn, Rangitata, and Ashley Rivers during May and September 2018.

	Sampling sites and substrates											
	'above intensive dairy'						'below intensive dairy'					
Sampling time	**Ashley water**	**Ashley sediment**	**Rangitata water**	**Rangitata sediment**	**Selwyn water**	**Selwyn sediment**	**Ashley water**	**Ashley sediment**	**Rangitata water**	**Rangitata sediment**	**Selwyn water**	**Selwyn sediment**
May (autumn)	45	230	20	7,100	10	200	440	730	175	273,300	160	230
September (spring)	20	400	20	5,000	505	200	2,000	3,600	40	1,600	2,250	12,200

Table 3.3 Presence of *Escherichia coli* control (*uidA*), STEC virulence (*stx*$_1$, *stx*$_2$, *eae*), serogroup (O26 *wzy* and O157 *rfbE*), and antibiotic resistance (*bla*$_{CTX-M}$) genes in water and sediment samples collected from the Selwyn, Rangitata, and Ashley rivers during May and September 2018.

	Sampling sites and substrates											
	'above intensive dairy'						'below intensive dairy'					
Gene locus	**Ashley water**	**Ashley sediment**	**Rangitata water**	**Rangitata sediment**	**Selwyn water**	**Selwyn sediment**	**Ashley water**	**Ashley sediment**	**Rangitata water**	**Rangitata sediment**	**Selwyn water**	**Selwyn sediment**
May (autumn)												
uidA	+	+	+	+	+	+	+	+	+	+	+	+
stx$_1$	–	–	–	–	–	–	+	+	+	–	–	–
stx$_2$	–	–	–	–	–	–	–	+	+	–	–	–
eae	–	–	–	–	–	–	+	+	+	–	–	–
*wzy*O26	–	–	–	–	–	–	+	+	+	–	–	–
*rfbE*O157	–	–	–	–	–	+	+	+	+	–	–	+
bla$_{CTX-M}$	–	–	–	–	–	–	–	–	–	–	–	–
September (spring)												
uidA	+	+	+	+	+	+	+	+	+	+	+	+
stx$_1$	–	+	–	+	–	–	–	–	+	–	+	+
stx$_2$	–	+	–	+	–	+	+	–	–	+	–	+
eae	–	+	+	+	–	+	+	+	+	+	+	+

	Sampling sites and substrates											
	‘above intensive dairy’						‘below intensive dairy’					
Gene locus	**Ashley water**	**Ashley sediment**	**Rangitata water**	**Rangitata sediment**	**Selwyn water**	**Selwyn sediment**	**Ashley water**	**Ashley sediment**	**Rangitata water**	**Rangitata sediment**	**Selwyn water**	**Selwyn sediment**
*wzy*026	–	–	–	–	–	–	–	–	+	–	–	–
*rfbE*O157	–	–	–	–	–	+	–	–	–	+	+	+
$bla_{\text{CTX-M}}$	–	–	–	+	–	–	–	–	+	–	+	+

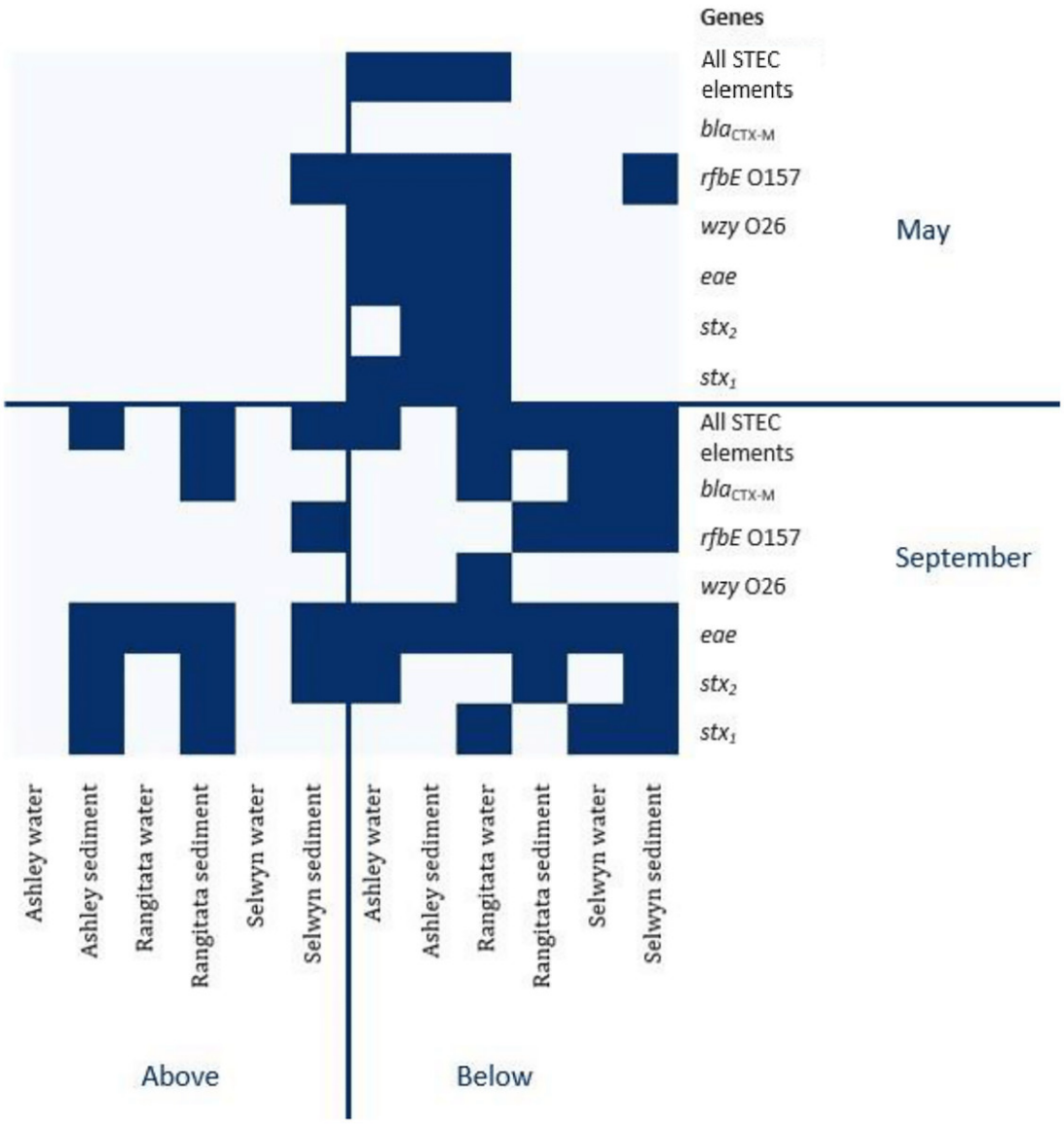

Figure 3.2 Heatmap depicting the presence or absence of the six genetic loci.
Heatmap depicting the presence or absence of the six genetic elements associated with human virulence in the Ashley, Rangitata, and Selwyn rivers in May and September of 2018, above and below intensive dairy sites. Locations at which all the elements necessary for human pathogenic STEC are signified by "All HP STEC elements'.

3.5 Discussion

Research is limited on the prevalence and distribution of AMR and zoonotic bacteria in Aotearoa waterways because growing, isolating, and identifying all potential target organisms from the potential millions contained in a single sample is time consuming and costly. In part, this is because identification through culturing is complicated by diverse metabolic requirements and identifying individual organisms present in a sample would require multiple

culture methods (Jaros et al., 2013; Irshad et al., 2016). In studies of ESBLs and STEC strains from ruminant faeces and environmental samples the microbial communities are generally enriched prior to DNA-based testing (Muirhead et al., 2004; Gluckman, 2017). The potential impacts of such enrichment on the microbial communities and the associated detection of zoonotic bacteria are difficult to quantify. In the present study, we examined samples for genes associated with AMR and STEC virulence without an initial enrichment step.

In our analyses the genetic markers indicative of *E. coli* (*uidA*) genes were detected in all three of the rivers sampled. Every sample tested positive for the *uidA* gene, consistent with *E. coli* being isolated at all six sites, from both substrates, and at both sampling times using traditional plating.

Using relatively small sample volumes we were able to detect genes associated with both AMR and human pathogenic STEC. Although all the genes associated with human pathogenic STEC virulence (stx_1, stx_2 and *eae*) and O serotype (O26 *wzy* and O157 *rfbE*) were present in every river, their presence varied by location, substrate, and season. Most importantly, all the genes necessary for human pathogenic STEC (e.g., one toxin gene - stx_1 or stx_2 – and *eae*) were detected within a single sample 11 times and AMR genes were only detected from samples that contained all the genes for human pathogenic STEC. These results suggest that explanations for the distribution of these genes across the landscape are likely to be complex and involve a range of factors.

Escherichia coli levels were higher and AMR and STEC associated genes more common at sites below intensive dairy. These results are consistent with previous reports suggesting that agricultural effluent is a major source of faecal contamination in Aotearoa waterways (Gluckman, 2017). Faecal bacteria may be transferred from pastures to waterways via run-off, carried either directly by the flow or indirectly as a result of adsorption to soil particles (Palmateer et al., 1993; Muirhead et al., 2004; Byappanahalli & Ishii, 2011). In the current study we sampled adjacent to intensive dairy operations but expected microbial communities to vary along each river as the diversity of land use changes downstream. Microbial communities are likely to be most strongly influenced by adjoining land use but will also be influenced by inputs from upstream. For example, the stx_1 and stx_2 genes were only detected at sites above intensive dairy during spring. One explanation for this may be inputs from smaller farms or non-farm inputs upstream of the reaches sampled. Importantly, the detection limit of the STEC assay at 10^3 CFU per ml (Anklam et al., 2012) is not so sensitive that a negligible presence of the targeted genetic elements would be over detected. A positive finding suggests that the genetic elements associated with STEC disease in humans were

present in the waterways in numbers significant enough to warrant further investigation to ensure public health safety.

The AMR and STEC associated genes occurred at greater frequency in the spring sampling than the autumn sampling. Specifically, the STEC were detected in eight of 12 samples (66%) in the spring and in three of 12 samples (25%) in the autumn whereas the AMR gene was only detected in the spring from two rivers. Detection of the AMR gene during the spring may reflect patterns of antibiotic use on the surrounding farms. Antibiotics are often administered to drying-off cows during winter as well as during spring calving and early milking (Bryan & Hea, 2017), potentially selecting for AMR bacteria in the cow microbiome. Additionally, spring calving likely increases the bacterial load on land bordering these rivers. As calves have poorly developed intestinal biomes, are stressed by weaning, or are removed from their mother prior to receiving colostrum they are prone to colonisation by, and heavy shedding of, bacteria, including AMR and STEC strains (Garzio-Hadzick et al., 2010; Gluckman, 2017). In addition to the increased faecal loading on pastures, spring rainfall patterns may lead to higher faecal or other, non-farm related inputs reaching rivers. However, further work including quantification of relevant bacterial genes in water and sediment is required to assess the influence of land-use upon waterways.

In all three of the sampled rivers bacterial colonies indicative of *E. coli* were consistently higher in sediments than the water column. This is a common finding frequently attributed to substrate stability. Sediments are less mobile than flowing water, therefore a longer-term and more stable habitat for bacteria (Boehm Jr et al., 2009; Fluke, González-Pinzón & Thomson, 2019). The AMR gene was equally likely to be found in sediment or water, but STEC associated genes were more commonly detected in sediment samples. These results are consistent with previous studies that indicate aquatic sediments may act as a store for *E. coli* (Anderson, Whitlock & Harwood, 2005; Bryan & Hea, 2017). Given that such stores may persist for months or years and that suspended sediments increase *E. coli* levels in the water column, this finding has potentially important implications for monitoring specific pathogenic and CTX-M producing *E. coli* strains (Davies-Colley, Valois & Milne, 2018; Weiskerger & Whitman, 2018). Generally, water testing by environment agencies is restricted to the water column. Although recreational use of waterways is discouraged when levels of suspended sediment are high (e.g., following precipitation) (Davies-Colley, Valois & Milne, 2018), disturbance of sediments by recreational users is not generally considered. Further work is needed to quantify the pathogens associated with the localised mixing of

sediment into the water column by recreational users and to determine whether zoonoses are being underestimated by the sampling of a single microhabitat.

3.6 Conclusion

Monitoring of recreational waterways for human pathogens is a complex but important task. In part this can be attributed to the metabolic diversity of the bacteria themselves and the apparent lack of a relationship between faecal indicator bacteria (e.g., counting colonies visually assessed to be *E. coli*) and the presence of genes associated with virulence and/or AMR. Moreover, the virulence or AMR status of isolated bacteria are not routinely assessed leading to delays in risk management. Understanding the relationship between pathogenic bacteria and AMR in recreational waterways would require more intensive sampling over a broader geographic range and the inclusion of multiple substrates in sampling protocols. In most cases monitoring is conducted on samples retrieved from the water column. Such samples may not accurately reflect the microbial community that recreational users of the waterway are potentially exposed to. Our results suggest sediments may act as an important reservoir of bacteria and resuspension of these sediments by waterway users could potentially increase exposure to pathogenic strains.

There is a growing appreciation of new technologies that enable the presence and persistence of zoonoses to be monitored without the need for microbial culturing (Palmateer et al., 1993; Pilliod et al., 2019). This represents a fundamental change in our approach to microbial monitoring allowing us to take a holistic view of the riverine environment and improving our ability to protect environmental, animal, and human health. While small, this study is the first step towards understanding zoonoses in waterways at a time when global health is under the microscope.

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References

Please see the full reference list at the end of this thesis.

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We, the candidate and the candidate's Primary Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

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Name/title of Primary Supervisor:	Prof. Russell G. Death
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MT Davis designed the study with input from supervisors, performed 100% of the experimental work, drafted and submitted the manuscript, created the figures, tables, and performed all statistical analyses.	
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(This form should appear at the end of each thesis chapter/section/appendix submitted as a manuscript/ publication or collected as an appendix at the end of the thesis)

Preface to Chapter 4

"Growth is limited by that necessity which is present in the least amount. And, naturally, the least favourable condition controls the growth rate."

Dune – Frank Herbert

4 Nitrate enrichment does not affect enteropathogenic *Escherichia coli* in aquatic microcosms but may affect other strains present in aquatic habitats

4.1 Abstract

In freshwater systems, nitrate-nitrogen – one of the nutrients responsible for eutrophication – is linked to biodiversity losses and ecosystem degradation. One of the main sources of freshwater nitrate pollution in Aotearoa is agriculture. Aotearoa's pastoral farming system relies heavily on the application of chemical fertilisers. These fertilisers in combination with animal urine, also high in nitrogen, result in high rates of nitrogen leaching into adjacent aquatic systems. In addition to nitrogen, livestock waste commonly carries human and animal enteropathogenic bacteria, many of which can survive in freshwater environments. Two strains of enteropathogenic bacteria found in Aotearoa's cattle, are K99 and Shiga-toxin-producing *Escherichia coli* (STEC). To better understand the effects of ambient nitrate concentrations in the water column on environmental enteropathogenic bacteria survival, a microcosm experiment with three nitrate-nitrogen concentrations (0, 1, and 3 mg NO_3-N/L), two enteropathogenic bacterial strains (STEC O26 - human - and K99 - animal), and two water types (sterile and containing natural microbiota) was run. Both STEC O26 and K99 reached 500 CFU/10 ml in both water types at all three nitrate concentrations within 24 hours and remained at those levels for the full 91 days of the experiment. Although enteropathogenic strains showed no response to water column nitrate concentrations, the survival of background *Escherichia coli*, imported as part of the in-stream microbiota did, surviving longer in 1 and 3 mg NO_3-N/L concentrations ($P<0.001$). While further work is needed to fully understand how nitrate enrichment and in-stream microbiota may affect the viability of human and animal pathogens in freshwater systems, it is clear that these two strains of STEC O26 and K99 can persist in river water for extended periods alongside some natural microbiota.

4.2 Introduction

Provision of clean water and sanitation for all by 2030 is one of the United Nations Sustainable Development Goals (United Nations, 2015, 2016). Although increased access to consistently sanitised water has reduced the number of drinking water related outbreaks in many developed countries, there are currently 1.8 billion people worldwide lacking safely managed drinking water supplies (United Nations, 2016) including portions of Aotearoa (Ministry of Health, 2018). In many regions, sources of drinking water are contaminated with

faeces and nutrients as a result of current and historical wastewater, agricultural, and industrial practices (Baron et al., 2002; Mallin et al., 2015; Jalliffier-Verne et al., 2016). Source water quality is particularly important in areas where water sanitation measures are absent or prone to failure, where microorganisms are resistant to the sanitation treatments available, and/or where recreational, food irrigating, and/or food harvesting waters are affected (Hrudey, Hrudey & Pollard, 2006; Jones et al., 2013; Viñas, Malm & Pettersson, 2019). To achieve the United Nation's 2030 goal, preventing and remediating source water pollution in combination with increased water sanitation infrastructure is necessary (United Nations General Assembly, 2015; United Nations, 2016).

Globally, agriculture uses more than 70% of all freshwater abstractions, with livestock farming using disproportionately more water than other agricultural products (e.g., grain and vegetables) by weight (Pimentel et al., 2004). Once used, that water commonly re-enters the environment polluted with both nutrients and faeces (Pimentel et al., 2004; Shah et al., 2020). In Aotearoa, land use has changed considerably over the last few decades, moving away from beef and sheep farming to a more intensive pastoral dairy model (Weeks et al., 2016; Julian et al., 2017). Between 1990 and 2015, Aotearoa's agriculture documented a 600% increase in fertiliser use, a 90% increase in irrigated agricultural land, and a 70% increase in the number of dairy cattle, with at least 65% of animal-derived nitrate leachate into freshwater ecosystems originating from dairy farms (Stats NZ, 2017). As a result, Aotearoa's freshwaters have amassed considerable levels of faecal and nutrient pollution(Gluckman, 2017; Stats NZ, 2020).

Waterborne disease is a not just a third world problem. The largest drinking water acquired campylobacteriosis outbreak ever documented happened in 2016 in Havelock North, Aotearoa (Gilpin et al., 2020). More than 5,500 people using an unchlorinated town water supply developed symptoms of gastroenteritis after the water was likely contaminated with sheep faeces (Gilpin et al., 2020).

In Aotearoa, between 2013 and 2018, just 32% of 364 monitored drinking water sites met the *Escherichia coli* (*E. coli*) standards at all times (Ministry of Health, 2018). Median nitrate-nitrogen (NO_3-N) levels at 44% of monitored sites were greater than 3 mg NO_3-N /L, a standard limit used by Regional Councils for reporting, and 19% of sites failed to meet safe drinking water standards for nitrate with levels greater than 11 mg NO_3-N /L, at least once (Stats NZ, 2020). Additionally, it is estimated that 50% of Aotearoa's rivers (by length) are above 0.44 mg NO_3-N/L (ANZG, 2018). A recent report by Environment Canterbury (New Zealand) Regional Council (2020) concluded that the observed deterioration of regional

ground water quality in the region was a direct result of agricultural land use. Of the 202 wells tested for NO_3-N across the region 47% were actively deteriorating and 28% were unchanged (Tregurtha, 2020). Of those, 32% had nitrate levels of greater than 5.65 mg NO_3-N/L, half of the maximum acceptable value (MAV) (Ministry of Health, 2018), and 6% were above the maximum acceptable value of 11.3 mg NO_3-N/L. In addition to high NO_3-N levels, half (102 wells) exceeded the maximum allowable levels of *E. coli* between 5% and 50% of the time (Tregurtha, 2020).

Allochthonous human or animal pathogenic organisms in aquatic habitats are a primary cause of disease in humans and animals (Uno, 2013; Jenkins, Ahmed & Barnes, 2021), commonly associated with faecal contamination and typically assessed by monitoring water column *E. coli* levels as a proxy (Odonkor & Ampofo, 2013). One cow/pig/sheep excretes approximately twice the *E. coli* per day a human does, some of which may be human and/or animal enteropathogenic strains (Jamieson et al., 2003; Avery, Moore & Hutchison, 2004). Shiga-toxin-producing *E. coli* (STEC) are one type of zoonotic bacteria commonly hosted asymptomatically by ruminants and shed through their faeces (Cooley et al., 2013). *Escherichia coli* strains, especially strains expressing Shiga-toxins capable of causing diarrhoea, enterohaemorrhagic disease or haemolytic uraemic syndrome in humans are clinically and economically significant emerging zoonoses both in Aotearoa and globally (European Center for Disease Prevention and Control, 2018).

Instead of identifying human or animal pathogenic organisms directly in aquatic systems, non-specific *E. coli* in the water column are monitored, as their presence is assumed to be representative of the extent of recent faecal contamination (Odonkor & Ampofo, 2013). However, *E. coli*, including enteropathogenic strains such as STECs, are able to persist for months or years outside a host (Fairbrother & Nadeau, 2006; Fremaux, Prigent-Combaret & Vernozy-Rozand, 2008; Ahmed, Gyawali & Toze, 2015). Two strains of STEC, responsible for the majority of human disease globally, O157 and O26 are frequently associated with environmental, produce, and/or water contamination (European Center for Disease Prevention and Control, 2018; Joseph et al., 2020).

Other *E. coli* strains hosted by ruminants (e.g., enteropathogenic K99) are a leading cause of diarrhoeal disease and mortality in neonatal livestock (Brunauer, Roch & Conrady, 2021). In addition to *E. coli*, other microorganisms (e.g., viruses, protozoa, and other bacteria) responsible for both human and animal disease present in livestock faeces can enter adjacent aquatic systems where environmental stores may be created (Soller et al., 2010b,a; Salman & Steneroden, 2015).

Understanding the mechanistic drivers of enteropathogenic bacteria in aquatic systems is complex. Livestock dominated catchments frequently display heavily modified geography, altered hydrological cycling, significant soil compaction, and/or increased erosion (Germer et al., 2010). Additionally, reduced riparian cover, agrochemical application, and nutrient leaching is common (Molina et al., 2017). These modifications may affect the movement, virulence, and/or persistence of human and animal pathogens (Strawn et al., 2013). This is concerning as many livestock hosted microorganisms are potentially zoonotic and shed through excreta, urine and faeces (Cleaveland, Laurenson & Taylor, 2001).

Of these changes, eutrophication is one of the most pervasive, impacting aquatic systems globally (Frumin & Gildeeva, 2014). Nutrient enrichment can affect aquatic microorganisms directly by relieving nutrient-limited growth constraints or indirectly through reduced predator suppression (Haller et al., 2009; Zimmer-Faust et al., 2017). Experimentally manipulating aquatic environmental conditions and assessing the growth and persistence of organisms of interest is a useful way to gain a better understanding of the individual drivers of waterborne bacteria.

Studies of in-stream microbial communities have suggested that predation by, and competition with, in-stream microbiota are the primary factors limiting *E. coli* survival in the water column (Wanjugi & Harwood, 2013; Korajkic et al., 2019). Despite extensive research into *E. coli,* including work showing that non-toxigenic *E. coli* can take up nitrate (Taabodi et al., 2019), the influence of concentrations of nitrate (the most abundant form of dissolved inorganic nitrogen in waterways (Sigman et al., 2001)) on the growth and persistence of *E. coli* in aquatic systems, remains largely unexamined.

This study aimed to use a microcosm experiment to investigate the effects of ambient nitrate concentrations in the water column on survival rates of two enteropathogenic strains of *E. coli*, one affecting calves (K99) and one affecting humans (STEC O26), in both a sterile environment and in the presence of in-stream microbiota. We hypothesised there would be a positive relationship between water column nitrate concentrations and pathogenic *E. coli* reproduction and persistence. We also anticipated that the presence of in-stream microbiota would mediate this relationship, as microbial predation and/or competition may limit the survival of the pathogenic *E. coli* (Wanjugi & Harwood, 2013). Although *E. coli* stores are most commonly found in benthic substrates (Havelaar & Melse, 2003; Muirhead et al., 2004), this experiment was focused on the effects of water column nitrate concentrations as this is the most common sample type used in monitoring drinking and recreational waters (World Health Organization, 2011; Ministry for the Environment, 2020).

4.3 Materials & Methods

4.3.1 *Escherichia coli* Strains

We used *E. coli* K99 (ESR 3020 (ESR - NZRM culture collection)) and STEC O26 (NZRM 3537 (Browne et al., 2018; ESR - NZRM culture collection)), as model organisms and monitored the number of colony-forming units (CFU) present in the water column, to measure persistence, over 91 days. A pilot experiment demonstrated that there was no measurable difference between the survival rates of two different STEC strains (e.g., STEC O157 and STEC O26 (Supplementary Information)), so a single STEC strain, O26, was chosen for use in the microcosm.

In addition to the artificially inoculated strains (i.e., K99 and STEC O26), background *E. coli*, imported as part of the microbiome in the stream water used, was present in all the wells using unsterilised water. The background *E. coli* were present in the control wells at each of the three NO_3-N concentrations and were monitored in the same manner as the inoculated wells to determine whether they responded to NO_3-N concentration.

4.3.2 Microcosm Experiment

The nitrate concentrations examined (1 and 3 mg NO_3-N/L) were selected to align with the proposed maximum nitrate levels for riverine ecosystem health in Aotearoa (i.e., 1 mg NO_3-N/L) and three times that level (Essential Freshwater Science and Technical Advisory Group, 2019; Canning, 2020).

Throughout the experiment the water temperature was maintained at ~10^{o} C, mimicking a typical river in Aotearoa's average winter water temperature (Scott & Poynter, 1991). The temperature-controlled room housing the microcosms had standard UV grow lights set on a timer to mimic a typical winter day length in Palmerston North, Aotearoa (-40.393560, 175.633072): 9.5 hr of light and 14.5 hr of dark. Aotearoa's streams experience the greatest rainfall in winter and consequently the greatest loading of faecal pollution (Phiri et al., 2020).

The experiment used 90 microcosm wells (30L ×10D ×20W cm, containing 3L of water) made in-house. Treatments were replicated five times and randomly assigned to wells.

Two types of water were used in the experiment, (1) sterile stream water, unfiltered and containing cellular debris and other native chemicals, and (2) intact stream water, with the water column microbiome intact. A pilot experiment demonstrated that there was no measurable difference between the use of highly filtered Milli-Q water and unfiltered sterile stream water on K99 or STEC O26 growth or persistence (Supplementary Information).

Stream water was collected from the Turitea stream, Palmerston North, Aotearoa (40.393728°S, 175.632937°W). The Turitea is a third order, stony bottom stream, with a five-year *E. coli* median attribution band rating of E (i.e., the lowest/worst ranking). It typifies the worst 25% of Aotearoa's streams (LAWA, 2020). Because it drains low intensity agriculture it was less likely these strains would be novel to the in-stream microbiota.
Stream water was left intact until NO_3-N levels measured less than 0.1 mg/L (within +/- 5%, +0.1mg/L) on a TriOS NICO nitrate meter (KISTERS AG, Germany) and then was sterilised or used intact. Stream water was autoclaved in a Getinge autoclave (Getinge AB, Sweden). Potassium nitrate (KNO_3) powder (Thermo Fisher Scientific, Waltham, MA) was mixed into each well until the target NO_3-N concentration was established (within +/- 5% + 0.1mg/L), measured with a TriOS NICO nitrate meter. The excess nitrate enriched stream water (intact and sterile) was saved in the cold room and used to replace the water removed for culturing. Once the nitrate levels were established, 30 of the wells were inoculated with ~300 CFU each of a single enteropathogenic *E. coli* strain (i.e., K99 or STEC O26), control wells were not inoculated. The *E. coli* levels and NO_3-N concentrations of the water column in each microcosm were examined on 35 occasions, every 24 hrs for the first seven days then every 72 hrs until day 91. Colony counts above 500 CFU per 10 ml of water were too many to count, for this reason a 500 CFU/10 ml maximum was instituted. Nitrate concentrations were maintained by adding KNO_3 as needed.

4.3.3 Sample Collection and Bacterial Culturing

All of the *E. coli* settled out of the water column in less than 24 hrs (as previously seen in the pilot experiment (Supplementary Information)), so all wells were briskly agitated with a sterile glass stirring spoon to resuspend the *E. coli* in the water column immediately prior to sample collection. Water column sample aliquots were diluted 1:10, 1:100, 1:1,000, 1:10,000, 1:100,000, and 1:1,000,000 with sterile MilliQ H_2O to a final volume of 100 ml. Water samples of 10 µl were processed for each K99 and STEC O26 well. For negative control wells in sterile stream water 100 ml was sampled to ensure there was no contamination/growth. In the control wells containing intact stream water, background *E. coli* levels were monitored in the same method as the experimental wells with between 5-100 ml of water sampled, in increasing volumes until there were no *E. coli* grown. An additional 100 ml sample was processed from each control well 24 hours after 0 CFU/100 ml was reached. All water samples less than 100 ml in size were diluted with sterile Milli-Q to a final volume

of 100 ml. That 100 ml of water was then vacuum filtered through a single sterile 0.45 µm cellulose ester membrane filter (Merck KGaA, Darmstadt, Germany) and cultured. Bacterial culturing followed United States Environmental Protection Agency method 1603 (EPA, 2015). Each filter was placed onto a Difco Modified mTEC Agar (VWR, Radnor, PA, USA) plate, incubated at 37.5ºC for two hours, and then incubated at 45ºC for 18-20 hours. Following incubation, colonies resembling *E. coli* (red/magenta colonies) were counted. Colony counts were calculated in CFU/10 ml and the amount removed from each vial or mesocosm for culture was replaced with an equal amount of the same water type containing the appropriate NO_3-N concentration.

4.3.4 Identification of *E. coli* Present in the Intact Stream Water

Enterobacter cloacae, commonly found in mammalian faeces is a bacterium that may produce the β-glucuronidase enzyme (Pearez, Berrocal & Berrocal, 1986). This enzyme is responsible for the red/magenta colony colour used to identify *E. coli* on Modified mTEC agar. It is also the gene typically targeted to identify *E. coli* using molecular methods. Therefore, they are easily mistaken for *E. coli* when using either of these methods. To ensure the identity of the colonies that were counted as *E. coli* were in fact *E. coli*, 96 colonies were randomly chosen from the background *E. coli* cultures across the 10-25 days for further characterisation. The selected colonies were purified on plate count agar (Merck KGaA, Darmstadt, Germany) and identified by matrix-assisted laser desorption ionisation-time of flight (MALDI-TOF) mass spectrometry (Bruker, Billerica, CA, USA) using the "on slide formic acid extraction" method (Lévesque et al., 2015).

4.3.5 Sample processing for molecular testing

To confirm the colonies grown from the inoculated intact river water microcosms were STEC O26 or K99, not imported background *E. coli*, 192 colonies (e.g., 96 potential STEC O26 and 96 potential K99) phenotypically identified as *E. coli* by their red/magenta colour on Modified mTEC Agar were randomly selected from across the cultures, up to and including day 91. Additionally, to ensure no contamination of the control wells had occurred during sampling, 90 colonies (e.g., 45 from STEC O26 intact control wells and 45 from K99 intact control wells) were randomly selected from across the intact control well cultures, up to and including day 25. Colonies were purified on Modified mTEC Agar. Genomic DNA was extracted from each purified colony using a boil preparation protocol; two or three colonies

were suspended in 1 ml of Milli-Q H_2O and heated at 100° C for 10 minutes then centrifuged at 13,000 rpm for 5 minutes. The supernatant was aliquoted and used as DNA template.

4.3.6 Molecular testing for target genes

We confirmed STEC O26 and K99 using a polymerase chain reaction (PCR) targeting *wzy* for O26 and the fimbril subunit for K99 (Table 4.1) (Roosendaal, Gaastra & de Graaf, 1984; Franck, Bosworth & Moon, 1998; Anklam et al., 2012). The detection limits of the STEC assays have been reported at 10^3 CFU/ml (Anklam et al., 2012) with a specificity to sensitivity ratio at 92%:91% for O26 strains (Browne et al., 2018). The K99 primer has been reported to be highly specific and sensitive (Franck, Bosworth & Moon, 1998) however no exact limits were published. Using a positive control we determined that detection was best when there was at least 2 ng/µl of DNA template per reaction.

Table 4.1. Details of oligonucleotide primers used in this study.

Gene	Primer sequences	Product size	References
uidA	Forward: 5′ AGTGTGATATCTACCCGCTT-3′ Reverse: 5′ AGAACGGTTTGTGGTTAATCAG-3′	84 bp	(Anklam et al., 2012)
wzy O26	Forward: 5′ AGCGTATGTTGATATATTTAATGTC-3′ Reverse: 5′ AATGTGGTCCCAAGGAATAAA-3′	141 bp	(Anklam et al., 2012)
K99	Forward: 5′ TATTATCTTAGGTGGTATGG-3′ Reverse: 5′ GGTATCCTTTAGCAGCAGTATTTC-3’	314 bp	(Roosendaal, Gaastra & de Graaf, 1984)

Amplification reactions were performed in 20 µl reaction volumes. STEC O26 reactions each contained 0.5 × iQ PerfeCTa® qPCR ToughMix™, ROX™ (QIAGEN, Düsseldorf, Germany), 1 pM of each primer, and 2.5 µl of DNA template. While K99 reactions contained 0.5 × iQ PerfeCTa® qPCR ToughMix™, ROX™ (QIAGEN, Düsseldorf, Germany), 0.5 µM of each primer, and 3 µl of DNA template.

Thermocycling for both reactions was performed in a SensoQuest labcycler (Biomedizinische Elektronik, Göttingen, Germany) using standard cycling conditions as described in Anklam *et al*. (2012) and Roosendall and deGraff (1984).

Amplification products were visualised using RedSafe ™(iNtRON Biotechnology, Daejeon, Korea) following electrophoresis in 2% Tris-acetate-ethylenediamine tetraacetic acid agarose gels.

4.3.7 Data analysis

Statistical analyses were performed in R (R Core Team, 2013). Generalised linear models (GLM; Poisson response) were used to examine the response of K99, STEC O26, and background *E. coli* (in the control wells containing intact stream water), concentrations to treatment with nitrate, water type (sterile or containing in-stream microbiome) and duration of treatment. *Post hoc* Tukey's honestly significant difference (HSD) test were performed on an ANOVA using the *AICcmodavg* package (Mazerolle & Mazerolle, 2017) to identify significant factors associated with background *E. coli* persistence. Plots were made in *ggplot2* (Wickham, 2016).

4.4 Results

4.4.1 Molecular testing and identification

All 96 potential STEC O26 colonies tested for *wzy* O26 contained the gene and all 96 K99 were positive for the fimbril subunit locus confirming their identities. The 90 background *E. coli* colonies were negative for *wzy* O26 and the K99 fimbril subunit but positive for *uidA*. Additionally, all but three of the 96 colonies chosen from the background *E. coli* cultures were confirmed as *E. coli* by MALDI-TOF. The three that were not *E. coli* were identified as *Enterobacter cloacae*.

4.4.2 Microcosm results

Duration, NO_3-N concentration, and water type (sterile or containing in-stream microbiota) had no measurable effect on STEC O26 or K99 growth or persistence (Table 4.2). Both strains attained 500 CFU/10 ml of water within 24 hours of inoculation and maintained that level for the full 91 days. However, the survival of the background *E. coli*/*E. cloacae* group, imported as part of the in-stream microbiome in the intact stream water, increased non-linearly with NO_3-N concentration and decreased with time (Figure 4.1 and 4.2).

Table 4.2 Summary of the effects experiment duration, strain (K99, STEC O26, or background), water type (intact or sterile), and NO_3-N concentration (0, 1, or 3 mg/l) had on *E. coli* growth and/or persistence using generalised linear models and post hoc Tukey's HSD. Bold denotes a significance >0.05.

GLM (Poisson)		Df.	Std. Error	Z-value	P value
STEC	(Intercept)	2099	2.434e-03	1971	**<2e-16**
	Strain (O26/O157)	2099	1.952e-03	0	1
	Day	2099	3.415e-05	0	1
	NO_3-N conc.	2099	7.825e-04	0	1
	Water type	2099	1.952e-03	0	1
E. coli/ E. cloacae group	(Intercept)	77	0.020	277.73	**<2e-16**
	Day	77	0.002	-58.98	**<2e-16**
	NO_3-N conc.	77	0.008	14.91	**<2e-16**

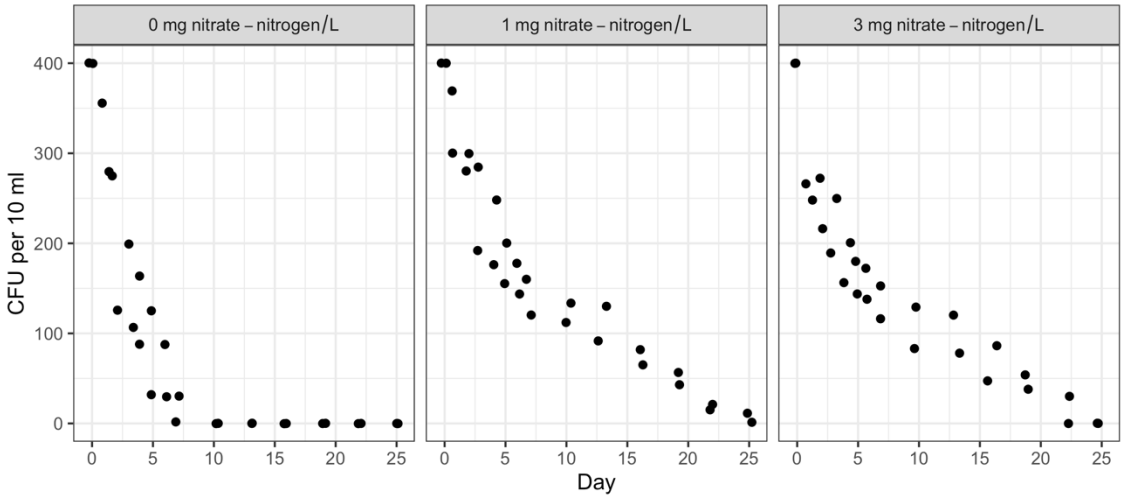

Figure 4.1 Averaged background *E. coli/E. cloacae* group die-off rates.
Averaged background *E. coli/E. cloacae* group die-off rates in the intact stream water at 0, 1, and 3 mg NO_3-N/L concentrations.

Post hoc comparisons using the Tukey HSD test identified differences in background *E. coli* and *E. cloacae* group survival in NO_3-N concentrations of 3 mg/L NO_3-N were and 1 mg/L NO_3-N were similar (Tukey's HSD: df= 2, P adj.=0.153); but that survival in 0 mg/L NO_3-N was significantly shorter than 1 mg/L (Tukey's HSD: df= 2, P adj.=0.000) and 3 mg/L NO_3-N (Tukey's HSD: df= 2, P adj.=0.000) (Figure 4.3, Table 4.2). The background *E. coli/E.*

cloacae group were either no longer culturable or dead by day 10 in 0 mg/L NO_3-N; but survived up to 15 days longer in 1 and 3 mg/L NO_3-N with significant differences in survival rate a result of duration.

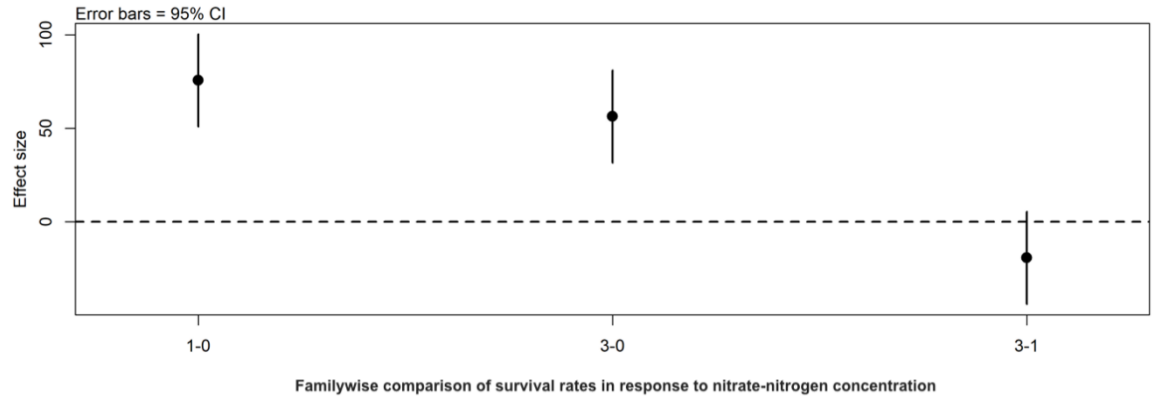

Figure 4.2 The mean differences in background *E. coli*/*E. cloacae* group survival. Familywise comparison performed using a *post hoc* Tukey's HSD test, of NO_3-N concentration on the mean differences in background *E. coli*/*E. cloacae* group survival. Persistence in 3 mg NO_3-N/L was similar to that in 1 mg NO_3-N/L (Tukey's HSD: df= 2, P adj.=0.153); but survival in 0 mg/L NO_3-N was shorter than 1 mg/L (Tukey's HSD: df= 2, P adj.=0.000) and 3 mg/L NO_3-N (Tukey's HSD: df= 2, P adj.=0.000).

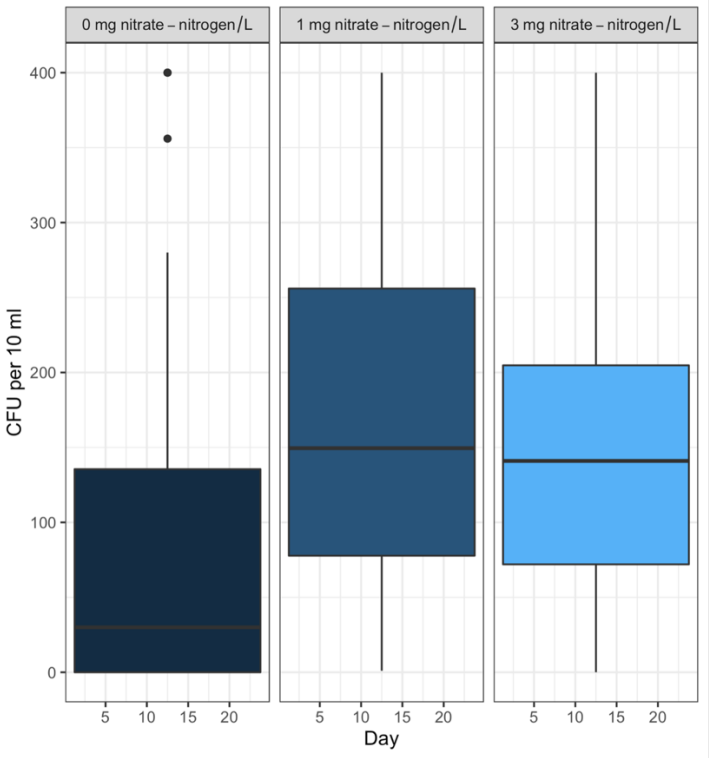

Figure 4.3 Mean *E. coli/E. cloacae* numbers at the different NO_3-N/L concentrations. Mean number of background *E. coli/E. cloacae* group in the intact stream water at 0, 1, and 3 mg NO_3-N/L concentrations.

4.5 Discussion

This study is novel in that it investigated the effects of nitrate enrichment on animal and human enteropathogenic *E. coli* strains as well as in-stream sourced *E. coli* and *E. cloacae* group survival in the water column. It is also novel in its use of Aotearoa sourced enteropathogenic *E. coli* strains in combination with Aotearoa's indigenous water column microbiota to determine whether in-stream microbiota do in fact remediate enteric/pathogenic bacterial pollution as has been hypothesised (Wanjugi & Harwood, 2013; Ravva, Sarreal & Mandrell, 2014). The effects of nutrient pollution and eutrophication on human and animal enteropathogenic *E. coli* strains in freshwater systems are poorly understood. One reason for this is that recreational water samples are rarely put through the additional testing necessary to identify specific enteropathogenic strains of *E. coli* as it can be time consuming and costly (Cooley et al., 2013; Tarr et al., 2019). However, enteropathogenic bacteria can be identified quickly and accurately using other techniques, including molecular and MALDI-TOF (Anklam et al., 2012; Ragupathi et al., 2018).

That there was no measurable difference in enteropathogenic *E. coli* growth in response to increased nitrate concentrations was surprising; extending the length of the experiment and/or reducing the number of bacteria introduced should be investigated further. That K99 and STEC O26 were able to persist and grow in the microcosms regardless of NO_3-N levels is an important finding supported by other studies where STECs, notably O157, have demonstrated extended persistence in aquatic environments (Korajkic et al., 2019; Wang, Deering & Kim, 2020).

Interestingly, the background *E. coli*/*E. cloacae* group imported as part of the microbiome in the intact stream water did respond to NO_3-N concentration. The response was not linear with increasing enrichment; treatments with 1 mg NO_3-N/L had the highest retention and longest persistence time, followed by 3 mg NO_3-N/L, and then 0 mg NO_3-N/L. This may be due to death or the organisms no longer being culturable. Viable but not culturable bacterial cells are understood to occur in laboratories (Ding et al., 2017; Liu et al., 2017). That said, further work on characterising the background *E. coli*/*E. cloacae* group to better understand their response to in-stream eutrophication is needed.

An unexpected finding was the speed at which the two enteropathogenic strains formed biofilms. Within 24 hours, water column sampling without resuspension of the bacterial biofilms through mechanical agitation resulted in zero *E. coli* colonies grown. This is important from a monitoring perspective as recreational water monitoring only uses samples from the water column for microbial water quality and recreational safety assessments. Streams and rivers are mobile systems where mixing occurs regularly. However, many streams are slow moving or intermittent, and even those with high flows have slower flowing runs and pools. In areas where mixing is reduced, *E. coli* quickly fall out of the water column and adsorb to substrates, periphyton, and/or form biofilms on the water surface (Moreira et al., 2012; Vogeleer et al., 2014). For this reason, the water column may not be the best substrate to monitor in recreational waterways when attempting to assess the risk of enteropathogenic *E. coli* strains (Davis et al., 2021). In this experiment water flow and substrates such as rocks or sediment were not used, to ensure the manipulated variables were responsible for any observed differences. Future work should focus on the addition of different substrates, oxygenation levels, and water flow regimes and characterisation of the biofilms and their role in enteropathogenic *E. coli* survival.

Finally, and most importantly, in-stream microbiota appeared to have no measurable effect on the growth or persistence of either K99 or STEC O26. Other studies have suggested that survival of enteric/faecal bacteria, both commensal and pathogenic, may be mediated by aquatic microbes through competition and/or predation (Ravva, Sarreal & Mandrell, 2014b; Korajkic et al., 2019a). A study by Wanjugi & Harwood (2013) found that the presence of in-stream microbiota was the most important factor in remediating *E. coli* in the water column and sediments, but that STEC O157 displayed extended persistence. While their study used a different STEC serotype, took place over a much shorter time frame, at higher temperatures (5-10^{o} C higher than in our experiments), and included sediments, a similar resilience to in-stream microbiota and extended persistence was demonstrated by the enteropathogenic *E. coli* in our study (Wanjugi & Harwood, 2013b). Reductions in colony counts were observed within the first five days Wanjugi and Harwood's (2013a) study. This did not happen in our study. There was no effect on either enteropathogenic strain that was attributable to in-stream microbiota. Our study used no substrate/sediment, only intact stream water. Considering the speed with which biofilm accumulation occurred, ongoing work is needed to determine whether the addition of benthic substrates could be important to the persistence/remediation of human and animal enteropathogenic *E. coli* strains in aquatic habitats. This next step may help identify an overlooked habitat for in-stream human and animal enteropathogenic *E. coli*

strain sequestration or an environment hosting species capable of remediating enteropathogenic *E. coli* from aquatic systems.

While international studies on human pathogens have mixed findings on whether predation and competition in aquatic systems are important limiting factors to persistence or drivers of virulence (Erken, Lutz & McDougald, 2013; Mauro et al., 2013; Schmidt, Shringi & Besser, 2016), neither was found to be a significant factor for human and animal enteropathogenic *E. coli* strains in this experiment. In a realistic scenario, rivers or lakes reaching and maintaining NO_3-N concentrations above 3 mg/L are likely to have other in-stream changes related to eutrophication and ecosystem distress, primarily, periphyton blooms or overgrowth of macrophytes (Camargo & Alonso, 2006). The ramifications of periphyton and macrophyte overgrowth (e.g., fluctuating dissolved oxygen levels and changes in the type/amount of food available) may benefit human and animal pathogenic bacteria that are metabolically diverse, and are capable of surviving in both anoxic and hyperoxic environments. Conversely, bactivorous organisms may not be as adaptable to these conditions, therefore reducing predation (Blount, 2015)

4.6 Conclusion

It is important we understand the potential impacts of rising nitrogen enrichment on our recreational and drinking water sources (Ward et al., 2018; Sprong et al., 2020). Nitrate-nitrogen concentrations at or exceeding those used in this study are being documented in waterways and aquifers, both nationally and globally (Canning, 2020; Tregurtha, 2020). The co-occurrence of elevated NO_3-N concentrations and livestock faeces potentially carrying human and animal pathogens in freshwater systems is a direct result of catchment management and should have us questioning what effect excess nitrogen in our waterways is having on aquatic microbial communities and how that may affect human health (Bertrand et al., 2009; Snelder, McDowell & Fraser, 2017). Therefore, aiming to reduce both nutrient and microbial pollution entering freshwater systems is the best way to protect all water for human and non-human life.

Acknowledgements

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References

Please see the full reference list at the end of this thesis.

Chapter 4 Appendix 1

Supplementary information

Pilot experiment

The primary aims of the pilot experiment were to: expand and refine the effects of a greater range of nitrate-nitrogen (NO_3-N) concentrations, determine whether there was any response to cellular debris in the water, and to quantify any STEC strain specific responses.

Methods

Bacteria strains

For the pilot, two strains of Shiga-toxin-producing *Escherichia coli* (STEC) O157 (NZRM 4274 (ESR - NZRM culture collection), and STEC O26 (NZRM 3537 (Browne et al., 2018; ESR - NZRM culture collection)) - were examined as model enteropathogenic bacteria and monitored for the number of colony forming units (CFU) present in the water column, to measure persistence, over 91 days.

Water types

Two types of water were used in the pilot, Milli-Q and sterile stream water, unfiltered and containing cellular debris and other native chemicals. Stream water was collected from same stream and processed in the same manner as the sterile stream water in the primary experiment.

Nitrate levels

A gradient range, from one to 13 mg NO_3-N/L in 0.5 NO_3-N/L increments (i.e., 0, 0.5, 1.0, 1.5 mg NO_3-N/L), was used for the pilot experiment.

Experimental setup

Once the 27 nitrate levels were established in 250 ml Schott bottles, 15 ml of the NO_3-N enriched water was decanted into four, 20 ml bijou bottles as a negative control or for inoculation (e.g., four bottles of each water type – a negative control and three to be inoculated with one of the three bacterial strains - at each NO_3-N concentration, n=162). The excess nitrate enriched water was saved in the cold room and used to replace the water removed for culturing. Due to the size of the bottles, testing NO_3-N throughout the experiment was not possible. The pilot study was run consecutively over 18 months, not concurrently, due to space limitations.

Three bijou bottles at each of the 27 NO_3-N concentrations were inoculated with ~100 CFU each of a single STEC strain (e.g., either O157 or O26). Negative control bottles were not inoculated. The bijou bottles with loosened tops were then held consecutively for 91 days in the dark at 10ºC (+/- 0.5ºC). Three replicates were performed for each treatment (n=486).

Sample collection and bacterial culturing

The STEC concentrations from each Bijou bottle were examined over the same time frame as the primary experiment. Similar to the primary experiment, both STECs formed biofilms in less than 24 hrs, so all bottles were briskly agitated to resuspend the STEC in the water column immediately prior to sample collection. Water samples ranged in volume from 2-500 µl, sample size was determined based on the previous culture results. For negative controls 2 ml was sampled to ensure there was no contamination/growth. All water samples were processed, cultured, and enumerated in the same manner as the primary experiment.

Data analysis

Statistical analyses were performed in R (R Core Team, 2013). An ANOVA (R Core Team, 2013) to ascertain whether colony forming units were affected by *E. coli* strain (e.g., STEC O157 or O26), NO_3-N concentration, water type, and/or duration of treatment. A *Post hoc* Tukey honestly significant difference (HSD) test was performed using the *AICcmodavg* package (Mazerolle & Mazerolle, 2017) to identify significant factors. Plots were made using the ggplot2 package version 3.3.3 (Wickham, 2016).

Results

Neither strain (P=0975) nor water type (P=0.987) affected STEC growth or persistence; however, NO_3-N concentration (P<0.001) and duration (P<0.001) strongly influenced survival (Table 1). All statistically significant variation in growth and persistence was limited to the first seven days (P<0.001) and was restricted to NO_3-N concentrations between 6.5 mg NO_3-N/L to 8.5 mg NO_3-N/L (P<0.001). Growth in 12.0 and 12.5 mg NO_3-N/L was noticeably slower in achieving 500 CFU/10 ml (taking ~48 hours (P<0.001)); but once it achieved 500 CFU/10 ml it remained there. All other NO_3-N concentrations reached 500 CFU/10 ml by day two and remained there till the end of the experiment (i.e., day 91).

Table 1. Summary of the effect duration, water type, and NO_3-N concentration had on STEC growth using ANOVA. Bold denotes a significance of 0.01 level or higher. NO_3-N concentrations and days that significantly affected STEC growth determined using *post hoc* Tukey's HSD tests are documented in the table.

ANOVA		**Z-value**	***p*-val**
K99 and O26 CFU	Day	5.386	**<0.001**
	NO_3-N concentration	56,577	**<0.001**
	Strain	0.005	0.975
	Water type	0.001	0.987
Tukey's HSD	6.5-8.5 mg NO_3-N/L		**<0.001**
	12-12.5 mg NO_3-N/L		**<0.001**
	Days 1-7 : 10-91		**<0.001**

Preface to Chapter 5

"It's a rule of ecology… The struggle between life elements is the struggle for the free energy of a system."

Dune – Frank Herbert

5 Aquatic archaeal communities and their drivers

5.1 Abstract

Often referred to as the third domain of life, archaea are the most recently characterised procaryotes. Consequently, they are the least understood and studied microorganisms, particularly in lotic environments and at the catchment scale. However, they are a diverse domain with high functional and genetic plasticity providing fundamental geochemical cycling services in aquatic ecosystems. To better understand the drivers of microbial community structure and their functions in lotic aquatic systems, a sophisticated diagnostic toolbox was employed (e.g., shotgun metagenomics, microbiological culturing, polymerase chain reaction -PCR-, and matrix assisted laser desorption ionisation-time of flight mass spectrometry -MALDI-TOF) to study the mainstem draining a well-defined rural land use catchment in Te Moana a Toi, Aotearoa. Community structure was strongly related to season in both entire communities and core biomes. Functional groups including methanogens, nitrifying, and denitrifying genera were discernible using metagenomics. Halophilic, acidophilic, and other thermally robust genera were also present. Further work understanding the relationships between aquatic archaea, their drivers, and their influence on climactic stability is needed.

5.2 Introduction

Rivers, as receiving bodies, naturally transport significant nutrient loads from land to the sea (Hansell & Carlson, 2002; Schlesinger, 2009; Yao et al., 2020). Importantly, it has been estimated that more than 50% of the carbon and nitrogen inputs are processed by microbial communities present in riverine sediments prior to reaching the sea (Clark et al., 2022; Zou et al., 2022). The United Nations Sustainable Development Goal 13 highlights the need to sequester carbon and mediate greenhouse gases to prevent catastrophic climate change and Guterres' (2023) progress report painted a bleak near future picture if extreme collective action isn’t taken.

Importantly, some of the same changes associated with the degradation of most of Aotearoa’s lakes, rivers, and estuaries (e.g., the conversion of native bush to pastoral farming) also drive climate change (Hamill & McBride, 2003; Monaghan, de Klein & Muirhead, 2008). Meaning that many of the same solutions for climate change (e.g., native forest, riparian vegetation, and wetland restoration and reducing ruminant livestock) can reverse aquatic degradation. Similar to greenhouse gases in the atmosphere, the aquatic pollutants associated with pastoral

farming (e.g., faecal bacteria such as *Escherichia coli – E. coli* – and nutrients such as nitrogen, carbon, and phosphorus) are diffuse, highly mobile, and difficult to remediate (Fitzpatrick et al., 2001; Wilcock et al., 2013; Porter et al., 2017). The physical changes associated with pastoral conversion drastically alter riverscapes through warming, straightening, deepening, reducing shade, and increasing water velocity affecting aquatic communities (Zeglin, 2015; Battin et al., 2016). However, quantifying the impacts of this environmental degradation on nutrient cycling by aquatic prokaryote communities in Aotearoa's rivers is lacking.

Archaea are a hardy domain with diverse metabolisms that contribute significantly to global aquatic biogeochemical cycling and ecosystem services (Kajale, Jani & Sharma, 2021; Llames, Quiroga & Schiaffino, 2022; Zhang et al., 2022; Tang et al., 2023). Although they are often overlooked, a result of their low relative abundance when compared to other microorganisms, archaea's presence in marine and freshwater, aquatic sediments, wetlands, and as plant symbiotes is essential to aquatic metabolism (Karner, DeLong & Karl, 2001; Ma et al., 2016; Lei et al., 2020; Tang et al., 2023). Furthermore, unlike bacteria, archaea are not associated with pathogenicity or the production of toxins, rendering them relatively innocuous in the environment (Gill & Brinkman, 2011; Yamaguchi, Park & Inouye, 2011). Even with this knowledge, archaea continue to be overlooked outside of extreme environments and gut biome studies (Bahram et al., 2019; Baehren et al., 2023).

Although archaea are classified as prokaryotes, genomic studies have determined that they are more closely related to eukaryotes than bacteria and that gene sharing between prokaryote domains was instrumental in eukaryogenesis (Brown & Doolittle, 1997; MacLeod et al., 2019; Long, Xue & Wong, 2020; Vigneron et al., 2022). Therefore, it is unsurprising that archaea and bacteria continue to share genes and that gene sharing is an important evolutionary mechanism for both domains (Paul, 1999; Ghaly et al., 2022; Gophna & Altman-Price, 2022). The prevalence of these genetic transactions, the constancy of their distribution within defined ecosystems or habitats (e.g., *intI* genes from all freshwater bacteria are different from those from marine bacteria), and the resulting phenotypic fluidity it affords prokaryotes beg the question: how else might communities be benefiting from this continuous genetic exchange (Auguet & Casamayor, 2008; Skippington & Ragan, 2011)? Aquatic habitats, particularly in sediments and/or at environmental interfaces (e.g., fresh:salt water, air:water, land:water), are known reservoirs of extracellular DNA readily available for prokaryotic transformation (Corinaldesi, Danovaro & Dell'Anno, 2005; Vlassov, Laktionov & Rykova, 2007). Importantly, the transfer of genes between domains may escalate in

environments where the temperature is high and/or oxygen is low (e.g., thermal waters and sediments, aquatic sediments, and thermal:aquatic interfaces common in geothermal areas of Aotearoa), confer antimicrobial resistance, and rely on the presence of integrons to reduce the transfer cost between domains (Fuchsman et al., 2017; Ghaly et al., 2022).

In addition to sharing genes, some bacteria and archaea are able to communicate using the density and/or distribution of signalling molecules to activate and/or regulate gene expression (e.g. bioluminescence in *Vibrio fischeri* governed by N-3-oxohexanoyl-homoserine lactone) (Seet & Zhang, 2011; Zhang et al., 2012; Rajput & Kumar, 2017). Signal molecules may cross domains, be silenced (e.g., broken down by chemicals produced by other organisms to impede communication), and/or have their reception inhibited (Kjelleberg et al., 1997; Manefield et al., 2002; Hmelo, 2017). Therefore, microbial communication is likely an important aspect of aquatic community recruitment (Ma et al., 2023; Zheng et al., 2023). Insight to archaeal communication and recruitment is likely to be pivotal in developing robust bioremediation strategies for anthropogenic pollutants in aquatic environments (e.g., wastewater, eutrophication, and petroleum spills) (Araújo et al., 2020; Wang et al., 2021; Zheng et al., 2023).

Current knowledge of aquatic archaeal communities is inadequate to form any global or nationally meaningful conclusions. However, important findings from studies on lentic freshwater archaeal communities show that they may be more diverse than marine and soil communities, physicochemical differences in the environment may explain some variation in the communities (Berdjeb et al., 2013; Hugoni et al., 2015; Zhang et al., 2015), and that some archaea, unlike bacteria, are able to produce methane in the presence of oxygen (Hu, Hou & Yu, 2015). The even fewer studies on lotic freshwater systems found ammonia-oxidising archaea were crucial for nitrification in rivers where ammonium levels acidified the water below the optimal pH for ammonia-oxidising bacteria (Liu et al., 2013; Sun et al., 2014) and in sediments, total nitrogen was positively correlated with methanogenic archaea presence and organic carbon was negatively correlated with ammonia-oxidising archaea (Yue et al., 2022). Though archaea compose a relatively small portion of the microbial ecosystem, understanding archaea communities is not only crucial in understanding aquatic emissions, affecting greenhouse gas calculations and predicting the extent of climate change, but also on the interdependencies archaea may have with aquatic plant and animal communities (Bogard et al., 2014).

That said, understanding the members and drivers of archaeal community structure in rivers is a necessary first step. Therefore, the aim of this study was to determine whether aquatic

archaeal community structure along a typical rural river in Aotearoa was associated with any environmental factors (e.g., land use, site, substrate). To achieve this, water and benthic substrates were sampled in a longitudinal study of the Waiotahe river and Te Ahiaua (the Waiotahe's terminal estuary), in Te Moana a Toi, Aotearoa.

5.3 Materials and Methods

5.3.1 Background

Ecological surveys, matrix-assisted laser desorption ionisation-time of flight (MALDI-TOF) mass spectrometry (Bruker, Billerica, CA, USA), and microbiological culturing were used for samples from each of the eight sites along the Waiotahe river mainstem, one tributary, and Te Ahiaua. Additionally, entire archaeal communities and core biomes were built from the shotgun metagenomic sequences obtained from the environmental DNA (eDNA) collected from both substrates at each of the ten sites. Key drivers were ascertained by analysing the relationships between archaeal community structure and substrate (i.e., benthic sediment and water), spatial and temporal variables (e.g., site, environmental, and land use factors), and human health measures (i.e., *E. coli* concentrations from both substrates).

Due to the varied land uses present in the catchment and lotic nature of rivers, bacterial inputs were expected to be composites, building in complexity from the headwaters to Te Ahiaua. That said, archaeal community structure was expected to be influenced more strongly by physicochemical characteristics of the site sampled. Allochthonous archaeal contributions were likely to be associated with: animals including, insects, domesticated and non-domesticated mammals (e.g., rats, mice, stoats, possum, cats, dogs, pastoral livestock, and deer), native and introduced avifauna, and humans; soil communities; and riparian vegetation communities.

5.3.2 Study Location

The Waiotahe catchment is ~15,000 ha in size and has more than 50% of its land in steep or very steep hill country that drains onto alluvial plains. Rainfall is typically between 400-900 mm per year and the primary land uses include native forest (58% - upper catchment), exotic forest (16% - upper/upper-mid catchment), a few beef and sheep paddocks (<1% - upper-mid catchment), and dairy farming (25% - mid/lower catchment) (Banks, 2011).

Nine sites were chosen along the Waiotahe river (S38.187134, E177.178642 to S37.991217, E177.205603) and one tributary, as well as one site from Te Ahiaua, before the water enters

Te Moana-nui-a-Kiwa (the Pacific Ocean). The sample sites were selected to coincide with reaches where the effects of the various land uses were most likely to be in evidence (Figure 5.1, Table 5.1).

Figure 5.1. Map of the Waiotahe river. Map of the ten sites along the Waiotahe river and one tributary in Te Moana a Toi, Aotearoa, where samples were collected seasonally (e.g., March, July, October, and December) in 2018.

5.1 Land uses associated with the ten sites sampled along the Waiotahe river and one tributary in Te Moana a Toi, Aotearoa, in 2018. Sites are listed in order, from the headwaters to the sea.

Site	Land use
10	Native Forest
9	Native Forest
8	Exotic Forest
7	Exotic Forest
6	Beef and Sheep
5	Dairy (low intensity)
4	Dairy (low intensity)
3	Intensive Dairy Farm Drain
2	Effluent Disposal Station (human and animal)
1	Te Ahiaua

5.3.3 Sample Collection

Two substrates, water and sediment, were sampled from each of the eight sites on the Waiotahe river, one tributary, and Te Ahiaua, seasonally in 2018 (March – autumn, July – winter, October – spring, and December – summer). Water (three, 1 L samples) and sediment (three, >25 g samples) were collected in separate sterile containers from each site, every season (n=240). All samples were packed on ice, transported to the laboratory, and processed within 24 hours of collection.

5.3.4 Sample Processing for Bacterial Culturing

Water column sample aliquots were diluted 1:10, 1:5, and 1:2.5 with sterile MilliQ H_2O to a final volume of 100 ml. Water samples with high levels of suspended sediment required extra dilution. Therefore, at sites where sediment was visible in the water column, an additional 1:50 dilution was prepared to ensure accurate enumeration. For each of the three biological replicates per site, three technical replicates were prepared at each dilution (i.e., 9-12 dilutions per sample).

For sediment samples, 2 g of wet sediment was transferred to a 5 ml microtube with 3 ml of sterile MilliQ H_2O and the mixture was agitated vigorously for ~30 seconds. Aliquots of the resulting supernatant were diluted 1:500, 1:200, 1:100, and 1:50 with MilliQ H_2O to a final volume of 100 ml. Again, for each sample three technical replicates were prepared at each dilution (i.e., 12 dilutions per sample).
For each dilution of both substrates, a final volume of 100 ml was vacuum filtered through a single, sterile 0.45 μm cellulose ester membrane filter (Merck KGaA, Darmstadt, Germany).

5.3.5 Bacterial Culturing and Colony Identification

Bacterial culturing followed United States Environmental Protection Agency method 1603 (EPA, 2015). Each filter was placed onto a Difco Modified mTEC Agar (VWR, Radnor, PA) plate, incubated at 37.5°C for two hours, and then at 45°C for 18-20 hours. Following incubation, colonies indicative of *E. coli* (red/magenta) were counted.
To ensure the identity of the colonies that were counted as *E. coli* were in fact *E. coli*, 192 colonies were randomly chosen for further characterisation. The selected colonies were isolated on plate count agar (Merck KGaA, Darmstadt, Germany) and identified using the MALDI-TOF (Bruker, Billerica, CA, USA) "on slide formic acid extraction" method (Lévesque et al., 2015).

5.3.6 Sample Processing for Molecular Testing

Three, 500 ml aliquots of water from each site were vacuum filtered through separate 0.45 μm cellulose ester membrane filters. eDNA was extracted from half of each filter using the NucleoSpin® soil kit (Machery-Nagel GmbH and Co. KG, Düren, Germany) following the manufacturer's instructions. For each sediment sample eDNA was extracted from three 0.5 g aliquots of wet sediment, also using the NucleoSpin® soil kit. Extracted eDNA was quantified using the NanoDrop™ Spectrophotometer (ThermoFisher Scientific, Waltham, MA, USA) and Qbit fluorometric quantisation (Invitrogen, ThermoFisher Scientific, Waltham, MA, USA). To identify any DNA contamination that may have been introduced during processing or from reagents, two negative control extractions (blank 1, from an unused sterile 0.45 μm cellulose ester membrane filter and MiliQ H_2O and blank 2, DNA extracted from the reagents alone) were performed and sent for sequencing with the environmental samples.

5.3.7 Metagenomic Preparation and Sequencing

The DNA extractions were transported to Massey Genome Service (Massey University, Palmerston North, Aotearoa) for high throughput sequencing library preparation using the Illumina Nextera™XT library kit (Illumina, San Diego, California, USA). The two DNA extractions with the highest quality and quantity, from both substrates, at each site, for each season, and the two negative controls were sent for library preparation (n=162). Library preparation uses enzymatic shearing to first randomly fragment the genomic DNA and then attach barcoded Illumina adapters to each end of the fragments during an enrichment PCR. Libraries were then checked for quality using LabChip® DNA HS assay (PerkinElmer, Waltham, Massachusetts, USA) and Quant-iT™ dsDNA HS assay (ThermoFisher Scientific, Waltham, MA, USA) and pooled by molarity. Both negative control's (i.e., blank 1 and 2) DNA concentrations were too low to pass quality control checks but were pooled with the other samples equimolarly. The pooled library was then paired-end sequenced by Novogene (Wan Chai, Hong Kong) using two lanes on an Illumina HiSeq 2500.

5.3.8 Quality Control and Analysis of Sequences

Quality control trimmed adapters and removed any sequences where: the sequence error rate was above 10%, where adapters were retained, where 10% or more of the read base couldn't be determined, and when the quality was less than 50% of the total base (resulting in a total read length of less than 150 bp). Reads were analysed without further trimming as there was no genome assembly, RNA sequenced, or single nucleotide polymorphism analysis (del Fabbro et al., 2013). The GC content and phred scores for each sample were recorded.
The majority of the reads were ~150 bp in length, unjoined. Metadata were arranged in tables and sequence reads were analysed as pairs using a local version of Kaiju (Menzel, Ng & Krogh, 2016) with the nr_euk database (version July 12, 2019, https://www.ncbi.nlm.nih.gov/). The database contained ~277 million sequences from the NCBI protein database composed of bacteria, archaea, viruses, fungi and microbial eukaryote genetic information – excluding human contamination accession numbers identified by Breitwieser et al. (2019) (https://github.com/bioinformatics-centre/kaiju). The most stringent parameters were chosen for taxa identification: maximum exact matches, a minimum match length of 11 proteins, and minimum read depth of 21.

5.3.9 Physical Characteristics

Ecological surveys were performed each time samples were collected and provided data on 15 site specific environmental variables (CH 5 Appendix 1 Table 1). Conductivity

(automatically adjusted to 25°C) and water temperature were measured with a EuTech Oakton Instruments meter (Metex Corporation, Toronto, Ontario, Canada) and a head rod was used to measure velocity. Thirty-three additional variables and indices were curated for each site (CH 5 Appendix 1 Table 1). These included *E. coli* concentrations, modelled nutrient inputs sourced from Unwin & Larned (2013), flow data from Booker & Woods (2014), and catchment geology, topography, and temperature from the FENZ database (Leathwick *et al.*, 2010b).

5.3.10 Molecular Testing for Target Genes

I evaluated the presence of specific antimicrobial resistance genes using a polymerase chain reaction (PCR) targeting the *bla* gene associated with group 1 CTX-M β-lactamases ($bla_{CTX\text{-}M}$) using the primers of Lalzampuia et al. (2013). Molecular characterisation of Shiga-toxin-producing *E. coli* (STEC) focused on genes associated with virulence. Specifically, we targeted genes associated with serogroup specific antigen biosynthesis, *rfbE* for O157 and *wzy* for O26, the stx_1 and stx_2 toxin genes, and the intimate attachment and effacing gene, *eae*, using the primers reported by Anklam et al. (2012). The detection limits of the STEC assays have been reported at 10^3 CFU/ml (Anklam et al., 2012) with a specificity to sensitivity ratio at 95%:92% for O157 strains 92%:91% for O26 strains (*eae*, stx_1, and stx_2 genes inclusive) (Browne et al., 2018). As an amplification control, we targeted the β-glucuronidase gene, *uidA*, which is present in most *E. coli* (Table 5.2) (Anklam et al., 2012).

Amplification reactions were performed in 20 µl reaction volumes containing 0.5 × iQ PerfeCTa® qPCR ToughMix™, ROX™ (QIAGEN, Düsseldorf, Germany), 1 pM of each primer, and 2.5 µl of DNA template. Thermocycling was performed in a T1 thermocycler (Biometra GmbH, Göttingen, Germany) using standard cycling conditions including an initial denaturation at 94°C for 3 mins, followed by 35 cycles of 94°C for 30 s, 60°C for 30 s and 72°C for 1 min, with a final extension at 72°C for 5 mins. Amplification products were visualised using SYBR Safe (ThermoFisher Scientific, Waltham, MA, USA) following electrophoresis in 2% Tris-acetate-ethylenediamine tetraacetic acid agarose gels.

Table 5.2 Details of oligonucleotide primers used in this study.

Gene	Primer sequences	Product size	References
bla_{CTX-M}	Forward: 5′ CCCATGGTTAAAAAACACTGC-3′ Reverse: 5′ CAGCGCTTTTGCCGTCTAAG-3′	950 bp	(Lalzampuia et al., 2013)
uidA	Forward: 5′ AGTGTGATATCTACCCGCTT-3′ Reverse: 5′ AGAACGGTTTGTGGTTAATCAG-3′	84 bp	(Anklam et al., 2012)
stx_1	Forward: 5′ GGATAATTTGTTTGCAGTTGATGTC-3′ Reverse: 5′ CAAATCCTGTCACATATAAATTATTTCGT-3′	107 bp	(Anklam et al., 2012)
stx_2	Forward: 5′ GGGCAGTTATTTTGCTGTGGA-3′ Reverse: 5′ GAAAGTATTTGTTGCCGTATTAACGA-3′	131 bp	(Anklam et al., 2012)
eae	Forward: 5′ CATTGATCAGGATTTTTCTGGTGATA-3′ Reverse: 5′ CTCATGCGGAAATAGCCGTTA-3′	102 bp	(Anklam et al., 2012)
wzy O26	Forward: 5′ AGCGTATGTTGATATATTTAATGTC-3′ Reverse: 5′ AATGTGGTCCCAAGGAATAAA-3′	141 bp	(Anklam et al., 2012)
rfbE O157	Forward: 5′ ATGCTGCCCACAAAAATAATGTAAA -3′ Reverse: 5′ CATAATCGGTTGGTGTGCTAA-3′	86 bp	(Anklam et al., 2012)

5.3.11 Data Analysis

All archaea names were based on Kaiju's outputs, not necessarily the latest nomenclature. All statistical analyses were performed in R (R Core Team, 2013). Because microbial communities are not normally distributed in either the water column or sediment, non-parametric tests were used for all analyses.

Metadata were analysed by season for each of the three taxonomic levels: class, order, and genus. Rarefactions were plotted to ensure species richness was not a result of sampling effort (i.e., taxonomic richness was similar across samples with significantly different read numbers). To correct for any bias introduced by the variation in total read numbers, relative abundances (i.e., the proportion of the total sample each taxa comprised) were calculated and used for all metagenomic analyses. In addition to using relative abundances, any taxa representing less than 0.001 (i.e., 0.1%) of the total abundance were removed from the metadata prior to statistical analysis.

Communities were visualised using heatmaps with dendrograms, hierarchical clustering using Bray-Curtis distances, with the gplots package (Warnes et al., 2015). Key differences in archaeal community structure associated with the 48 curated variables (e.g., diversity metrics, *E. coli* concentrations, and environmental factors) were visualised for each taxonomic level

using principal coordinates analyses (PCoA) using Bray-Curtis distances. PCoAs for seasonal and annual variation in community structure were plotted in ggplot2 (Wickham, 2016) with significant (p-value <0.05) explanatory variables ($R^2 \geq 0.6$) were projected onto the plots with the R^2 value.

Core biomes (e.g., taxa present in all the samples of a particular type) were constructed at the class, order, and genus levels. Within taxonomic level, substrate, sampling time, water type, and land use were used, in a stepwise manner, to determine the parameters of each core biome type (CH 5 Appendix 1 Table 2). Because there were fewer estuarine samples (e.g., two for each substrate per season, n=16), seasonal patterns were not investigated for Te Ahiaua. Heatmaps using core biome Z-Scores, to highlight each taxa's relationship to the average relative abundance, were plotted in gplots (Warnes et al., 2015) to illustrate key compositional differences. Dendrograms, using Bray-Curtis dissimilarity, were projected onto the opposite/parallel axis of each heatmap for core biome types (y-axis) and taxa (x-axis). Order and class core biome heatmaps contained all taxa ≥0.1% of the relative abundance. However, due to the number of taxa present in genera core biomes, taxa were limited to those that comprised ≥1% or more of the total core biome for the core biome heatmap.

Finally, for each taxonomic level, the significant drivers of community structure and core biome type were investigated using per-mutational multivariate analysis of variances (PERMANOVA) in the vegan package (Oksanen *et al.*, 2007).

5.4 Results

5.4.1 Bacterial Culturing and Colony Identification

All but 15 of the 192 colonies chosen at random from the *E. coli* cultures across the year were confirmed as *E. coli* by MALDI-TOF. The 15 that were not *E. coli* were identified as *Enterobacter cloacae*. *Escherichia coli*/*Enterobacter cloacae* (*E. coli*/*E. cloacae*) group concentrations were highly variable in both water and sediment samples, ranging between 2-19,640 CFU/100 ml in water compared to 0-206,400 CFU/100 ml in sediment (Figure 5.2). Concentrations were typically higher in sediment than water from the same site and time. The exceptions to this were in the lower native forest (site 9) in winter and the Te Ahiaua (site 1) in spring.

Sediment *E. coli*/*E. cloacae* concentrations were highest in the autumn at sites associated with native forest (9 and 10), beef and sheep (6), and effluent disposal (2). The intensive

dairy drain (site 3) *E. coli*/*E. cloacae* concentrations peaked in winter with ~458,000 CFU/100 ml. The remaining sites, Te Ahiaua (1), exotic forest (7 and 8), and dairy (4 and 5), had the highest concentrations over summer.

E. coli/*E. cloacae* concentrations in water had a different pattern. Concentrations were highest in the winter at the intensive dairy drain (3), Te Ahiaua (1), and the native and exotic forest sites (7-10). Inversely, the effluent station (2), dairy (4 and 5), and the beef and sheep (6) site's concentrations were highest in summer.

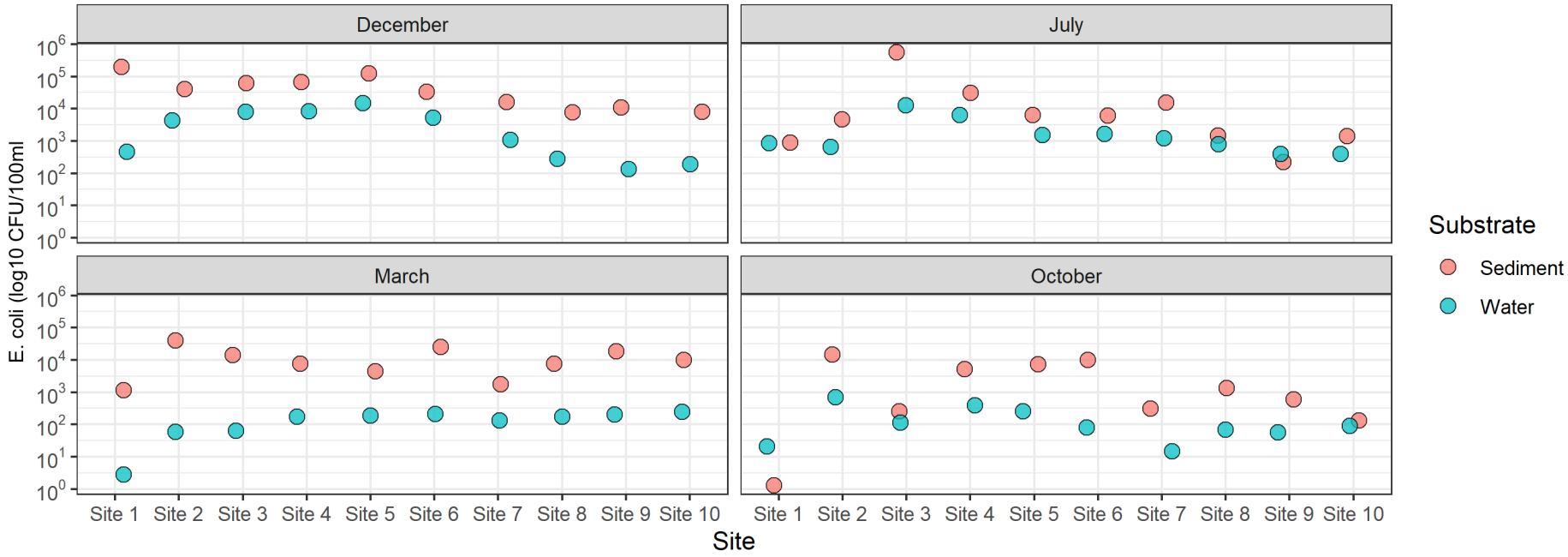


Figure 5.2 *E. coli*/*E. cloacae* concentrations at each of the ten sites in the Waiotahe catchment.

Log_{10} transformed *E. coli*/*E. cloacae* group concentrations, seasonally, from both sediment and water column samples from each of the eight sites along the Waiotahe river mainstem, one tributary site, and one site in Te Ahiaua, seasonally (e.g., autumn, winter, spring, and summer) in 2018.

5.4.2 eDNA characteristics and quality control

Even with preponderant pooling, blank one and blank two returned less than 350,000 reads each (CH 5 Appendix 1 Table 3). Single base pair error rates within the samples were typically less than 0.02% across all the samples and in all but nine samples 80-90% of the sequences had phred scores ≥30. Phred scores indicate the accuracy of every base identified within the reads, with a phred ≥30 corresponding to the probability of each base being correct 999 out of 1000 times. In the nine samples where the phred score was ≤30, the sequence phred scores were ≥20, correct 99 out of 100 times. Rarefaction curves (Figure 5.3) indicated that all taxa present in a sample were represented within ~10,000 reads, below the minimum read number achieved in every sample.

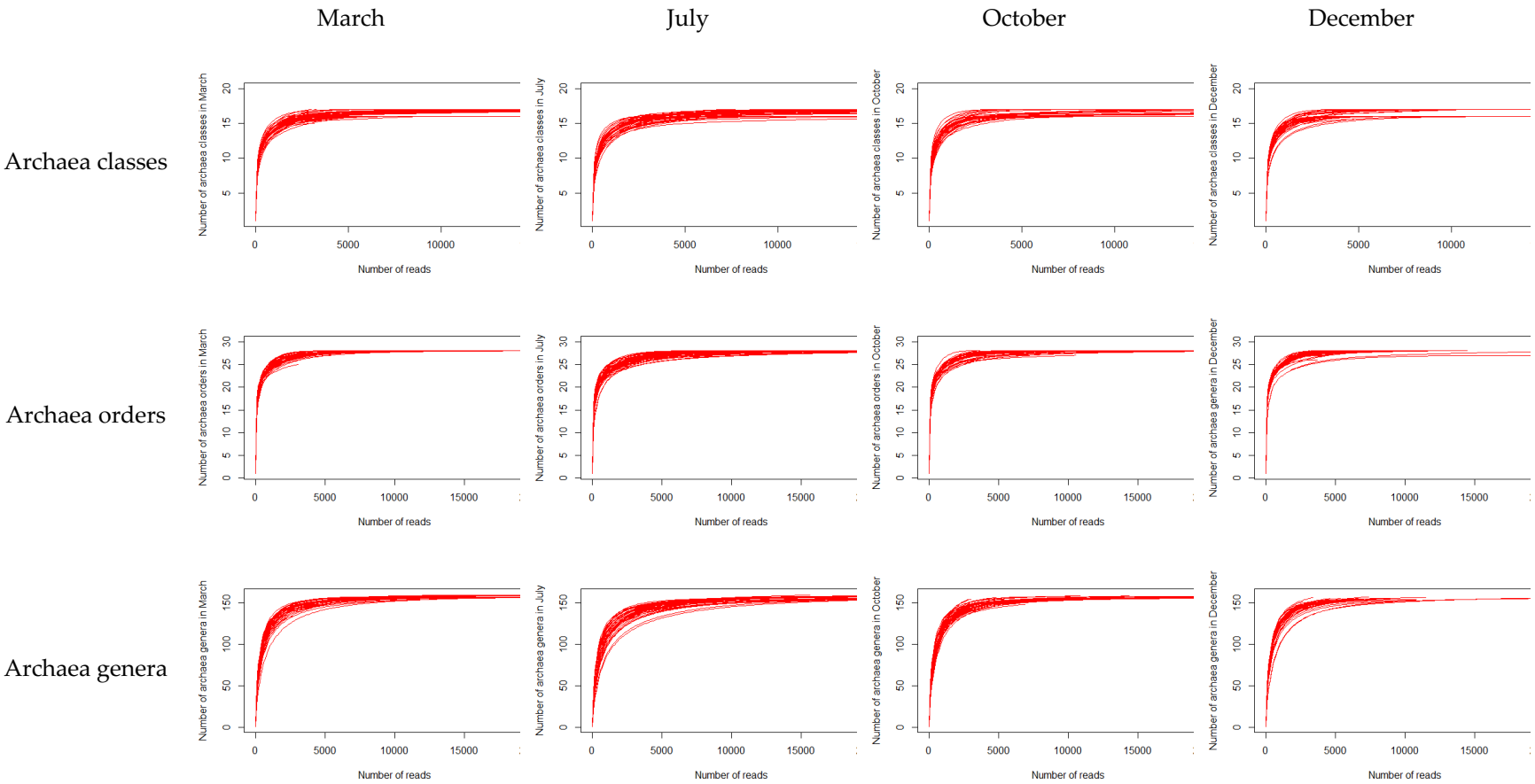


Figure 5.3. Rarefaction curves for archaea.

Rarefaction curves for archaeal classes, orders, and genera from each of the eight sites along the Waiotahe river mainstem, one tributary site, and one site in Te Ahiaua, seasonally (e.g., March, July, October, and December) in 2018.

5.4.3 Archaeal Community Structure

Of the 48 variables tested, season was the best at explaining the spatio-temporal variability observed within archaeal communities (PERMANOVA: Order Season $F_{(3)}$ =109.69, R^2 = 0.235, p-value = 0.001, Genera Season $F_{(3)}$ =142.08, R^2 = 0.345, p-value = 0.001) (CH 5 Appendix 1 Table 4-9).

Communities were dominated by acidophiles and nitrogen, sulfur, and carbon cycling taxa with diverse metabolic and lifecycle characteristics (Table 5.3, Figure 5.4). The most abundant classes were Methanomicrobia and Halobacteria, with their relative abundances commonly inverted. Methanomicrobia, abundance was highest in winter samples (e.g., sediments from exotic forest and water communities), spring sediments from intensive dairy and exotic forest, and autumn sediments from the effluent station, and water from dairy sites. While Halobacteria were typically more abundant in sediments than water and abundance was highest in summer sediments in native forest. Thermoprotei were ubiquitous and the third most abundant taxa for all samples except for spring (sites 7 and 3) and summer sediment (site 3) where Thermoplasmata was more abundant.

The order Methanosarcinales, was the most abundant in most samples. The primary exceptions to this were spring water from Te Ahiaua, where Nitrosopumiales dominated, and spring sediments from the intensive dairy site (3) and lower exotic forest site (7) where Methanomicrobiales was more abundant. Other abundant orders included Halobacteriales, Haloferacales, Natrialbales, and Sulfolobales (Figure 5.5).

Methanosarcina was the principal genus in most samples, comprising 10 – 70% (averaging 30%) of the total relative abundance in each sample. However, spring and summer sediments from the lower exotic forest site (7) and the intensive dairy farm were typified by *Methanoregula* and *Methanothrix* co-dominated communities. Other taxa with community defining abundance shifts away from *Methanosarcina* included: *Nitrosopumilus* and *Candidatus* Nitrosopelagicus in estuarine water in spring; *Nitrosarchaeum* in spring sediment at the effluent station (2); *Candidatus* Methanoperedens, *Candidatus* Nitrosotenius, and *Candidatus* Nitrosotalea in summer sediment at the lower dairy site (4). Individually these taxa typically comprised less than 5% of the total relative abundance samples where *Methanosarcina* was the principal; however, when combined with *Halorubrum* and *Sulfolobus*, they were the most abundant archaea genera (Figure 5.6, Table 5.3).

Table 5.3. Characteristics of key archaea genera from each of the eight sites along the Waiotahe river mainstem, one tributary site, and one site in Te Ahiaua, seasonally (e.g., March, July, October, and December) in 2018.

Archaea genus	Motility	Description	References
Candidatus Methanoperedens	capacity for motility	Anaerobic, pairs nitrate reduction and methane oxidising	(Guerrero-Cruz et al., 2018)
Candidatus Methanoperedins	capacity for motility	Anaerobic, methanotrophs with one species coupling methane oxidation + nitrate reduction using a bacterial nitrite reductase enzyme	(Stein, 2023)
Candidatus Nitrosopelagicus	unknown, likely non-motile	Aerobic, ammonia oxidising, marine habitats	(Santoro et al., 2015)
Candidatus Nitrosotalea	capacity for motility	Aerobic, autotrophic, acidophilic, ammonia oxidising, mercuric reductase gene from bacteria	(Lehtovirta-Morley et al., 2011; Herbold et al., 2015)
Candidatus Nitrosotenuis	capacity for motility	Aerobic, ammonia oxidising, carbon fixer, saline sensitive - freshwater, potential gene transfer events	(Sauder et al., 2018)
Halorubrum	capacity for motility	Halophile, species show evidence of frequent recombination and symbiotic gene and metabolite transfer	(Papke et al., 2004; Hamm et al., 2019; Gong et al., 2022)
Methanocella	non-motile	Anaerobic, methanogenesis, associated with agricultural soils and estuarine water, evidence of horizontal gene transfer and symbiotic metabolism	(Sakai et al., 2010, 2011; Angel, Claus & Conrad, 2012; Bertrand et al., 2019; Chen et al., 2020)
Methanoregula	non-motile	Anaerobic, methanogenesis at low pH,	(Bräuer et al., 2006; Lal & Lal, 2010)
Methanosarcina	non-motile	Anaerobic (but may adapt to withstand oxygen stress) methanogenesis by all pathways, multiple horizontal gene transfer events	(Garushyants, Kazanov & Gelfand, 2015; Jasso-Chávez et al., 2015; Lambie et al., 2015)
Methanothrix	non-motile	Anaerobic, methanogenesis, strictly acetate metabolism, electron transfers with *Geobacter metallireducens*	(Jetten, Stams & Zehnder, 1992; Holmes et al., 2017)

Archaea genus	Motility	Description	References
Nitrosarchaeum	capacity for motility	Aerobic, meso- and neutrophilic, ammoia oxidising, carbon fixers, estuarine and freshwater habitats, tubulin from gene transfer event	(Yutin & Koonin, 2012; Jung et al., 2018)
Nitrosopumilus	capacity for motility	Low oxygen habitats, chemoautotroph, ammonia oxidising with copper dependence	(Walker et al., 2010; Martens-Habbena & Stahl, 2011; Ren & Wang, 2022)
Sulfolobus	capacity for motility	Associated with volcanic springs, acidophile and thermophiles, sulphur oxidising, aggregate in response to UV, high frequency recombination via conjugation	(Quehenberger et al., 2017; Charles-Orszag et al., 2023; Sanchez-Nieves, Zhang & Whitaker, 2023)

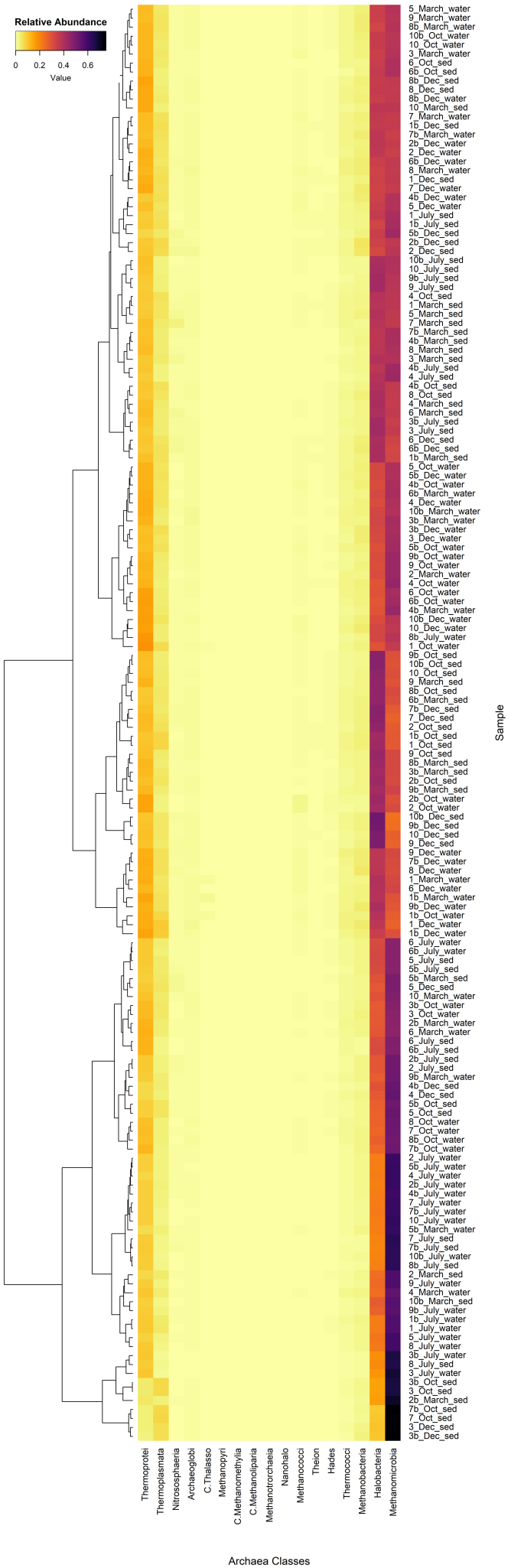


Figure 5.4. Heatmap of archaea classes. Heatmap depicting archaea classes, with relative abundances ≥0.1%, from the 160 eDNA samples collected from each of the eight sites along the Waiotahe river mainstem, one tributary site, and one site in Te Ahiaua, seasonally (e.g., March, July, October, and December) in 2018. A dendrogram, representing hierarchical clustering using Bray-Curtis dissimilarity, was projected onto the opposite/parallel axis for each sample (y-axis).

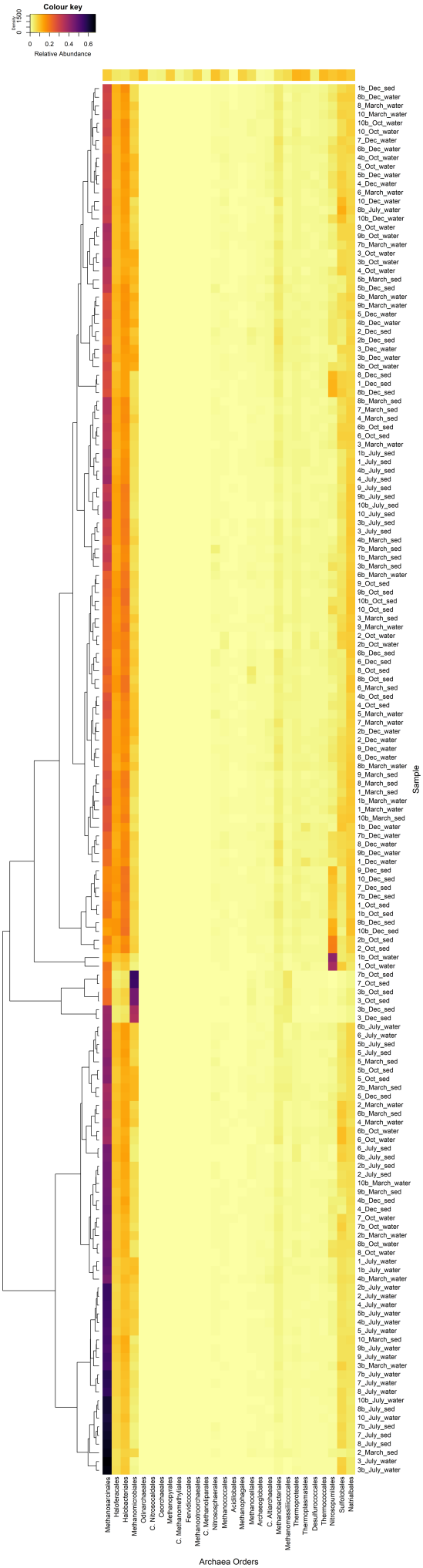


Figure 5.5 Heatmap of archaea orders. Heatmap depicting archaea orders, with relative abundances ≥0.1%, from the 160 eDNAsamples collected from each of the eight sites along the Waiotahe river mainstem, one tributary site, and one site in Te Ahiaua, seasonally (e.g., March, July, October, and December) in 2018. A dendrogram, representing hierarchical clustering using Bray-Curtis dissimilarity, was projected onto the opposite/parallel axis for each sample (y-axis).

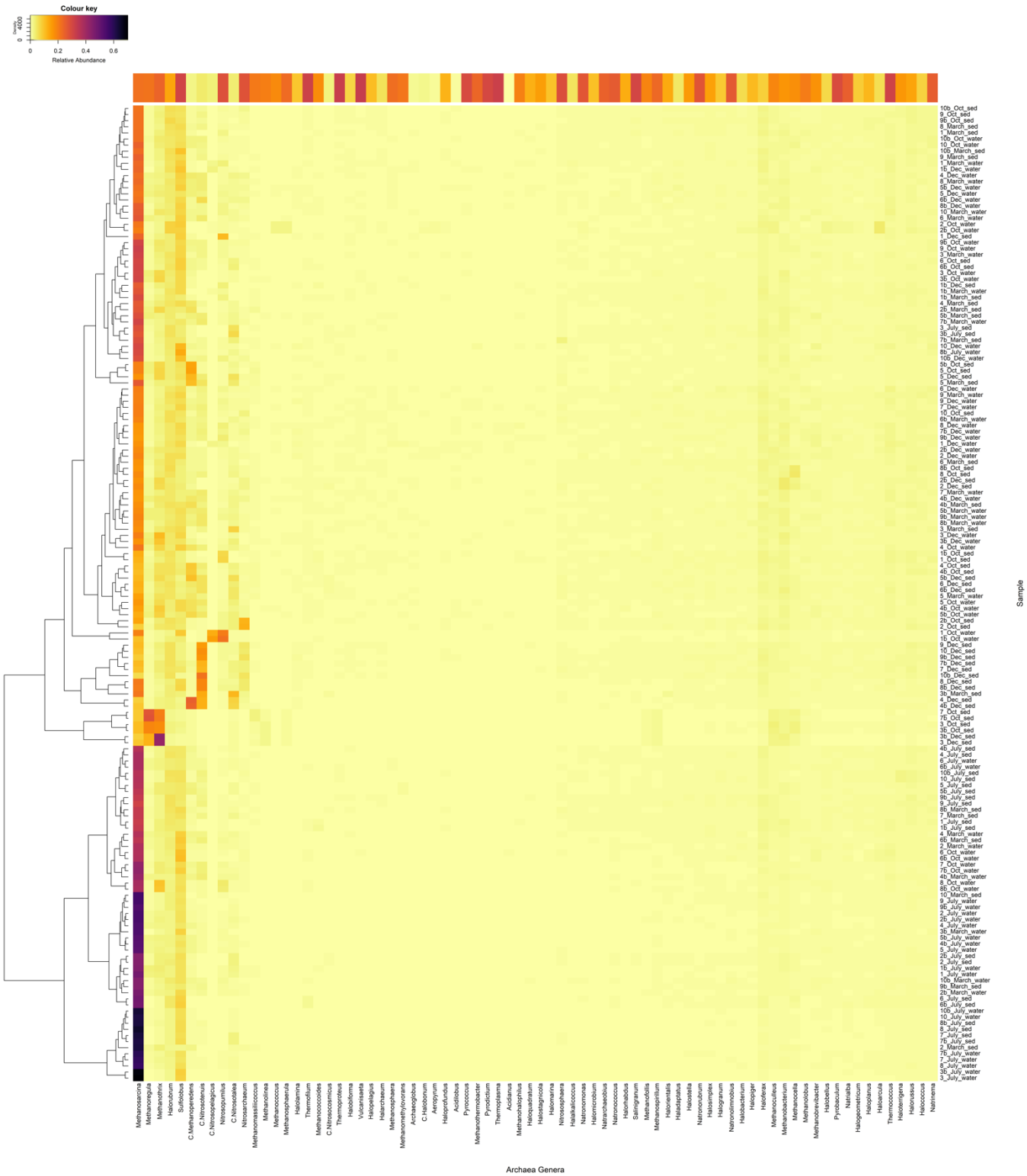
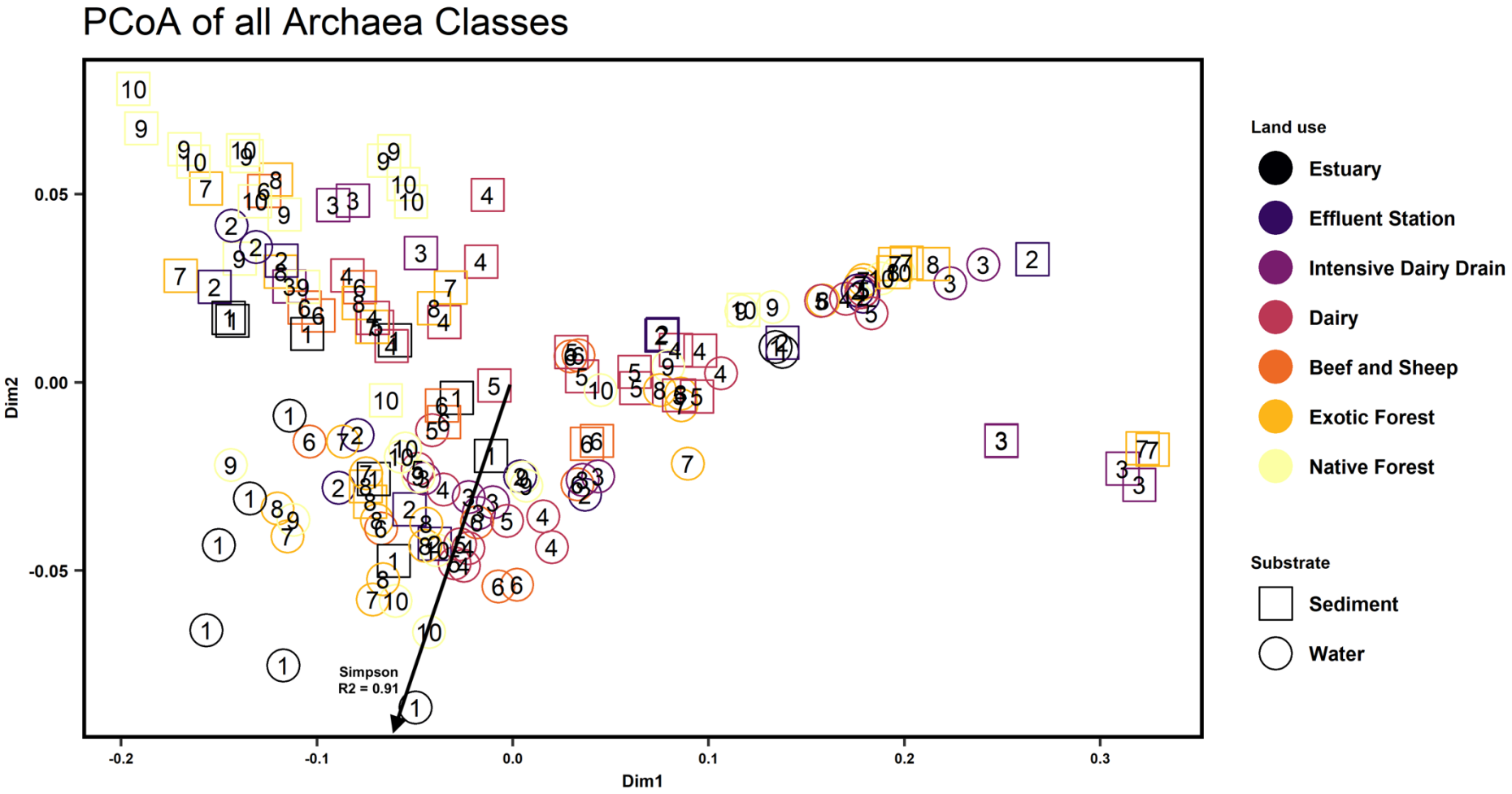


Figure 5.6 Heatmap of archaea genera.

Heatmap depicting archaea genera, with relative abundances ≥0.1%, from the 160 eDNA samples collected from each of the eight sites along the Waiotahe river mainstem, one tributary site, and one site in Te Ahiaua, seasonally (e.g., March, July, October, and December) in 2018. A dendrogram, representing hierarchical clustering using Bray-Curtis dissimilarity, was projected onto the opposite/parallel axis for each sample (y-axis).

5.4.4 Environmental Factors Associated with Communities

Of the 48 variables evaluated for their relationship to community structuring, many were significant (p-value <0.05) in differentiating communities. Overall, spatio-temporal variables (e.g., season or land use) explained archaeal communities better than environmental variables. However, important environmental variables included slope (upstream, downstream, and reach), upstream hardness, river width, riparian shade, upstream indigenous forest, and macrophyte cover (CH 5 Appendix 1 Table 4 – 9, Figures 5.7, 5.8, and 5.9).


PCoA of all Archaea Classes
Dim2
Dim1
0.05
0.00
-0.05
-0.2
-0.1
0.0
0.1
0.2
0.3
Simpson
R2 = 0.91
Land use
Estuary
Effluent Station
Intensive Dairy Drain
Dairy
Beef and Sheep
Exotic Forest
Native Forest
Substrate
Sediment
Water

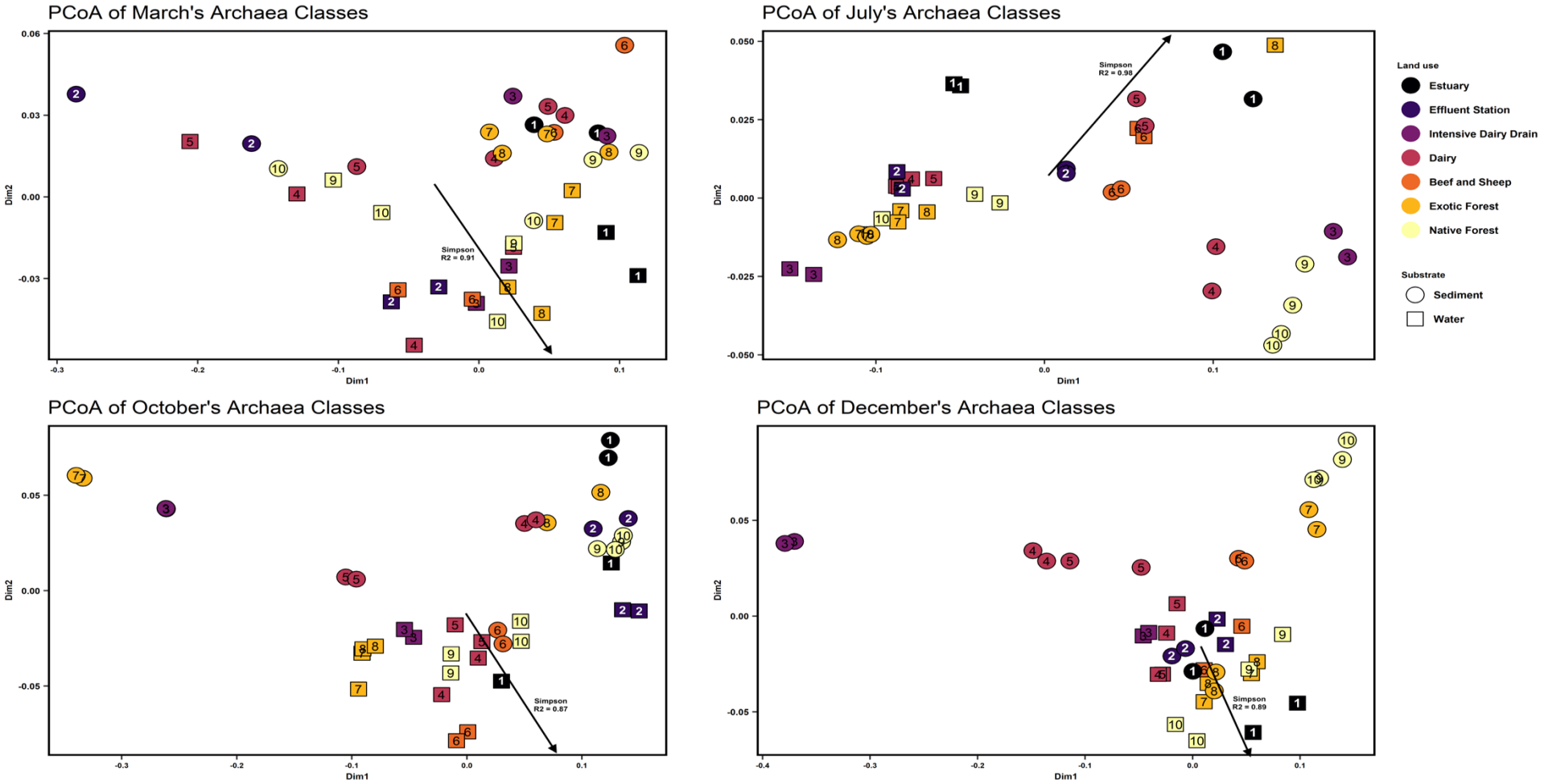
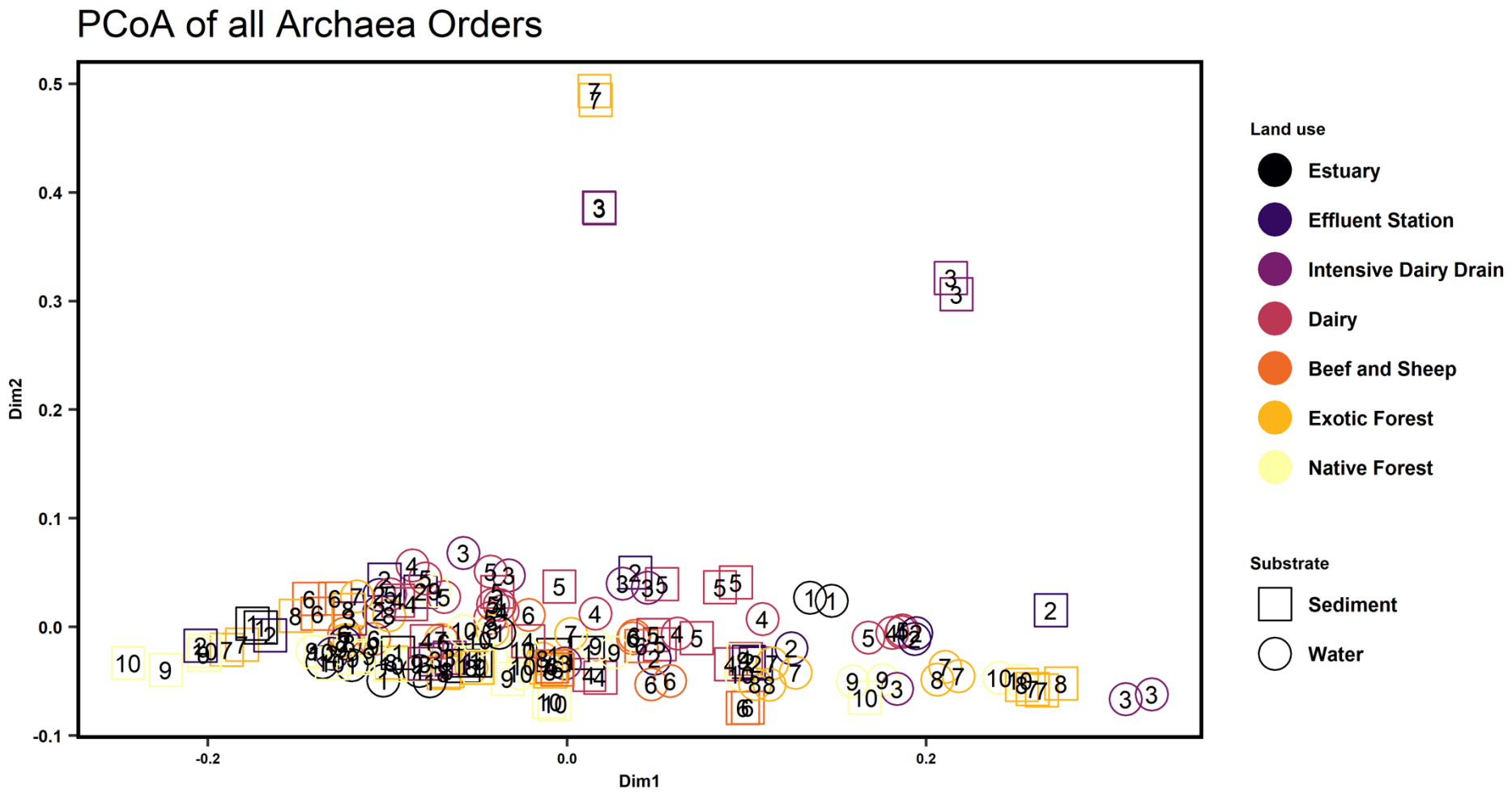


Figure 5.7 Class principal coordinates analysis, as an annual composite and individually by season.

Principal coordinates analysis, as an annual composite and individually by season (e.g., March, July, October, and December), of archaea class communities from the 160 eDNA samples collected from each of the ten sites from the Waiotahe river study in 2018. Environmental variables and metrics with an $R^2 \geq 0.6$ are projected onto the plots with the R^2 value and p-value.


PCoA of all Archaea Orders
Dim2
Dim1
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
0.0
0.2
Land use
Estuary
Effluent Station
Intensive Dairy Drain
Dairy
Beef and Sheep
Exotic Forest
Native Forest
Substrate
Sediment
Water

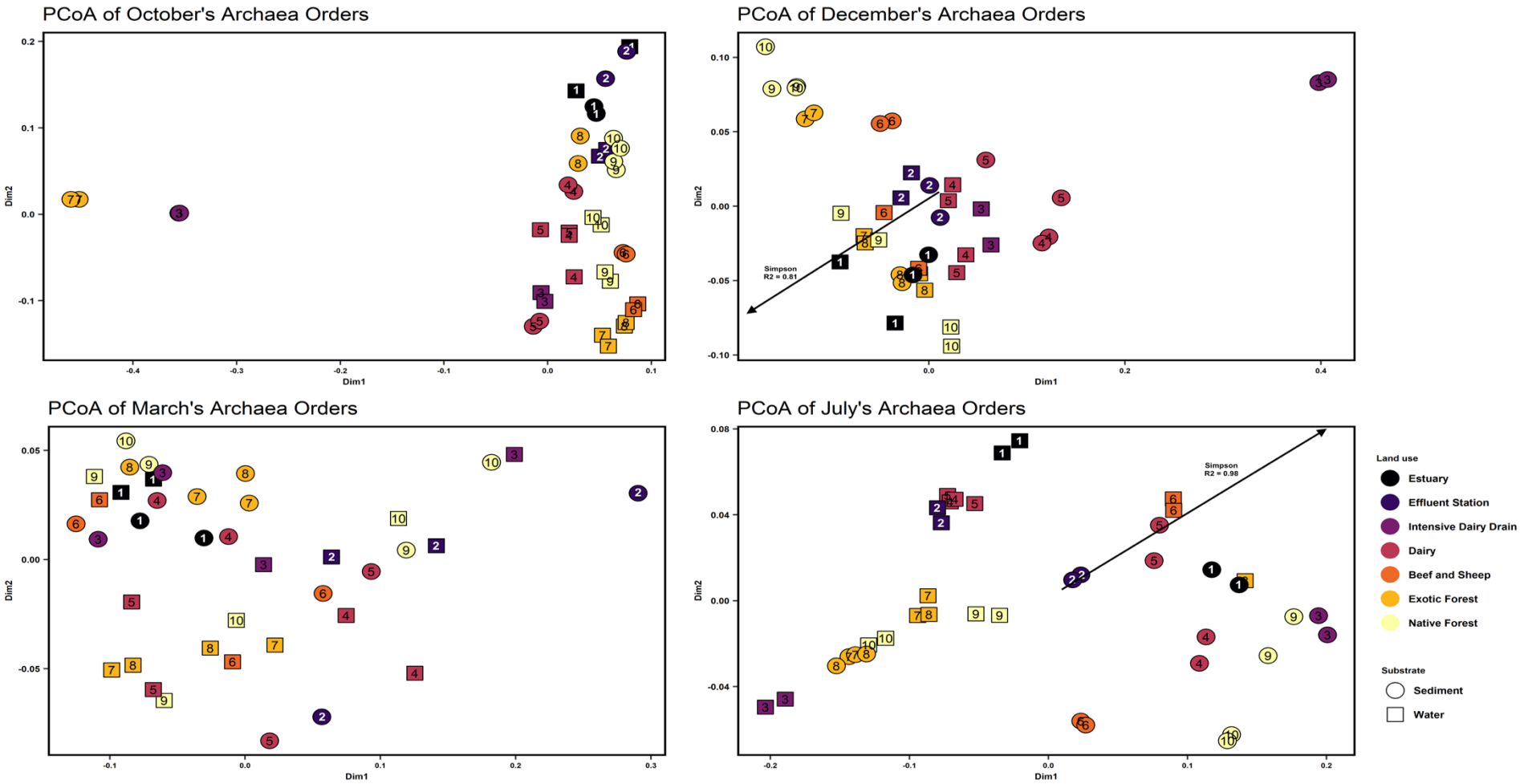
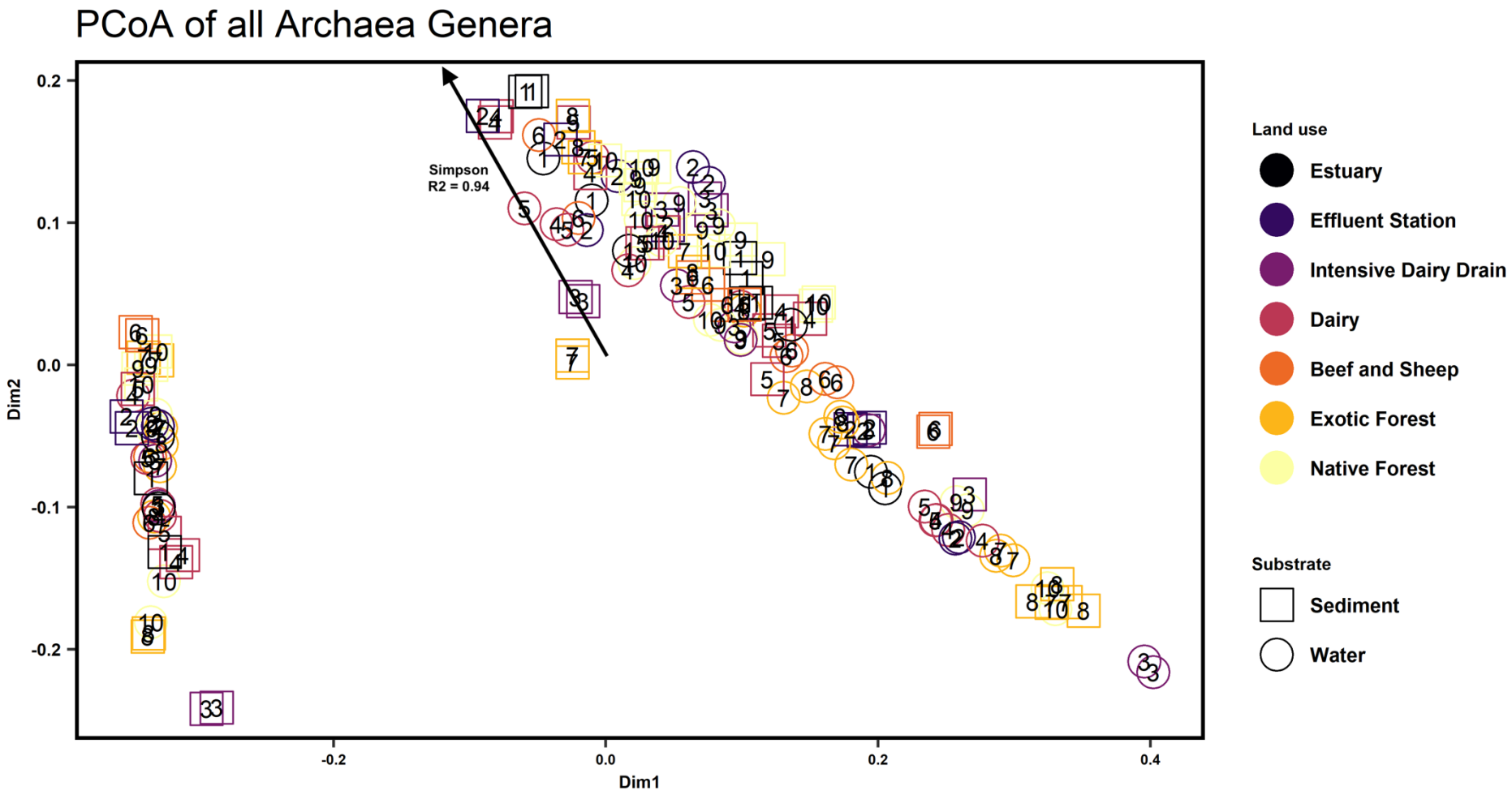


Figure 5.8 Orders principal coordinates analysis as an annual composite and individually by season.

Principal coordinates analysis as an annual composite and individually by season (e.g., March, July, October, and December) of archaea order communities from the 160 eDNA samples collected from each of the ten sites from the Waiotahe river study in 2018. Environmental variables and metrics with an $R^2 \geq 0.6$ are projected onto the plots with the R^2 value and p-value.


PCoA of all Archaea Genera
Simpson
R2 = 0.94
Dim1
Dim2
0.2
0.1
0.0
-0.1
-0.2
-0.2
0.0
0.2
0.4
Land use
Estuary
Effluent Station
Intensive Dairy Drain
Dairy
Beef and Sheep
Exotic Forest
Native Forest
Substrate
Sediment
Water

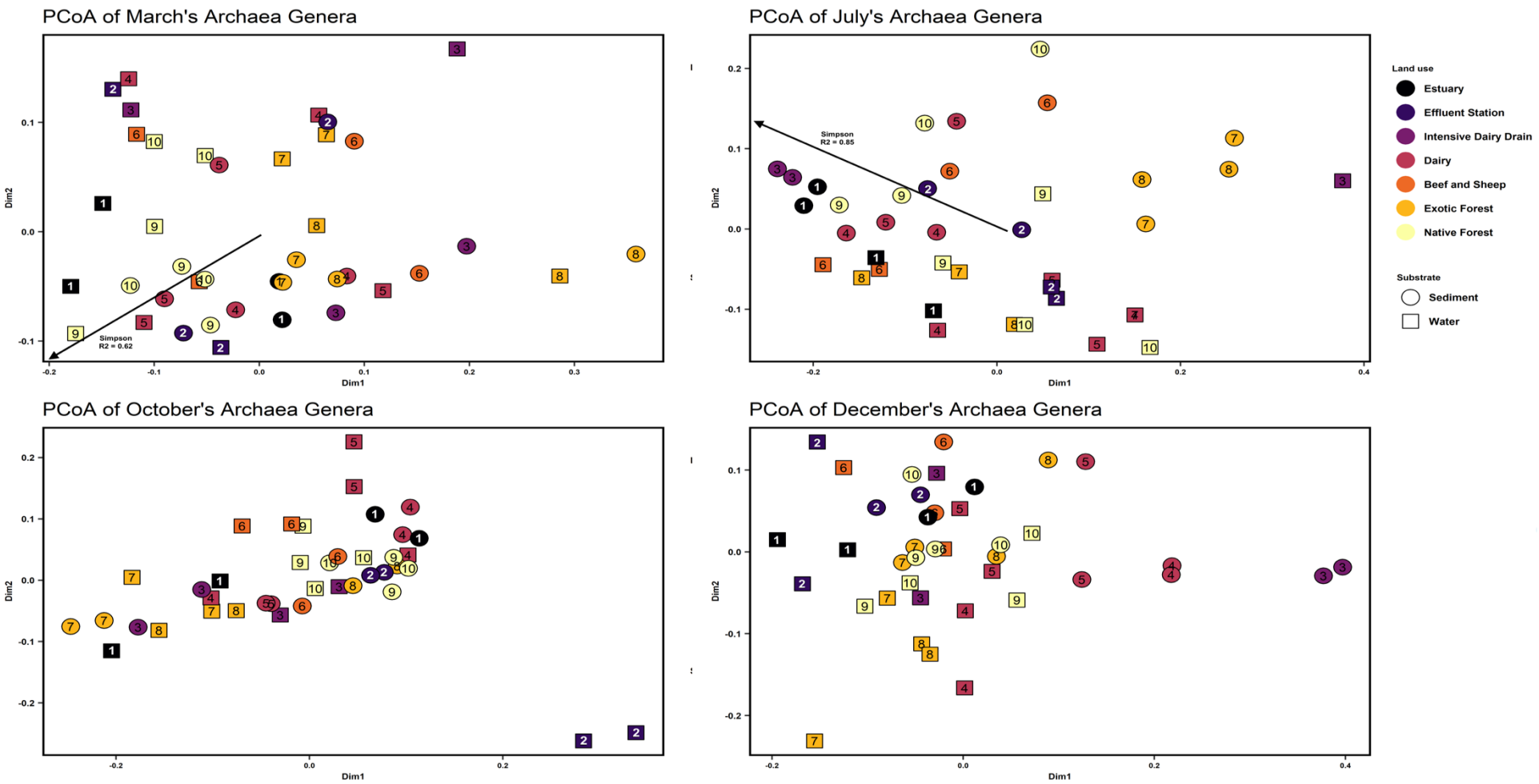


Figure 5.9 Genera principal coordinates analysis as an annual composite and individually by season.

Principal coordinates analysis as an annual composite and individually by season (e.g., March, July, October, and December) of archaea genera communities from the 160 eDNA samples collected from each of the ten sites from the Waiotahe river study in 2018. Environmental variables and metrics with an $R^2 \geq 0.6$ are projected onto the plots with the R^2 value and p-value.

5.4.5 Core Biomes

Similar to community structure, core biome composition was best explained by season (PERMANOVA: Season class $F_{(4)} = 4.86$, $R^2 = 0.378$, p-value = 0.011, Season order $F_{(4)} = 6.89$, $R^2 = 0.313$, p-value = 0.010, Season genera $F_{(4)} = 14.59$, $R^2 = 0.544$, p-value = 0.001) (CH 5 Appendix 1 Table 10, 11, and 12). Archaea class core biomes were similar to each other and generally reflected the most abundant taxa with a few, highly abundant taxa providing clear differences in community structure.

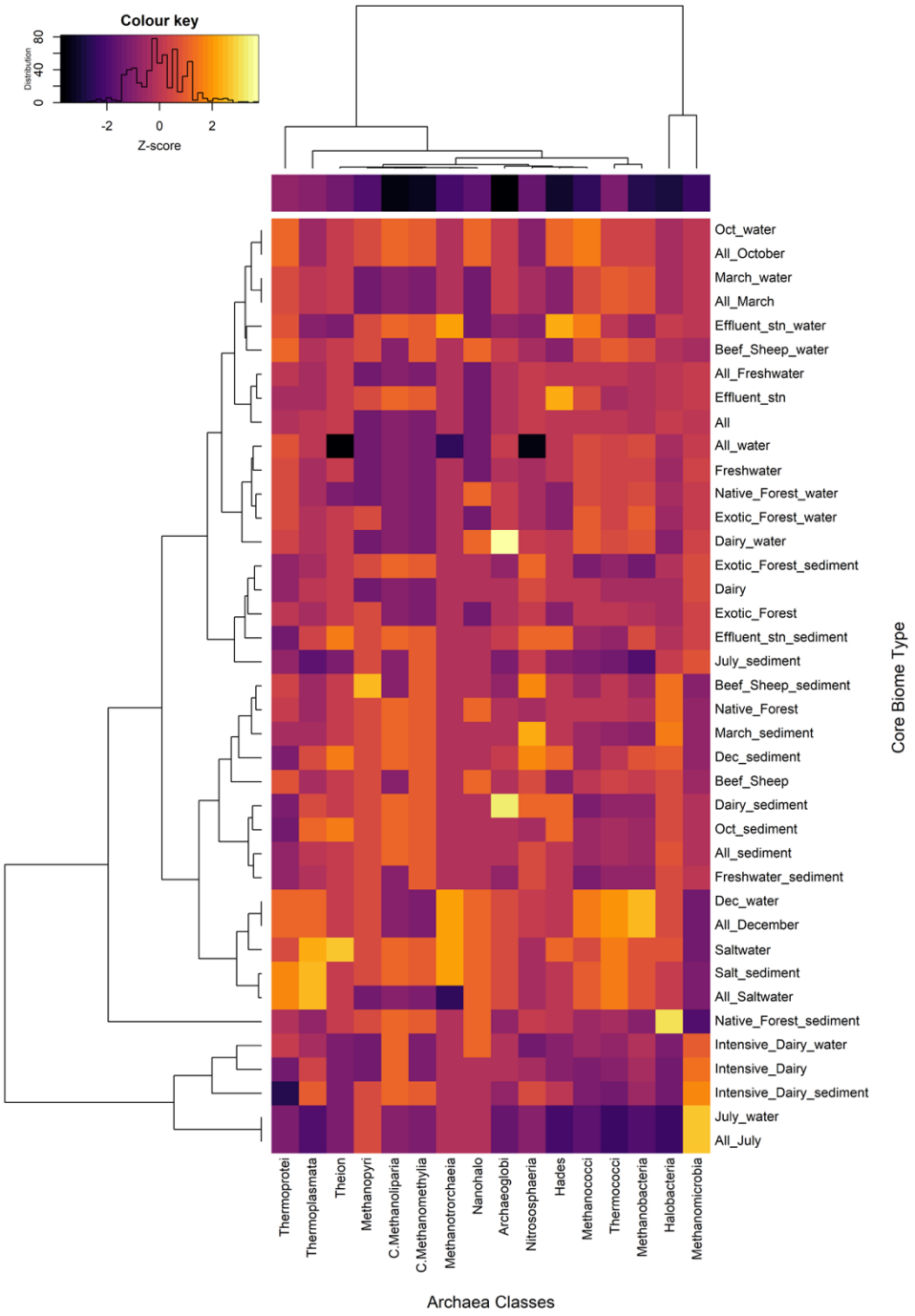


Figure 5.10 Heatmap of archaea class Z-scores.

Heatmap depicting archaeal class core biome Z-scores from the 160 eDNA samples collected from each of the eight sites along the Waiotahe river mainstem, one tributary site, and one site in Te Ahiaua, seasonally (e.g., March, July, October, and December) in 2018. Dendrograms, representing hierarchical clustering using Bray-Curtis dissimilarity, were projected onto the opposite/parallel axis for each core biome type (y-axis) and taxa (x-axis).

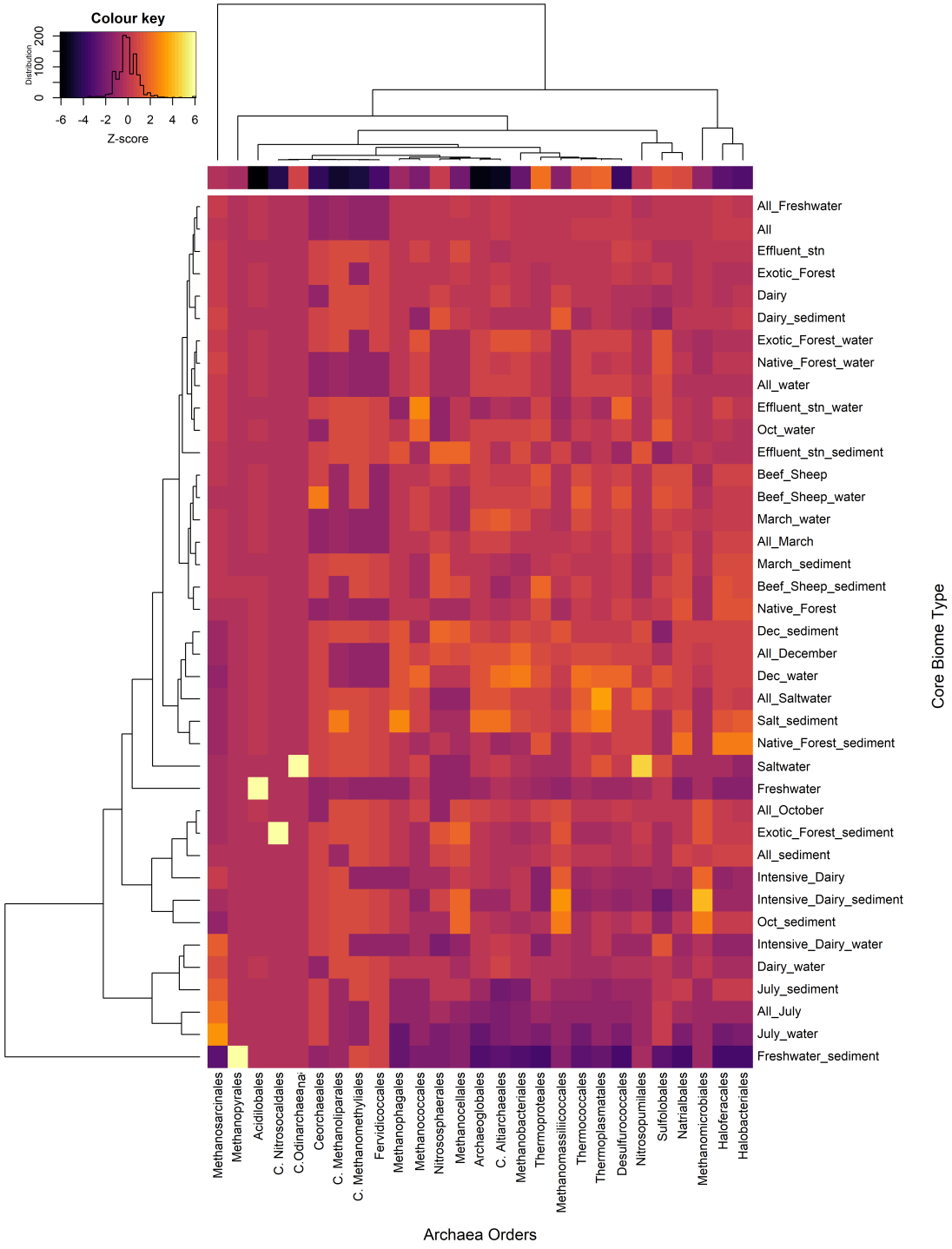


Figure 5.11 Heatmap of archaea order Z-scores.

Heatmap depicting archaeal order core biome Z-scores from the 160 eDNA samples collected from each of the eight sites along the Waiotahe river mainstem, one tributary site, and one site in Te Ahiaua, seasonally (e.g., March, July, October, and December) in 2018. Dendrograms, representing hierarchical clustering using Bray-Curtis dissimilarity, were projected onto the opposite/parallel axis for each core biome type (y-axis) and taxa (x-axis).

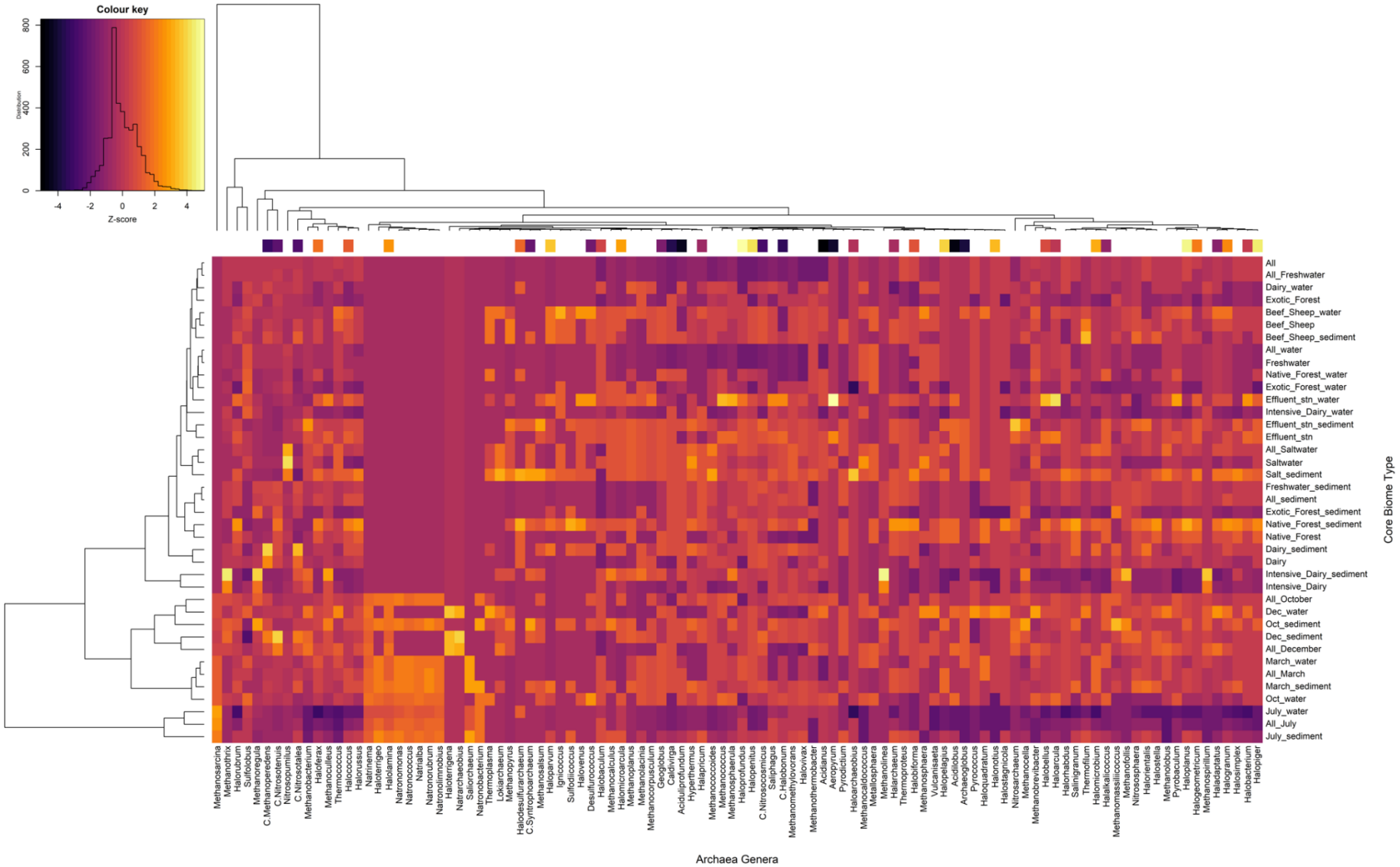


Figure 5.12 Heatmap of archaea genera Z-scores.

Heatmap depicting archaeal genera core biome Z-scores from the 160 eDNA samples collected from each of the eight sites along the Waiotahe river mainstem, one tributary site, and one site in Te Ahiaua, seasonally (e.g., March, July, October, and December) in 2018. Dendrograms, representing hierarchical clustering using Bray-Curtis dissimilarity, were projected onto the opposite/parallel axis for each core biome type (y-axis) and taxa (x-axis).

5.4.6 Molecular Testing for Target Genes

The *uidA* locus associated with *E.* coli was successfully amplified from every sample. In contrast, the three gene loci associated with pathogenic STEC (*rfbE* for O157, *wzy* for O26, stx_1, stx_2 and *eae*) were not amplified from any of the 160 samples. This finding was validated by searching the metagenomic results for the identification of any STEC strains and none were found. A gene associated with antibiotic resistance ($bla_{\text{CTX-M}}$) was detected in both substrates, on every occasion from Te Ahiaua, the effluent station, and the intensive dairy sites (n=24). The $bla_{\text{CTX-M}}$ gene was not identified from any other sample.

5.5 Discussion

Abundant archaea orders included methanogenic (e.g., Methanomicrobiales and Methanosarcinales), ammonia-oxidising (e.g., Nitrosopumilales), and sulfur-metabolising (e.g., Sulfolobales) taxa (Whitman et al., 2001; Leigh et al., 2011). The presence of methanogens along the full length of the Waiotahe river and Te Ahiaua reflects their role in global carbon cycling and greenhouse gas production, organic waste digestion, and association with agricultural drivers. The high relative abundance of *Methanosarcina*, a methyltrophic genera linked to effluents (both human and ruminant (Lambie et al., 2015; Tiirik et al., 2021)) and aquatic (fresh and saline) sediments, and responsible for the majority of global methane production (Guss et al., 2008), in riverine and estuarine samples is consistent with other studies (Whitman et al., 2001; Wang et al., 2019). *Methanothrix*, another methanogen, and *Candidatus* Nitrosotenuis, an aerobic ammonia-oxidising genera (Herbold et al., 2015), though present in lower in abundances, are equally important genera in agricultural catchments and those where effluents enter waterways (Li et al., 2016; Wang et al., 2019; Liu et al., 2021; Kabaivanova et al., 2022). Their presence as a community suggests that ruminants, their effluents, and the nutrients their farming rely on, strongly influence in-stream archaeal communities in the Waiotahe catchment (Whitman et al., 2001; Auguet, Barberan & Casamayor, 2010; Angelidaki et al., 2011). This is further bolstered by the explanatory power of season and upstream nutrient inputs for archaeal community structure observed in this study.

Previous studies on the effects of wastewater and nitrate enrichment on archaeal communities documented increased abundances of *Methanosarcina* (Li et al., 2021; Tiirik et al., 2021). Wastewater and effluents entering waterways are also associated with increased abundances of human pathogenic microorganisms and outbreaks of waterborne disease (Huffman, Quinter-Betancourt & Rose, 2003; Gilpin et al., 2020; Reaser et al., 2021; Tiirik et al., 2021).

Importantly, though no archaea are known human pathogens, the potential for them to be pathogenic exists (Cavicchioli et al., 2003) and some already carry genes capable of causing harm to humans (Guan et al., 2022). Two such archaea are *Methanothrix* and *Methanosarcina*, known to carry multiple antimicrobial resistance genes that they are able, and may be predisposed, to share with other archaea and bacteria through horizontal gene transfer (Fuchsman et al., 2017; Guan et al., 2022). While there are no documented cases of ESBL genes being found in archaea, it is unclear whether they have been looked for in environmental samples from Aotearoa. Additionally, the environmental pressures associated with polluted and/or anthropogenically impacted aquatic systems may encourage the sharing of these mobile genetic elements, particularly in sediments (Fuchsman et al., 2017; Guan et al., 2022). When combined with the documented functional succession of bacteria to archaea in months with temperature extremes (e.g., both high and low temperatures in winter and summer) and reversion back to bacteria in more temperate conditions, there is ample opportunity for interaction and gene transmission (Diao et al., 2023). Furthermore, remediation of antimicrobial resistance in aquatic systems may be insurmountable if archaea, highly resilient microorganisms capable of withstanding incredible environmental pressures, are indeed the primary drivers and reservoirs of these genes (Whitman et al., 2001; Cavicchioli et al., 2003; Auguet, Barberan & Casamayor, 2010; Fuchsman et al., 2017; Fan et al., 2020)

5.6 Conclusion

Seasonal variation explains aquatic archaeal community structure best. Due to archaea's low relative abundances and the difficulty isolating and culturing them, little is known about aquatic archaea outside of their functional aptitude. Here I describe the archaeal communities and explored their drivers in a longitudinal, source to sea study in Te Moana a Toi, Aotearoa. Further work will be necessary to determine how these hardy organisms compare across landscapes (e.g., urban, marine, and horticultural) and continents.

Chapter 5 Appendix 1

Table 1. The 48 curated variables and indices for each of each of the eight sites along the Waiotahe river mainstem, one tributary site, and one site in Te Ahiaua, seasonally (e.g., March, July, October, and December) in 2018.

Curated Environmental Variables and Indices	Definition
Depth (cm)	The average of 5 equidistant depth measurements along the middle of the sampled reach
Velocity (m/s)	The average of 5 equidistant measurements along the middle of the sampled reach using a velocity head rod
Temperature (°C)	A single measure at each site sampled
Conductivity (uS/cm)	A single measure at each site sampled
% Fine Sediment	The estimated percent of fine sediment deposited in/on the benthic substrate along the reach sampled
% Pool	The estimate percent of the sampled reach where water is pooled (area of low flow)
% Riffle	The estimate percent of the sampled reach where water is riffling (area of high flow with rocky protrusions)
% Run	The estimate percent of the sampled reach where water is running (area of high/moderate flow without protrusions)
% Macrophyte Cover	The estimate percent of the sampled reach where aquatic plants are present (not including algae)
% Debris Jam	The estimate percent of the sampled reach where debris is obstructing the usual flow of the river (e.g., a fallen tree or branch)
Periphyton	The estimated amount of periphyton (i.e., algae) present in a sampled reach; an ascending scale from 0-5, none to heaps
Substrate size	The estimated size of the benthic substrate present in a sampled reach; an ascending scale from 0-5, finest to coarsest (e.g., mud/silt, sand, fine gravel, coarse gravel, cobble, boulder)
Embeddedness	The estimated embeddedness of the benthic substrate present in a sampled reach; an ascending scale from 0-5 loose to unmovable
Stream Shade	The estimate percent of the sampled reach shaded (typically by trees and shrubs but may include bridges and/or other structures)
Land use	Dominant use of the land adjacent to the site
Substrate	Benthic substrate (sediment/stone) or water

Curated Environmental Variables and Indices	Definition
Taxonomic/Taxa richness	The number of taxa
Simpson's Diversity Index	$D = 1 - \left(\frac{\left(N(N-1)\right)}{\sum\left(n(n-1)\right)}\right)$ N = total number of organisms n = number of individuals of a species
Water column *E. coli*/*E. cloacae*	Cultured *E. coli*/*E. cloacae* concentrations from within the water column
Sediment *E. coli*/*E. cloacae*	Cultured *E. coli*/*E. cloacae* concentrations from the benthic substrates
January air temperature	Summer (January) air temperature (degrees C) – used in the absence of robust estimates of water temperature
Upstream hardness ($CaCO_3$)	Average hardness (induration) of surface rocks using values derived from the underlying LENZ layers – refer LENZ documentation for details
Water type	Based on potential salinity – freshwater, brackish, or saline
Flow	Mean annual flow (m^3/sec), derived from hydrological models, provided by Jochen Schmidt, NIWA, 2006
Low flow	Mean annual 7-day low flow (m^3/sec), derived from hydrological models, provided by Jochen Schmidt, NIWA, 2006
Flow 4^{th}	Segment mean annual 7-day low flow (m^3/s), fourth root transformed
Flow variability	Ratio of annual low flow/annual mean flow – indicates long-term stability of flow through the year
Slope	Segment slope (degrees), derived from GIS calculation using length and difference between upstream and downstream elevation for each segment
Slope sqrt	Square root transformed segment slope
Upstream calcium	Calcium concentrations in surface rocks using values derived from the underlying LENZ layers – refer LENZ documentation for details
Upstream phosphorus	Phosphorus concentrations in surface rocks using values derived from the underlying LENZ layers – refer LENZ documentation for details
Site	The reach where samples were collected
Historic shade	Estimated shade assuming complete vegetation cover as could be expected during pre-human conditions
Riparian native	Proportion of native riparian vegetation within a 100 m buffer of the river, calculated using landcover information contained in version one of the Landcover Database (LCDB)

Curated Environmental Variables and Indices	Definition
CLUES Nitrate	Nitrogen concentration (ppb) as estimated from CLUES, a leaching model combined with a regionally-based regression model, implemented within a catchment framework (Woods et al., 2006)
Downstream distance to coast	Distance to coast (km), from mid-point of each river segment, recomputed in Hamilton and differing from the original REC estimates of downstream distance that were computed from the upstream end; change made so values indicate average distance from a segment to the coast, rather than the maximum
Downstream average slope	Average slope (degrees), from mid-point of each river segment to the coast, differing from the original REC estimates of downstream slope that were computed from the upstream end; change made so values indicate average slope from within a segment
Downstream average slope sqrt	Square root transformed values of DSAvgSlope , i.e. $(slope+1)^{0.5}$.
Upstream days rain	Days/year with rainfall greater than 25 mm in the upstream catchment to indicate the likely frequency of elevated flows, rainday frequencies were provided by Brett Mullan (NIWA) and were derived by averaging across estimated daily rainfalls over the 10 year period from 1990 to 2000 – indicates short-term stability of flow through the year
Upstream indigenous Forest	Flow-weighted area of indigenous forest in upstream catchment (proportion), computed using cover estimates from LCDB1
Upstream pasture	Flow-weighted area of pasture in upstream catchment (proportion), computed using cover estimates from LCDB1
Reach sediment	Weighted average of proportional cover of bed sediment using categories of: 1– mud; 2–sand; 3–fine gravel; 4–coarse gravel; 5–cobble; 6–boulder; 7–bedrock, predicted from a boosted regression tree model – details of model fitting are provided in Leathwick et al. (2008)
Reach habitat	Weighted average of proportional cover of local habitat using categories of: 1– still; 2–backwater; 3–pool; 4–run; 5–riffle; 6–rapid; 7–cascade, predicted from a boosted regression tree model – details of model fitting are provided in Leathwick et al. (2008)
Riparian shade	Riparian shading (proportion), the likely degree of riparian shading derived by using national, satellite image-based vegetation classification to identify riparian shading in each segment, with the degree of shading then estimated from river size and expected vegetation height
Downstream max local slope	Maximum downstream slope (degrees), local slopes at 100m intervals along each river segment where calculated and maximum value encountered

Curated Environmental Variables and Indices	Definition
	recorded. Each segment was traversed downstream from its mid-point to the coast to identify the maximum downstream value encountered
Upstream native	Flow-weighted area of indigenous vegetation in upstream catchment (proportion), computed using cover estimates from LCDB1
Upstream average slope	Average slope in the upstream catchment (degrees), describes catchment-driven modification of flow variability
Season/Annual	Sample collection timeframe as a season or annual instead of months; December : summer, March : autumn, July : winter, October : spring, all : annual

Table 2. Delineation of the core biome stepwise progression.

Stepwise progression	Core biome type	Sample type contained
All	All samples	All samples (n-=160)
Substrate	All water	All water samples (n=80)
	All sediment	All sediment samples (n=80)
Water type	All freshwater	Both freshwater substrates (n=144)
	Freshwater	Water from freshwater sites (n=76)
	Freshwater sediment	Sediment from freshwater sites (n=76)
	All saltwater	Both estuarine substrates, site 1 (n=16)
	Saltwater	Water from the estuary, site 1 (n=8)
	Saltwater sediment	Sediment from the estuary, site 1 (n=8)
Season – Freshwater sites only	All autumn	Both freshwater substrates autumn (n=36)
	Autumn water	Water from freshwater sites in autumn (n=18)
	Autumn sediment	Sediment from freshwater sites in autumn (n=18)
	All winter	Both freshwater substrates winter (n=36)
	Winter water	Water from freshwater sites in winter (n=18)
	Winter sediment	Sediment from freshwater sites in winter (n=18)
	All Spring	Both freshwater substrates spring (n=36)
	Spring water	Water from freshwater sites in spring (n=18)
	Spring sediment	Sediment from freshwater sites in spring (n=18)
	All summer	Both freshwater substrates summer (n=36)
	Summer water	Water from freshwater sites in summer (n=18)

Stepwise progression	Core biome type	Sample type contained
	Summer sediment	Sediment from freshwater sites in summer (n=18)
Land use (where more than 1 site)	Native forest	Both substrates (n=32)
	Exotic forest	Both substrates (n=32)
	Beef and sheep	Both substrates (n=16)
	Dairy	Both substrates (n=32)
	Intensive dairy	Both substrates (n=16)
	Effluent station	Both substrates (n=16)
Land use by substrate	Native forest water	Freshwater (n=16)
	Native forest sediment	Sediment (n=16)
	Exotic forest water	Freshwater (n=16)
	Exotic forest sediment	Sediment (n=16)
	Beef and sheep water	Freshwater (n=8)
	Beef and sheep sediment	Sediment (n=8)
	Dairy water	Freshwater (n=16)
	Dairy sediment	Sediment (n=16)
	Intensive dairy water	Freshwater (n=8)
	Intensive dairy sediment	Sediment (n=8)
	Effluent station water	Freshwater (n=8)
	Effluent station sediment	Sediment (n=8)

Table 3. eDNA quality control statistics for all samples, including the number of raw reads and cellular organisms identified, the percent of reads identified and their contribution to each of the three domains (i.e., bacteria, eukaryote, and archaea), and the percent effective, error, phred scores > 20 and >30 and GC, at each of each of the eight sites along the Waiotahe river mainstem, one tributary site, and one site in Te Ahiaua, seasonally (e.g., March, July, October, and December) in 2018.

Sample	Raw reads	ID cellular orgs	% ID	bac % of ID org	euk % of ID org	arch % of ID org	Effective (%)	Error(%)	Q20(%) phred	Q30(%) phred	GC(%)
B1	389135	389,134	88.7	84.3	2	0.5	99.97	0.02	93.96	88.94	54.62
B2	229950	217,803	94.7	92.7	0.8	0.1	99.97	0.02	94.14	89.44	55.23
S_DEC_1_1	3534877	2419177	68.4	92	4	0.9	99.99	0.04	92.28	83.91	54.2
S_DEC_1_2	4106942	2910522	70.9	92	3	0.9	99.98	0.05	90.14	80.61	55.02
S_DEC_10_1	2640667	2073466	78.5	94	2	0.6	99.98	0.05	90.81	80.94	61.2
S_DEC_10_2	4242760	3256701	76.8	94	2	0.7	99.97	0.06	88.39	77.32	60.5
S_DEC_2_1	2692694	2047175	76	94	2	0.9	99.98	0.04	91.75	82.77	59.43
S_DEC_2_2	2907490	2210442	76	94	2	0.9	99.98	0.04	92.49	84.01	59.54
S_DEC_3_1	2453252	1862313	75.9	93	2	2	99.98	0.04	92.56	84.02	58.33
S_DEC_3_2	2310624	1739711	75.3	92	2	2	99.98	0.05	90.43	80.52	57.97
S_DEC_4_1	2877974	1950443	67.8	92	4	1	99.98	0.05	90.38	80.66	58.3
S_DEC_4_2	2470779	1697673	68.7	93	4	1	99.98	0.04	91.54	82.42	58.61

Sample	Raw reads	ID cellular orgs	% ID	bac % of ID org	euk % of ID org	arch % of ID org	Effective (%)	Error(%)	Q20(%) phred	Q30(%) phred	GC(%)
S_DEC_5_1	3008564	2391575	79.5	94	2	1	99.97	0.06	88.76	78.34	61.68
S_DEC_5_2	2114165	1656714	78.4	98	0.7	0.3	99.97	0.05	89.66	79.31	61.31
S_DEC_6_1	3177359	2490580	78.4	94	2	0.7	99.97	0.04	91.75	82.79	61.18
S_DEC_6_2	2943879	2299303	78.1	94	2	0.7	99.97	0.05	90.44	80.67	61.37
S_DEC_7_1	3361816	2614603	77.8	94	2	0.5	99.97	0.05	90.67	80.97	60.31
S_DEC_7_2	2676372	2085092	77.9	94	2	0.6	99.98	0.05	90.59	80.81	60.25
S_DEC_8_1	2437885	1649775	67.7	94	3	0.6	99.98	0.03	93.1	85.28	52.08
S_DEC_8_2	2306728	1570870	68.1	94	3	0.6	99.98	0.03	93.62	86.06	52.28
S_DEC_9_1	3138026	2406843	76.7	94	2	0.6	99.96	0.05	90.16	80.03	60.22
S_DEC_9_2	2634413	2023480	76.8	94	2	0.6	99.98	0.04	91.44	82.22	60.07
S_JUL_1_1	9291491	6870127	73.9	92	3	0.9	99.96	0.03	94.27	87.81	58.22
S_JUL_1_2	2421299	1730202	71.5	92	3	0.9	99.98	0.05	90.11	80.3	56.66
S_JUL_10_1	5399446	3904444	72.3	93	3	0.7	99.97	0.01	95.78	90.55	61.38
S_JUL_10_2	1982349	1416836	71.5	93	3	0.8	99.98	0.03	92.97	84.94	61
S_JUL_2_1	5062497	4022853	79.5	94	2	0.7	99.97	0.02	95.75	90.75	61.02

Sample	Raw reads	ID cellular orgs	% ID	bac % of ID org	euk % of ID org	arch % of ID org	Effective (%)	Error(%)	Q20(%) phred	Q30(%) phred	GC(%)
S_JUL_2_2	4535764	3572061	78.8	94	2	0.8	99.98	0.03	93.17	85.5	60.7
S_JUL_3_1	4021578	3184476	79.2	94	2	0.6	99.96	0.03	94.18	87.56	63.28
S_JUL_3_2	3297698	2560076	77.6	94	2	0.6	99.98	0.06	89.36	79.03	62.95
S_JUL_4_1	7459377	6066620	81.3	94	3	0.5	99.96	0.03	94.82	88.69	62.84
S_JUL_4_2	5560689	4374491	78.7	93	3	0.5	99.98	0.04	91.1	81.74	61.05
S_JUL_5_1	9447627	7362709	77.9	94	2	0.8	99.96	0.02	95	89.13	62.2
S_JUL_5_2	2606182	1990243	76.4	93	2	0.9	99.98	0.04	91.38	82.29	61.28
S_JUL_6_1	5533745	3169664	57.3	88	4	1	99.97	0.02	95.95	91.3	57.97
S_JUL_6_2	3824202	2175545	56.9	88	4	1	99.98	0.03	93.65	86.55	57.73
S_JUL_7_1	6989833	5659121	81	92	2	1	99.96	0.02	95.31	89.78	62.19
S_JUL_7_2	5266759	4215161	80	92	2	1	99.98	0.04	92.08	83.59	61.95
S_JUL_8_1	7214241	4751226	65.9	91	3	1	99.97	0.01	95.54	90.29	57.66
S_JUL_8_2	7134355	4529313	63.5	90	3	1	99.98	0.04	92.56	84.38	57
S_JUL_9_1	3438059	3432191	99.8	94	2	0.6	99.96	0.03	94.59	88.3	62.55
S_JUL_9_2	6046011	4836091	80	94	2	0.6	99.97	0.05	90.41	80.79	63.52

Sample	Raw reads	ID cellular orgs	% ID	bac % of ID org	euk % of ID org	arch % of ID org	Effective (%)	Error(%)	Q20(%) phred	Q30(%) phred	GC(%)
S_MAR_1_1	6424994	4677546	72.8	93	3	0.8	99.96	0.03	94.69	88.74	57.37
S_MAR_1_2	7565330	5488287	72.5	92	3	0.9	94.48	0.03	93.45	86.21	58.45
S_MAR_10_2	5753185	4650130	80.8	94	2	0.6	88.14	0.03	93.55	86.39	60.33
S_MAR_10_3	6223552	5004308	80.4	95	2	0.5	99.95	0.03	93.18	86.19	60.22
S_MAR_2_1	6090403	4905399	80.5	92	2	1	86.93	0.03	94.03	87.12	62.11
S_MAR_2_3	3739104	2959050	79.1	94	2	1	99.98	0.05	89.97	80.19	60.86
S_MAR_3_1	6353126	5114788	80.5	94	2	0.6	99.95	0.03	93.56	86.58	62.81
S_MAR_3_3	6867359	5488188	79.9	94	2	0.8	93.75	0.03	94.59	87.91	63.25
S_MAR_4_1	7791735	6027239	77.4	94	2	0.6	91.29	0.03	93.88	86.98	60.77
S_MAR_4_2	6906776	5407512	78.3	94	2	0.6	91.82	0.03	94.04	87.14	62.1
S_MAR_5_1	6345125	5203533	82	94	1	1	93.86	0.03	93.75	86.63	62.95
S_MAR_5_2	7006839	5567320	79.5	95	2	0.8	94.79	0.03	94.37	87.7	61.53
S_MAR_6_1	7641989	6090922	79.7	95	2	0.5	95.96	0.03	94.15	87.33	61.17
S_MAR_6_3	8811981	6978273	79.2	94	2	0.6	94.2	0.03	94.47	87.92	62.19
S_MAR_7_1	6447813	5155487	80	94	2	0.7	93.46	0.03	94.39	87.78	62.23

Sample	Raw reads	ID cellular orgs	% ID	bac % of ID org	euk % of ID org	arch % of ID org	Effective (%)	Error(%)	Q20(%) phred	Q30(%) phred	GC(%)
S_MAR_7_2	5867603	4821525	82.2	94	2	1	93.62	0.03	94	86.86	63.7
S_MAR_8_1	5738331	4589326	80	95	2	0.4	92.87	0.02	94.57	88.16	59.28
S_MAR_8_2	5740331	4696976	81.8	94	2	0.7	99.98	0.05	90.44	80.73	62.12
S_MAR_9_1	7525098	5745693	76.4	94	2	0.5	92.58	0.03	94.27	87.63	59.66
S_MAR_9_2	5720592	4376080	76.5	95	2	0.5	91.92	0.03	94.25	87.53	60.08
S_OCT_1_1	2720282	1949968	71.7	93	3	0.9	99.98	0.04	90.93	81.48	56.34
S_OCT_1_2	2641699	1898982	71.9	93	3	0.9	99.98	0.04	91.29	81.94	56.39
S_OCT_10_1	5283778	4176652	79	94	2	0.5	99.96	0.03	94.24	87.68	59.76
S_OCT_10_2	5758109	4569414	79.4	94	2	0.5	99.98	0.05	90.11	80.18	61.78
S_OCT_2_1	1986252	1553952	78.2	94	2	0.7	99.98	0.05	89.98	79.88	60.61
S_OCT_2_2	3553394	2800954	78.8	94	2	0.7	99.98	0.04	92.09	83.36	60.78
S_OCT_3_1	4358885	3323696	76.3	93	2	1	99.96	0.03	92.42	84.73	59.34
S_OCT_3_2	3508557	2612923	74.5	93	2	1	99.97	0.06	88.36	77.69	58.75
S_OCT_4_1	3451656	2718605	78.8	95	2	0.7	99.98	0.04	91.69	82.75	60
S_OCT_4_2	2714060	2139115	78.8	95	2	0.6	99.98	0.04	91.12	81.82	60.36

Sample	Raw reads	ID cellular orgs	% ID	bac % of ID org	euk % of ID org	arch % of ID org	Effective (%)	Error(%)	Q20(%) phred	Q30(%) phred	GC(%)
S_OCT_5_1	6410030	5074468	79.2	94	2	1	99.96	0.03	94.58	88.41	60.78
S_OCT_5_2	4334872	3379871	78	94	2	1	99.98	0.04	90.79	81.41	60.58
S_OCT_6_1	4700460	3758947	80	94	3	0.5	99.96	0.03	94.56	88.53	58.42
S_OCT_6_2	2909083	2299678	79.1	94	3	0.5	99.98	0.04	90.84	81.73	58.41
S_OCT_7_1	4528060	3486543	77	92	2	3	99.96	0.03	94.21	87.63	59.27
S_OCT_7_2	6031470	4574510	75.8	92	2	3	99.98	0.04	90.87	81.28	58.83
S_OCT_8_1	5515032	4329053	78.5	94	2	0.7	99.96	0.03	93.94	87.11	61.16
S_OCT_8_2	11572818	9131942	81	94	2	0.6	99.97	0.05	89.55	79.16	63.57
S_OCT_9_1	5543214	4293822	77.5	94	2	0.6	99.96	0.03	94.87	88.85	60.09
S_OCT_9_2	6512160	5097991	78.3	94	2	0.5	99.99	0.04	92.11	83.35	62.55
W_DEC_1_1	2660521	1436562	54	92	4	1	99.98	0.04	91.62	82.88	47.89
W_DEC_1_2	2863045	1682145	58.8	92	4	1	99.98	0.03	92.79	84.85	47.75
W_DEC_10_1	599838	488940	81.5	97	1	0.3	99.98	0.03	92.91	85.44	52.8
W_DEC_10_2	2988634	2358755	78.9	97	1	0.4	99.98	0.04	90.59	81.56	50.64
W_DEC_2_1	2789567	2044977	73.3	96	2	0.4	99.98	0.04	91.98	83.12	52.22

Sample	Raw reads	ID cellular orgs	% ID	bac % of ID org	euk % of ID org	arch % of ID org	Effective (%)	Error(%)	Q20(%) phred	Q30(%) phred	GC(%)
W_DEC_2_2	3120457	2334763	74.8	96	2	0.4	99.98	0.05	90.55	80.86	52.25
W_DEC_3_1	2551838	1924805	75.4	94	3	0.6	99.98	0.04	92.41	83.98	56.45
W_DEC_3_2	2289759	1688737	73.8	95	3	0.7	99.98	0.04	91.52	82.44	53.98
W_DEC_4_1	2709331	2280372	84.2	97	0.9	0.4	99.99	0.03	93.28	85.38	53.09
W_DEC_4_2	2869601	2357752	82.2	97	1	0.4	99.98	0.04	90.89	81.38	53.28
W_DEC_5_1	3148086	2703659	85.9	98	0.9	0.3	99.98	0.04	91.62	82.61	54.43
W_DEC_5_2	3035073	2615031	86.2	98	0.7	0.3	99.98	0.03	92.72	84.57	52.14
W_DEC_6_1	1956434	1680235	85.9	98	0.8	0.2	99.98	0.04	91.48	82.47	53.18
W_DEC_6_2	2548971	2052930	80.5	97	1	0.4	99.99	0.03	93.12	85.12	51.92
W_DEC_7_1	2468424	1954871	79.2	97	1	0.4	99.98	0.04	91.46	82.44	49.83
W_DEC_7_2	3017217	2440661	80.9	97	1	0.3	99.98	0.04	91.67	82.82	51.25
W_DEC_8_1	2784093	2334726	83.9	98	1	0.3	99.99	0.04	91.69	82.68	49.86
W_DEC_8_2	2412656	1976945	81.9	97	1	0.3	99.99	0.03	93.05	85.06	52.26
W_DEC_9_1	2509267	2004191	79.9	97	1	0.4	99.99	0.04	92.53	84.06	50.89
W_DEC_9_2	2111018	1696763	80.4	97	1	0.3	99.98	0.05	90.85	81.34	52.69

Sample	Raw reads	ID cellular orgs	% ID	bac % of ID org	euk % of ID org	arch % of ID org	Effective (%)	Error(%)	Q20(%) phred	Q30(%) phred	GC(%)
W_JUL_1_1	6646282	4871596	73.3	92	3	1	99.96	0.03	94.75	88.8	56.98
W_JUL_1_2	3225730	2312121	71.7	92	3	1	99.97	0.04	91.09	82.03	56.11
W_JUL_10_1	5939479	4664921	78.5	93	2	0.8	99.97	0.02	95.02	89.27	58.52
W_JUL_10_2	2788973	2162918	77.6	93	2	0.8	99.98	0.04	92	83.52	58.07
W_JUL_2_1	10237580	8288578	85	94	2	0.8	99.96	0.03	94.57	88.44	59.03
W_JUL_2_2	3314540	2641580	79.7	94	2	0.8	99.98	0.04	91.66	82.99	58
W_JUL_3_1	5461956	2520371	46.1	81	8	2	99.97	0.03	93.67	87.31	53.2
W_JUL_3_2	5103342	2306216	45.2	81	8	2	99.99	0.05	90.19	80.91	53.09
W_JUL_4_1	6739333	5331699	79.1	94	2	0.8	99.96	0.03	94.53	88.28	57.97
W_JUL_4_2	4391429	3417245	77.8	94	2	0.8	99.98	0.05	90.73	81.28	57.29
W_JUL_5_1	5235691	4458255	85.2	96	1	0.5	99.96	0.03	94.32	87.86	58.56
W_JUL_5_2	2587729	2171930	83.9	96	2	0.5	99.99	0.04	91.25	82.02	57.61
W_JUL_6_1	3667657	2775461	75.7	93	3	0.7	99.94	0.04	91.26	82.8	58.33
W_JUL_6_2	3398823	2500933	73.6	93	3	0.7	99.97	0.07	86.8	75.27	57.53
W_JUL_7_1	5233157	4100542	78.4	93	2	0.8	99.96	0.03	94.61	88.5	58.35

Sample	Raw reads	ID cellular orgs	% ID	bac % of ID org	euk % of ID org	arch % of ID org	Effective (%)	Error(%)	Q20(%) phred	Q30(%) phred	GC(%)
W_JUL_7_2	5168763	4049287	78.3	93	2	0.8	99.98	0.04	90.73	81.5	59.5
W_JUL_8_1	3284906	2557497	77.9	93	2	0.8	99.97	0.01	95.83	90.66	56.98
W_JUL_8_2	3734866	2906537	77.8	94	2	0.6	99.98	0.04	91.56	83.11	56.88
W_JUL_9_1	4832157	3782501	78.3	93	2	0.8	99.96	0.03	94.18	87.73	58.69
W_JUL_9_2	3948065	3032844	76.8	93	2	0.8	99.98	0.05	89.66	79.68	58.31
W_MAR_1_1	4548344	3271015	71.9	87	6	5	90.64	0.03	93.15	85.97	50.7
W_MAR_1_3	5175342	3707971	71.6	87	6	4	89.68	0.03	93.06	85.84	51.02
W_MAR_10_1	7586044	6641913	87.6	98	0.8	0.2	99.96	0.03	93.69	86.94	55.73
W_MAR_10_3	943813	809675	85.8	97	1	0.3	99.97	0.04	91.72	82.91	56.97
W_MAR_2_2	2137636	1863699	87.2	97	1	0.4	99.99	0.05	90.22	80.62	55.2
W_MAR_2_3	4958627	4398040	88.7	97	0.9	0.3	99.99	0.06	89.28	79.01	55.83
W_MAR_3_1	1153578	1003385	87	98	0.9	0.3	99.96	0.05	89.86	79.96	56.05
W_MAR_3_3	1493804	1279439	85.6	97	1	0.4	99.98	0.04	91.36	82.41	56.91
W_MAR_4_1	1701816	1495400	87.9	98	0.8	0.3	99.98	0.05	89.66	80.05	54.5
W_MAR_4_2	2611547	2206437	84.5	97	1	0.5	99.98	0.05	89.89	80.03	53.82

Sample	Raw reads	ID cellular orgs	% ID	bac % of ID org	euk % of ID org	arch % of ID org	Effective (%)	Error(%)	Q20(%) phred	Q30(%) phred	GC(%)
W_MAR_5_2	2949662	2502750	84.8	97	1	0.4	99.97	0.07	87.48	76.25	55.01
W_MAR_5_3	4481537	3808311	85.0	97	1	0.3	99.97	0.06	88.38	77.72	55.27
W_MAR_6_3	6544931	5467015	83.5	97	1	0.3	84.01	0.03	93.82	87.04	56.66
W_MAR_7_1	7460893	5790388	77.6	95	2	0.6	95.87	0.03	92.82	85.24	55
W_MAR_7_3	6587724	5054239	76.7	94	2	0.7	94.92	0.03	93.01	85.53	55.61
W_MAR_8_1	5557905	4356696	78.4	95	2	0.6	88.25	0.03	93.56	86.6	54.48
W_MAR_8_3	840287	609191	72.5	94	3	0.7	99.98	0.07	87.78	76.84	54.75
W_MAR_9_2	9571451	8135548	85.0	97	1	0.4	87.06	0.029	4.38	87.92	56.2
W_MAR_9_2	6441680	5240510	81.4	96	2	0.5	92.72	0.02	94.31	87.78	56.2
W_MAR_9_3	5214453	4450787	85.4	97	1	0.4	99.95	0.03	92.61	85.13	54.63
W_OCT_1_1	3886677	3038103	78.2	96	2	1	99.99	0.04	91.61	82.98	46.28
W_OCT_1_2	3374137	2648110	78.5	96	2	1	99.98	0.05	90.13	80.26	47.25
W_OCT_10_1	5863482	4235257	72.2	94	3	0.6	99.96	0.03	93.8	87.18	54.93
W_OCT_10_2	2522978	1784110	70.7	93	3	0.6	99.98	0.05	90.48	80.94	54.05
W_OCT_2_1	2984357	1187112	39.8	83	12	1	99.98	0.04	92.22	83.68	52.2

Sample	Raw reads	ID cellular orgs	% ID	bac % of ID org	euk % of ID org	arch % of ID org	Effective (%)	Error(%)	Q20(%) phred	Q30(%) phred	GC(%)
W_OCT_2_2	2778044	1131677	40.7	83	11	1	99.98	0.04	91.48	82.47	52.19
W_OCT_3_1	4404297	2978742	67.6	93	3	0.8	99.96	0.03	94.77	89	54.67
W_OCT_3_2	2988251	1973913	66.1	93	3	0.8	99.98	0.05	90.61	81.28	54.14
W_OCT_4_1	2290493	1964714	85.8	98	0.9	0.3	99.99	0.04	92.35	83.91	53.05
W_OCT_4_2	2956705	2558760	86.5	98	0.9	0.2	99.99	0.04	92.12	83.51	53.31
W_OCT_5_1	2211294	1865238	84.4	97	1	0.3	99.98	0.05	90.86	81.46	53.24
W_OCT_5_2	2238499	1868485	83.5	97	1	0.4	99.99	0.05	90.52	80.79	52.25
W_OCT_6_1	6002402	3601168	60	86	11	0.5	99.97	0.03	93.5	86.93	49.69
W_OCT_6_2	2763353	1614276	58.4	85	11	0.6	99.98	0.05	90.33	81.07	49.13
W_OCT_7_1	13561532	10885587	78.9	96	2	0.5	99.97	0.02	94.86	89.13	54.12
W_OCT_7_2	2726614	2166408	79.5	95	2	0.5	99.98	0.04	91.48	82.75	53.81
W_OCT_8_1	5854140	4971115	84.9	97	1	0.3	99.97	0.02	94.54	88.48	54.66
W_OCT_8_2	6801462	5733780	84.3	97	1	0.3	99.98	0.04	90.85	81.65	54.8
W_OCT_9_1	5155161	3947204	76.6	95	2	0.5	99.97	0.02	94.94	89.34	53.64
W_OCT_9_2	3985571	3008977	75.5	94	2	0.5	99.98	0.04	91.49	82.78	53.12

Table 4. PERMANOVA results identifying variables associated with archaeal class communities from each of the eight sites along the Waiotahe river mainstem, one tributary site, and one site in Te Ahiaua, seasonally (e.g., March, July, October, and December) in 2018. **Bold** denotes p-value <0.05%. The variable that best represented the variance in community structure has a red R^2.

Variable	**Df**	***F*-statistic**	**R^2**	***p*-val**
Land use	6	26.64	0.119	**0.001**
Substrate	1	45.85	0.034	**0.001**
Season	3	88.77	0.199	**0.001**
Simpson	1	1080.97	0.426	**0.001**
Land use : Substrate	6	13.55	0.032	**0.001**
Land use : Season	18	8.55	0.061	**0.001**
Substrate : Season	3	8.99	0.011	**0.001**
Land use : Simpson	6	7.39	0.018	**0.001**
Substrate : Simpson	1	9.20	0.004	**0.003**
Season : Simpson	3	9.32	0.011	**0.001**
Land use : Substrate : Month	18	5.51	0.039	**0.001**
Land use : Substrate : Simpson	6	4.83	0.011	**0.001**
Land use : Season : Simpson	18	1.098	0.008	0.366
Substrate : Month : Simpson	3	2.68	0.003	**0.034**
Land use : Substrate : Month : Simpson	18	0.81	0.006	0.707

Table 5. PCoA results, including distances, R^2, and p-values for Archaea classes present in each of the 160 samples collected from each of the eight sites along the Waiotahe river mainstem, one tributary site, and one site in Te Ahiaua, seasonally (e.g., March, July, October, and December) in 2018. **Bold** denotes a p-value <0.05%. The metric that best represented the variance in community structure is red.

Archaea classes										
Sample Time	**Annual**		**March / autumn**		**July / winter**		**October / spring**		**December / summer**	
	R^2	**p-val**	**R^2**	**p-val**	**R^2**	**p-val**	**R^2**	**p-val**	**R^2**	**p-val**
Spatial/Temporal Factors										
Season	0.202	**0.001**								
Land use	0.126	**0.003**	0.382	**0.006**	0.190	0.284	**0.508**	**0.001**	**0.571**	**0.002**
Site	0.050	**0.017**	0.009	0.849	0.227	**0.008**	0.035	0.521	0.212	**0.011**
Substrate	0.290	**0.029**	0.072	**0.028**	0.309	**0.001**	0.060	0.105	0.074	0.075
Water Type	0.035	**0.047**								
Metrics										
Richness	0.031	0.07	0.236	**0.005**	0.079	**0.013**	0.186	**0.028**	0.017	0.746
Simpson's Diversity Index	0.909	**0.001**	0.908	**0.001**	0.979	**0.001**	0.873	**0.001**	0.889	**0.001**
Water column *E. coli*/*E. cloacae*	0.549	**0.001**	0.391	**0.028**	0.219	0.508	0.472	**0.001**	0.562	**0.001**
Sediment *E. coli*/*E. cloacae*	0.378	**0.001**	0.419	**0.028**	0.215	0.409	0.367	**0.021**	0.418	0.114
Environmental Variables										
Jan Air Temp.	0.033	0.068	0.037	0.517	0.242	**0.006**	0.191	**0.02**	0.244	**0.014**

Archaea classes										
Sample Time	Annual		March / autumn		July / winter		October / spring		December / summer	
	R^2	p-val	R^2	p-val	R^2	p-val	R^2	p-val	R^2	p-val
Flow	0.043	**0.027**	0.034	0.511	0.180	**0.023**	0.014	0.773	0.244	**0.003**
Low Flow	0.033	0.066	0.040	0.491	0.250	**0.004**	0.196	**0.02**	0.229	**0.018**
Flow4th	0.034	0.067	0.034	0.546	0.224	**0.007**	0.187	**0.022**	0.263	**0.012**
Flow Variability	0.037	0.056	0.001	0.989	0.025	0.617	0.034	0.545	0.494	**0.001**
Slope	0.012	0.393	0.091	0.166	0.089	0.172	0.023	0.67	0.075	0.219
Slope Sqrt	0.040	**0.043**	0.080	0.213	0.013	0.785	0.203	**0.014**	0.077	0.257
Riparian Shade	0.069	**0.004**	0.004	0.933	0.364	**0.001**	0.010	0.798	0.158	**0.036**
Historic Shade	0.048	**0.026**	0.013	0.8	0.070	0.258	0.167	**0.032**	0.442	**0.001**
Riparian Native	0.058	**0.009**	0.007	0.877	0.213	**0.009**	0.002	0.967	0.194	**0.014**
Clues N	0.048	**0.028**	0.012	0.81	0.066	0.283	0.163	**0.033**	0.440	**0.001**
Distance to Coast	0.051	**0.016**	0.021	0.651	0.191	**0.014**	0.030	0.544	0.260	**0.003**

Archaea classes										
Sample Time	Annual		March / autumn		July / winter		October / spring		December / summer	
	R^2	p-val	R^2	p-val	R^2	p-val	R^2	p-val	R^2	p-val
Downstream Average Slope	0.014	0.333	0.017	0.729	0.156	**0.041**	0.200	**0.017**	0.082	0.191
Downstream Average Slope Sqrt	0.014	0.34	0.016	0.733	0.152	**0.044**	0.197	**0.022**	0.083	0.188
Downstream Max Local Slope	0.043	**0.027**	0.034	0.511	0.180	**0.023**	0.014	0.773	0.244	**0.003**
Upstream Days Rain	0.056	**0.017**	0.003	0.95	0.011	0.815	0.114	0.101	0.501	**0.001**
Upstream Average Slope	0.052	**0.021**	0.006	0.905	0.025	0.629	0.135	0.057	0.475	**0.001**
Upstream Calcium	0.061	**0.004**	0.021	0.659	0.152	**0.040**	0.003	0.934	0.306	**0.001**
Upstream Hardness	0.042	**0.038**	0.043	0.44	0.254	**0.004**	0.036	0.502	0.098	0.130
Upstream Phosphorus	0.064	**0.008**	0.001	0.972	0.005	0.908	0.087	0.177	0.526	**0.001**
Upstream Indigenous Forrest	0.063	**0.009**	0.002	0.95	0.003	0.959	0.113	0.102	0.510	**0.001**
Upstream Native	0.063	**0.009**	0.002	0.948	0.003	0.953	0.117	0.095	0.507	**0.001**
Upstream Pasture	0.050	**0.023**	0.006	0.899	0.032	0.561	0.137	0.055	0.461	**0.001**

Archaea classes										
Sample Time	Annual		March / autumn		July / winter		October / spring		December / summer	
	R^2	p-val	R^2	p-val	R^2	p-val	R^2	p-val	R^2	p-val
Reach Sediment	0.042	**0.041**	0.019	0.722	0.069	0.265	0.158	**0.037**	0.385	**0.002**
Reach Habitat	0.015	0.322	0.003	0.942	0.242	**0.006**	0.018	0.731	0.257	**0.006**
Width in m	0.031	0.091	0.259	**0.009**	0.042	0.456	0.005	0.916	0.060	0.327
Depth in cm	0.017	0.262	0.174	**0.029**	0.010	0.824	0.038	0.492	0.081	0.174
Velocity m/s	0.016	0.303	0.099	0.133	0.034	0.511	0.074	0.234	0.186	**0.016**
Temperature	0.012	0.352	0.028	0.595	0.283	**0.003**	0.041	0.446	0.136	0.078
Conductivity	0.076	**0.006**	0.130	0.066	0.253	**0.012**	0.128	0.061	0.127	0.072
% Fine Sediment	0.035	0.063	0.089	0.153	0.030	0.566	0.002	0.966	0.288	**0.001**
% Riffle	0.072	0.004	0.029	0.551	0.140	0.051	0.004	0.914	0.313	**0.003**
% Run	0.078	**0.002**	0.163	**0.029**	0.115	0.119	0.100	0.134	0.224	**0.01**
% Macrophyte Cover	0.052	**0.02**	0.019	0.731	0.087	0.177	0.212	**0.013**	0.411	**0.001**
% Debris Jam	0.066	**0.005**	0.015	0.733	0.080	0.206	0.025	0.662	0.149	**0.04**

Archaea classes										
Sample Time	**Annual**		**March / autumn**		**July / winter**		**October / spring**		**December / summer**	
	R^2	**p-val**	**R^2**	**p-val**	**R^2**	**p-val**	**R^2**	**p-val**	**R^2**	**p-val**
Periphyton	0.034	0.071	0.026	0.630	0.065	0.286	0.167	**0.031**	0.335	**0.002**
Substrate size	0.052	**0.015**	0.044	0.390	0.115	0.094	0.021	0.670	0.294	**0.002**
Embeddedness	0.080	**0.001**	0.007	0.878	0.208	**0.007**	0.018	0.703	0.314	**0.001**
Stream Shade	0.009	0.501	0.049	0.399	0.075	0.225	0.103	0.128	0.053	0.368

Table 6. PERMANOVA results identifying variables associated with archaeal order communities from each of the eight sites along the Waiotahe river mainstem, one tributary site, and one site in Te Ahiaua, seasonally (e.g., March, July, October, and December) in 2018. **Bold** denotes p-value <0.05%. The variable that best represented the variance in community structure has a red R^2.

Variable	**Df**	***F*-statistic**	**R^2**	***p*-val**
Land use	6	23.43	0.100	**0.001**
Substrate	1	56.56	0.040	**0.001**
Season	3	109.69	0.235	**0.001**
Simpson	1	261.65	0.187	**0.001**
Land use : Substrate	6	11.75	0.05	**0.001**
Land use : Season	18	8.01	0.103	**0.001**
Substrate : Season	3	16.25	0.035	**0.001**
Land use : Simpson	6	10.32	0.044	**0.001**
Substrate : Simpson	1	22.33	0.016	**0.001**
Season : Simpson	3	20.21	0.043	**0.001**
Land use : Substrate : Season	18	6.06	0.078	**0.001**
Land use : Substrate : Simpson	6	2.69	0.012	**0.006**
Land use : Season : Simpson	18	0.76	0.010	0.803
Substrate : Season : Simpson	3	0.97	0.002	0.449
Land use : Substrate : Season : Simpson	18	0.76	0.010	0.789

Table 7. PCoA results, including distances, R^2, and p-values for Archaea orders present in each of the 160 samples collected from each of the eight sites along the Waiotahe river mainstem, one tributary site, and one site in Te Ahiaua, seasonally (e.g., March, July, October, and December) in 2018. **Bold** denotes a p-value <0.05%. The variable that best represented the variance in community structure is red.

Archaea orders										
Sample Time	**Annual**		**March / autumn**		**July / winter**		**October / spring**		**December / summer**	
	R^2	**p-val**	**R^2**	**p-val**	**R^2**	**p-val**	**R^2**	**p-val**	**R^2**	**p-val**
Spatial/Temporal Factors										
Season	0.271	**0.001**								
Land use	0.089	**0.005**	0.293	**0.041**	0.211	0.186	0.405	**0.003**	0.564	**0.001**
Site	0.029	0.091	0.013	0.802	0.249	**0.006**	0.133	0.071	0.193	**0.016**
Substrate	0.025	**0.025**	0.017	0.427	0.250	**0.001**	0.125	**0.005**	0.590	0.114
Metrics										
Richness	0.003	0.786	0.13	0.788	0.051	**0.415**	0.044	0.404	0.115	0.106
Simpson's Diversity Index	0.396	**0.001**	0.065	**0.027**	0.977	**0.001**	0.066	0.285	0.808	**0.001**
Water column *E. coli*/*E. cloacae*	0.532	**0.001**	0.31	0.096	0.24	0.389	0.361	**0.015**	0.555	**0.001**
Sediment *E. coli*/*E. cloacae*	0.397	**0.001**	0.339	0.103	0.235	0.301	0.366	**0.013**	0.414	**0.014**
Environmental Variables										
Jan Air Temp.	0.021	0.203	0.067	0.272	0.235	0.008	0.191	**0.02**	0.239	**0.01**

Archaea orders										
Sample Time	**Annual**		**March / autumn**		**July / winter**		**October / spring**		**December / summer**	
	R^2	**p-val**	**R^2**	**p-val**	**R^2**	**p-val**	**R^2**	**p-val**	**R^2**	**p-val**
Flow	0.078	**0.006**	0.023	0.666	0.235	0.012	0.014	0.773	0.019	0.678
Low Flow	0.080	**0.005**	0.067	0.269	0.247	0.005	0.196	**0.02**	0.243	**0.009**
Flow4th	0.084	**0.003**	0.066	0.274	0.216	0.011	0.187	**0.022**	0.233	**0.012**
Flow Variability	0.091	**0.002**	0.030	0.56	0.021	0.679	0.034	0.545	0.064	0.263
Slope	0.002	0.83	0.109	0.135	0.294	**0.002**	0.023	0.67	0.022	0.681
Slope Sqrt	0.038	**0.039**	0.043	0.456	0.063	0.296	0.203	**0.014**	0.193	**0.018**
Riparian Shade	0.006	0.662	0.011	0.836	0.214	**0.016**	0.010	0.798	0.034	0.542
Historic Shade	0.125	**0.001**	0.042	0.448	0.059	0.319	0.167	**0.032**	0.205	**0.023**
Riparian Native	0.009	0.495	0.003	0.949	0.052	0.416	0.002	0.967	0.020	0.689
Clues N	0.125	**0.001**	0.044	0.437	0.054	0.353	0.163	**0.033**	0.199	**0.026**
Distance to Coast	0.037	**0.042**	0.026	0.635	0.221	**0.015**	0.030	0.544	0.115	0.11
Downstream Average Slope	0.004	0.691	0.064	0.302	0.161	**0.039**	0.200	**0.017**	0.428	**0.001**

Archaea orders										
Sample Time	**Annual**		**March / autumn**		**July / winter**		**October / spring**		**December / summer**	
	R^2	**p-val**	**R^2**	**p-val**	**R^2**	**p-val**	**R^2**	**p-val**	**R^2**	**p-val**
Downstream Average Slope Sqrt	0.005	0.672	0.064	0.296	0.159	**0.039**	0.197	**0.022**	0.424	**0.001**
Downstream Max Local Slope	0.021	0.203	0.023	0.666	0.235	**0.012**	0.014	0.773	0.019	0.678
Upstream Days Rain	0.128	**0.001**	0.030	0.574	0.005	0.909	0.114	0.101	0.147	0.059
Upstream Average Slope	0.129	**0.001**	0.038	0.492	0.017	0.74	0.135	0.057	0.168	**0.044**
Upstream Calcium	0.045	**0.015**	0.019	0.713	0.184	**0.032**	0.003	0.934	0.007	0.87
Upstream Hardness	0.004	0.751	0.056	0.367	0.297	**0.003**	0.036	0.502	0.020	0.697
Upstream Phosphorus	0.122	**0.001**	0.012	0.803	0.007	0.876	0.087	0.177	0.112	0.1
Upstream Indigenous Forrest	0.131	**0.001**	0.022	0.666	0.001	0.979	0.113	0.102	0.142	0.064
Upstream Native	0.132	**0.001**	0.023	0.655	0.001	0.978	0.117	0.095	0.146	0.06
Upstream Pasture	0.127	**0.001**	0.043	0.446	0.022	0.663	0.137	0.055	0.171	**0.041**
Reach Sediment	0.114	**0.001**	0.055	0.361	0.072	0.254	0.158	**0.037**	0.190	**0.031**

Archaea orders										
Sample Time	**Annual**		**March / autumn**		**July / winter**		**October / spring**		**December / summer**	
	R^2	**p-val**	**R^2**	**p-val**	**R^2**	**p-val**	**R^2**	**p-val**	**R^2**	**p-val**
Reach Habitat	0.035	0.065	0.089	0.173	0.151	**0.042**	0.018	0.731	0.049	0.386
Width in m	0.002	0.839	0.234	**0.009**	0.046	0.436	0.005	0.916	0.015	0.75
Depth in cm	0.028	0.107	0.215	**0.01**	0.014	0.795	0.038	0.492	0.107	0.135
Velocity m/s	0.005	0.69	0.080	0.228	0.020	0.686	0.074	0.234	0.202	**0.014**
Temperature	0.009	0.484	0.046	0.44	0.141	**0.044**	0.041	0.446	0.076	0.237
Conductivity	0.042	0.026	0.134	0.064	0.098	0.134	0.128	0.061	0.255	**0.002**
% Fine Sediment	0.051	0.016	0.138	0.053	0.003	0.958	0.002	0.966	0.021	0.683
% Riffle	0.035	0.047	0.028	0.602	0.136	0.072	0.004	0.914	0.032	0.534
% Run	0.074	0.001	0.161	**0.027**	0.037	0.514	0.100	0.134	0.176	**0.02**
% Macrophyte Cover	0.133	**0.001**	0.043	0.437	0.078	0.22	0.212	**0.013**	0.251	**0.008**
% Debris Jam	0.029	0.088	0.013	0.784	0.043	0.44	0.025	0.662	0.022	0.692
Periphyton	0.100	**0.004**	0.040	0.466	0.088	0.175	0.167	**0.031**	0.202	**0.024**

Archaea orders										
Sample Time	**Annual**		**March / autumn**		**July / winter**		**October / spring**		**December / summer**	
	R^2	**p-val**	**R^2**	**p-val**	**R^2**	**p-val**	**R^2**	**p-val**	**R^2**	**p-val**
Substrate size	0.056	**0.006**	0.042	0.457	0.237	**0.011**	0.021	0.670	0.066	0.268
Embeddedness	0.050	**0.012**	0.001	0.992	0.156	0.049	0.018	0.703	0.042	0.429
Stream Shade	0.016	0.272	0.030	0.581	0.039	0.475	0.103	0.128	0.115	0.100

Table 8. PERMANOVA results identifying variables associated with archaeal genera communities from each of the eight sites along the Waiotahe river mainstem, one tributary site, and one site in Te Ahiaua, seasonally (e.g., March, July, October, and December) in 2018. Bold denotes p-value <0.05%. The variable that best represented the variance in community structure has a red R^2.

Variable	**Df**	***F*-statistic**	**R^2**	***p*-val**
Land use	6	20.74	0.101	**0.001**
Substrate	1	61.42	0.050	**0.001**
Season	3	142.08	0.345	**0.001**
Simpson	1	130.03	0.105	**0.001**
Land use : Substrate	6	9.88	0.048	**0.001**
Land use : Season	18	7.12	0.104	**0.001**
Substrate : Season	3	12.25	0.030	**0.001**
Land use : Simpson	6	5.74	0.028	**0.001**
Substrate : Simpson	1	14.85	0.012	**0.001**
Season : Simpson	3	10.64	0.026	**0.001**
Land use : Substrate : Season	18	5.14	0.075	**0.001**
Land use : Substrate : Simpson	6	2.27	0.011	**0.004**
Land use : Season : Simpson	18	0.91	0.013	0.667
Substrate : Season : Simpson	3	1.41	0.003	0.174
Land use : Substrate : Season : Simpson	18	0.77	0.011	0.850

Table 9. PCoA results, including distances, R^2, and p-values for Archaea genera present in each of the 160 samples collected from each of the eight sites along the Waiotahe river mainstem, one tributary site, and one site in Te Ahiaua, seasonally (e.g., March, July, October, and December) in 2018. **Bold** denotes a p-value <0.05%. The metrics that best represented the variance in community structure are red.

Archaea genera										
Sample Time	**Annual**		**March / autumn**		**July / winter**		**October / spring**		**December / summer**	
	R^2	**p-val**	**R^2**	**p-val**	**R^2**	**p-val**	**R^2**	**p-val**	**R^2**	**p-val**
Spatial/Temporal Factors										
Season	0.793	**0.001**								
Land use	0.029	0.658	0.258	0.072	0.221	0.180	0.405	**0.003**	0.492	**0.001**
Site	0.004	0.734	0.002	0.967	0.242	0.140	0.133	0.071	0.213	**0.007**
Substrate	0.016	0.113	0.001	0.972	0.235	**0.002**	0.125	**0.005**	0.231	**0.001**
Metrics										
Richness	0.031	0.07	0.103	0.124	0.071	**0.264**	0.186	**0.028**	0.143	0.089
Simpson's Diversity Index	0.909	**0.001**	0.938	**0.001**	0.983	**0.001**	0.873	**0.001**	0.524	**0.002**
Water column *E. coli*/*E. cloacae*	0.840	**0.001**	0.302	0.112	0.252	0.379	0.361	**0.015**	0.518	**0.001**
Sediment *E. coli*/*E. cloacae*	0.690	**0.001**	0.35	0.073	0.241	0.316	0.366	**0.013**	0.387	**0.008**
Environmental Variables										
Jan Air Temp.	0.018	0.244	0.016	0.746	0.268	**0.002**	0.186	**0.023**	0.409	**0.002**

Archaea genera										
Sample Time	Annual		March / autumn		July / winter		October / spring		December / summer	
	R^2	p-val	R^2	p-val	R^2	p-val	R^2	p-val	R^2	p-val
Flow	0.006	0.256	0.015	0.746	0.256	**0.005**	0.182	**0.025**	0.428	**0.002**
Low Flow	0.019	0.64	0.022	0.659	0.254	**0.008**	0.038	0.474	0.174	**0.023**
Flow4th	0.017	0.268	0.015	0.747	0.236	**0.007**	0.179	**0.027**	0.458	**0.002**
Flow Variability	0.001	0.901	0.011	0.824	0.026	0.599	0.059	0.316	0.626	**0.001**
Slope	0.014	0.364	0.138	0.07	0.377	**0.001**	0.185	**0.023**	0.023	0.671
Slope Sqrt	0.019	0.228	0.024	0.641	0.053	0.383	0.284	**0.004**	0.120	0.089
Riparian Shade	0.001	0.928	0.006	0.911	0.233	**0.01**	0.001	0.982	0.095	0.148
Historic Shade	0.012	0.391	0.007	0.876	0.065	0.282	0.177	**0.038**	0.658	**0.001**
Riparian Native	0.002	0.873	0.007	0.872	0.048	0.409	0.011	0.827	0.212	**0.007**
Clues N	0.012	0.389	0.009	0.856	0.060	0.312	0.177	**0.038**	0.661	**0.001**
Distance to Coast	0.001	0.92	0.016	0.751	0.223	**0.016**	0.065	0.273	0.272	**0.001**
Downstream Average Slope	0.001	0.937	0.098	0.137	0.116	0.098	0.077	0.214	0.202	**0.035**

Archaea genera										
Sample Time	**Annual**		**March / autumn**		**July / winter**		**October / spring**		**December / summer**	
	R^2	**p-val**	**R^2**	**p-val**	**R^2**	**p-val**	**R^2**	**p-val**	**R^2**	**p-val**
Downstream Average Slope Sqrt	0.001	0.952	0.097	0.14	0.115	0.099	0.077	0.213	0.203	**0.032**
Downstream Max Local Slope	0.006	0.64	0.022	0.659	0.254	**0.008**	0.038	0.474	0.174	0.023
Upstream Days Rain	0.007	0.568	0.008	0.881	0.008	0.87	0.144	0.056	0.686	0.001
Upstream Average Slope	0.009	0.472	0.009	0.852	0.021	0.678	0.161	**0.042**	0.688	0.001
Upstream Calcium	0.002	0.843	0.033	0.547	0.214	**0.014**	0.028	0.576	0.229	**0.004**
Upstream Hardness	0.010	0.468	0.063	0.306	0.348	**0.001**	0.033	0.525	0.015	0.759
Upstream Phosphorus	0.004	0.754	0.008	0.875	0.011	0.819	0.112	0.112	0.628	**0.001**
Upstream Indigenous Forrest	0.007	0.587	0.007	0.899	0.003	0.946	0.146	0.061	0.671	**0.001**
Upstream Native	0.007	0.573	0.007	0.899	0.003	0.948	0.150	0.054	0.673	**0.001**
Upstream Pasture	0.010	0.457	0.012	0.82	0.026	0.607	0.163	**0.041**	0.685	**0.001**
Reach Sediment	0.014	0.327	0.014	0.776	0.082	0.204	0.176	**0.037**	0.638	**0.001**

Archaea genera										
Sample Time	**Annual**		**March / autumn**		**July / winter**		**October / spring**		**December / summer**	
	R^2	**p-val**	**R^2**	**p-val**	**R^2**	**p-val**	**R^2**	**p-val**	**R^2**	**p-val**
Reach Habitat	0.002	0.866	0.049	0.417	0.194	**0.02**	0.020	0.703	0.398	**0.002**
Width in m	0.001	0.935	0.324	**0.001**	0.075	0.244	0.029	0.574	0.039	0.482
Depth in cm	0.007	0.611	0.210	**0.024**	0.016	0.751	0.041	0.451	0.231	**0.018**
Velocity m/s	0.001	0.951	0.108	0.12	0.032	0.529	0.019	0.715	0.320	**0.001**
Temperature	0.054	**0.008**	0.194	**0.023**	0.105	0.129	0.020	0.665	0.185	**0.029**
Conductivity	0.012	0.372	0.344	**0.001**	0.055	0.359	0.008	0.908	0.158	0.053
% Fine Sediment	0.008	0.532	0.138	0.055	0.009	0.958	0.060	0.353	0.238	**0.005**
% Riffle	0.001	0.939	0.028	0.675	0.173	0.072	0.052	0.388	0.166	**0.028**
% Run	0.014	0.326	0.161	0.001	0.023	0.514	0.018	0.713	0.190	**0.031**
% Macrophyte Cover	0.015	0.31	0.043	0.89	0.082	0.22	0.220	**0.018**	0.635	**0.001**
% Debris Jam	0.009	0.467	0.013	0.773	0.061	0.44	0.039	0.485	0.038	0.437
Periphyton	0.016	0.285	0.040	0.918	0.094	0.175	0.172	**0.04**	0.514	**0.001**

Archaea genera										
Sample Time	**Annual**		**March / autumn**		**July / winter**		**October / spring**		**December / summer**	
	R^2	**p-val**	**R^2**	**p-val**	**R^2**	**p-val**	**R^2**	**p-val**	**R^2**	**p-val**
Substrate size	0.002	0.841	0.042	0.426	0.270	**0.011**	0.126	0.09	0.208	**0.007**
Embeddedness	0.001	0.923	0.001	0.966	0.163	**0.049**	0.052	0.38	0.293	**0.001**
Stream Shade	0.010	0.481	0.030	0.419	0.031	0.475	0.131	0.077	0.190	**0.009**

Table 10. PERMANOVA results identifying variables associated with archaeal class core biomes from each of the eight sites along the Waiotahe river mainstem, one tributary site, and one site in Te Ahiaua, seasonally (e.g., March, July, October, and December) in 2018. **Bold** denotes p-value <0.05%. The variable that best represented the variance in community structure has a red R^2.

Variable	**Df**	***F*-statistic**	**R^2**	***p*-val**
Core biome richness	1	3.69	0.072	0.083
% of total biome	1	4.25	0.083	0.057
Substrate	2	0.89	0.035	0.476
Water type	2	1.95	0.076	0.197
Season	4	4.86	0.378	**0.011**
Core biome richness : % of total biome	1	1.08	0.021	0.327
Core biome richness : Substrate	2	0.18	0.007	0.933
% of total biome : Substrate	1	0.19	0.004	0.846
Core biome richness : Water type	2	0.08	0.003	0.991
Substrate : Water type	2	0.96	0.046	0.562
Core biome richness : Season	3	0.786	0.046	0.562
Substrate : Season	5	0.19	0.018	0.991
Core biome richness: % total biome : Substrate	1	0.40	0.008	0.658

Table 11. PERMANOVA results identifying variables associated with archaeal order core biomes from each of the eight sites along the Waiotahe river mainstem, one tributary site, and one site in Te Ahiaua, seasonally (e.g., March, July, October, and December) in 2018. **Bold** denotes p-value <0.05%. The variable that best represented the variance in community structure has a red R^2.

Variable	**Df**	***F*-statistic**	**R^2**	***p*-val**
Core biome richness	1	2.70	0.031	0.140
Substrate	2	4.01	0.091	**0.026**
Water type	2	1.67	0.038	0.196
Season	4	6.89	0.313	**0.010**
Core biome richness : % of total biome	1	3.98	0.045	0.074
Core biome richness : Substrate	2	0.95	0.022	0.477
Core biome richness : Water type	2	4.47	0.051	0.068
Substrate : Water type	2	0.82	0.019	0.489
Core biome richness : Season	4	0.56	0.025	0.622
Substrate : Season	4	4.62	0.051	0.084
Core biome richness: % total biome : Substrate	1	0.38	0.009	0.816

Table 12. PERMANOVA results identifying variables associated with archaeal genera core biomes from each of the eight sites along the Waiotahe river mainstem, one tributary site, and one site in Te Ahiaua, seasonally (e.g., March, July, October, and December) in 2018. **Bold** denotes p-value <0.05%. The variable that best represented the variance in community structure has a red R^2.

Variable	Df	*F*-statistic	R^2	*p*-val
Core biome richness	1	13.62	0.127	**0.001**
Substrate	2	5.268	0.098	**0.004**
Water type	2	4.13	0.077	**0.014**
Season	4	14.59	0.544	**0.001**
Core biome richness : % of total biome	1	0.72	0.007	0.533
Core biome richness : Substrate	2	0.04	0.001	1.0
Core biome richness : Water type	2	0.63	0.012	0.679
Substrate : Water type	2	0.31	0.006	0.919
Core biome richness : Season	4	0.03	0.001	1.0
Substrate : Season	4	0.04	0.002	1.0
Core biome richness: % total biome : Substrate	1	0.83	0.015	0.538

Preface to Chapter 6

"What Darwin was too polite to say, my friends, is that we came to rule the Earth not because we were the smartest, or even the meanest, but because we have always been the craziest, most murderous motherfuckers in the jungle."

Cell – Stephen King

6 Microbial community structure reflects and shapes aquatic ecosystems

6.1 Abstract

Microbial communities are the foundation for all life on Earth. Therefore, the structure of these communities is likely to reflect and shape the ecosystems they inhabit. However, as an intact community, understanding the strength of their dominance, their drivers, and the scale of their reach has been underprioritised. This neglect has been magnified in the disparate way microbes are studied, typically in highly specialised domain silos with a predilection for those that interact with multicellular organisms and chemicals. For this reason, few studies capture the importance of shared space, morphology, metabolic fluidity, genetic mobility, and function in their design.

To better understand the drivers of microbial community structure and their functions in lotic aquatic systems, a sophisticated diagnostic toolbox was employed (e.g., shotgun metagenomics, microbiological culturing, polymerase chain reaction -PCR-, and matrix assisted laser desorption ionisation-time of flight mass spectrometry -MALDI-TOF) to study the mainstem, draining a well-defined, rural land use catchment in Te Moana a Toi, Aotearoa. Community structure was strongly related to microbial mobility although spatio-temporal drivers were unable to explain much of the variation observed in entire communities or core biomes. Bacteria currently used as water quality indicators/proxies for faecal contamination were poorly related to each other, to the organisms they are proxies for, or to community structure as a whole. Functional groups were discernible using genomic approaches and reshaping Aotearoa's aquatic environments in response to nutrient and faecal pollution. Further work understanding the relationships between aquatic microbial communities, their drivers, and human pathogens is necessary.

6.2 Introduction

Microbial communities, prokaryotes, archaea, and eukaryotes, are the functional and genetic scaffolding upon which all ecosystems and life on earth are built. They play essential roles in: preserving biodiversity (Lake et al., 2000; Danovaro & Pusceddu, 2007), habitat engineering (Breitburg et al., 2010; Passarelli et al., 2014); population control, through resource limiting (Huryn, 1998; Gandiwa, 2013), fitness moderation (Zilber-Rosenberg & Rosenberg, 2008), and disease mortality (el Bcheraoui et al., 2018); and nutrient cycling (Madsen, 2011; Rousk & Bengtson, 2014). In addition to the services they provide, microbial communities are capable of responding to, and may be a biological record of, environmental fluctuations that

last seconds (e.g., gene activity in response to light (Wang et al., 2011)) and/or decades (e.g., genetic changes related to chronic environmental acidification (Wise, Roane & Mosier, 2020)). In spite of their importance, our understanding of how these important domains are structured is perfunctory at best.

Aquatic ecosystems are receiving environments that support diverse microbial communities (Allan, Castillo & Capps, 2021). These microbial aquatic communities are often the first to respond to in-stream and land use changes (Labbate et al., 2016); their diversity and functionality reflecting the abiotic (e.g., nutrients, pH, salinity, temperature (DeLorenzo, Scott & Ross, 2001; Paerl et al., 2003; Bricheux et al., 2013)) and biotic (e.g., predation and competition (Wanjugi & Harwood, 2013)) pressures present. Furthermore, they are important tools in remediating pollutants, both organic and inorganic (e.g., nutrients, petrochemicals, and plastics (Brooijmans, Pastink & Siezen, 2009; Denaro et al., 2020)). This remediatory process is directly responsible for the observable degradation in aquatic ecosystems suffering eutrophication (Sigee, 2005).

However, few ecological studies of waterways directly address microbial communities, choosing to instead focus on measuring macroscopic responses to anthropogenic induced changes (e.g., algal blooms (Mishra & Mishra, 2012), fish populations (Pont, Hugueny & Rogers, 2007; Joy, 2015), or macroinvertebrate communities (Stark, 1993)). For this reason, only the most pronounced microbial responses to ecosystem degradation are well documented (Paerl et al., 2003). This leaves our understanding, of the less obvious but equally pervasive effects anthropogenic intrusions (e.g., deforestation, land use changes, medical waste, and chemicals (Lee et al., 2016; Effendi, Nedi & Pakpahan, 2017)) may have on lotic and benthic microbial communities, lacking. Specifically, there is a dearth of research with an authentic multidisciplinary lens (e.g., use of ecological, molecular, and/or microbiological techniques and systems thinking (Winkworth, 2013; Meziti et al., 2016; Gray et al., 2021)).

Understanding microbial communities is complicated by their size and versatility; they are microscopic yet containing thousands of poorly differentiated and undescribed species (Stackebrandt, 2006; Doolittle & Zhaxybayeva, 2009; Woodward, Gray & Baird, 2013). Microbial diversity is enhanced by an inherent functional mutability, with many able to change metabolic pathways in response to environmental pressures (Fuhrman, Cram & Needham, 2015; Rodriguez-R et al., 2020; Oren & Garrity, 2021) and a penchant for gene sharing (e.g., resistance, toxin production, metal resistance, and/or virulence genes) (Liu et al., 2010; Van Etten & Bhattacharya, 2020; Haverkamp et al., 2021). Though poorly

understood, the drivers and frequency of sharing genetic material between microorganisms are decidedly important in aquatic systems (Van Etten & Bhattacharya, 2020), notably for those inhabiting environmental interfaces (e.g., terrestrial:aquatic (Tytgat et al., 2014; Wilhelm, 2018), saline:freshwater (Chen et al., 2019; Neubauer et al., 2019), light:shade (Sulzberger et al., 2019), and water column:sediment (Logares et al., 2009; Wang et al., 2020)). Factor in the extensive range of potential interactions between microbes (Ponce-Soto et al., 2015; Feichtmayer, Deng & Griebler, 2017; Bižić-Ionescu, Ionescu & Grossart, 2018), between microbes and their environment (Perkins et al., 2014; Feichtmayer, Deng & Griebler, 2017), and the intricacies associated with lotic systems – multiple environmental and biological interfaces in a state of constant flux (e.g., air:land:water – fresh/brackish/salt – plants:other animals (Marques et al., 2006; Neubauer et al., 2019)) – and it's unsurprising that our understanding of these complex communities is – at best – superficial.

Identifying and ensuring key microorganisms and extra-domain dependencies associated with ecosystem functioning are conserved in aquatic systems has become imperative. While reducing future ecosystem failures is critical, the quality and quantity of water are important now in supporting and maintaining both terrestrial and aquatic biodiversity (Knapp et al., 2017), ecosystem functioning (Hjalten et al., 2016), and for both human and animal health (Daszak et al., 2020; Almeria, Robertson & Santin, 2021; Richards et al., 2021).

Aquatic 'ecosystem/ecological health', a magniloquent way of describing functionality, attempts to quantify how capable a system is of supporting the communities reliant on it by quantifying its ability to perform the processes expected of it (Shrader-Frechette, 1994; Ross et al., 1997; Lancaster, 2000; Howard, 2004). 'Ecosystem health' measures are often skewed towards conserving anthropocentric benefits and are calculated by measuring multiple observable elements in the system, compiling them, like evidence, into a final statement of health, generally a single number (Scrimgeour & Wicklum, 1996; Kleynhans, 1999; Weigel, Henne & Martinez-Rivera, 2002). This is problematic because 'ecosystem/ecological health' measures assume microbial ecosystem services are intact without quantifying what that entails or how to identify dysregulation.

This is especially pertinent to lotic, benthic sediment communities of which very little is known but ecosystem integrity relies upon (Hauer et al., 2018; Boeraş et al., 2021). Microbial sediment communities are equivalent to the gut-brain axis in humans. Although both structure and function are necessary to retain ecosystem integrity, many anthropogenic 'management' strategies for lotic systems (e.g., dredging, streambed gravel mining, and damming) undermine and disrupt benthic microbial communities responsible for maintaining

rivers' natural tendency towards balance and health (Hauer et al., 2018; Boeraş et al., 2021). Benthic microbial communities are the primary in-stream bioremediators of anthropogenic pollutants and responsible for the majority of riverine respiration (Craft, Stanford & Pusch, 2002; Coll et al., 2020).

Pivotal studies using genomics are beginning to fill these knowledge gaps by identifying key functional species (Zhang et al., 2019; Chen et al., 2020). These studies also lead the discourse on the confluence of function, morphology, and hierarchy in microbial communities (DeLong et al., 2006; Raes et al., 2011; Louca, Parfrey & Doebeli, 2016). Studies, focused on the effects of nutrient enrichment, have linked eutrophication to homogenisation and increased β-diversity of microbial communities by favouring generalist species (Geng et al., 2022). Eutrophication, through nitrogen loading, has also been associated with increased detection of ammonia oxidising archaea and bacteria in receiving rivers; with bacteria being more adaptable, diverse, and abundant than archaea (Cai et al., 2022). These studies are important because nitrate pollution is significant issue affecting waters in Aotearoa and globally (Parliamentary Commissioner for the Environment, 2013; Gluckman, 2017; Richards et al., 2021). How these studies may relate to Aotearoa's unique aquatic environments (e.g., ecosystem functioning capacity and in-stream metabolism) and microbial communities is as yet, underexplored.

As a geographically isolated island nation, Aotearoa's indigenous life and ecosystems are unique and vulnerable to environmental changes (Young, Townsend & Matthaei, 2004; Stewart-Harawira, 2020) and exotic invaders (Wallis & Trewick, 2009); plants (Kelly & Hawes, 2005), animals (Tempero et al., 2006), and microbial (Beville, Kerr & Hughey, 2012; Champion, 2018). For this reason, the transition in primary land cover from forest to pastoral cultivation impacts all of Aotearoa's indigenous life. Rural waterways in Aotearoa are subjected to altered hydrology (e.g., increased over land flow and drainage) and diffuse pollutants (e.g., excess nutrients, herb– and pesticides, effluents, sediments, and solid wastes (Duncan, 2017; Gluckman, 2017; Monaghan et al., 2021)) that are likely affecting aquatic microorganisms (Bryan & Hea, 2017; Kurenbach et al., 2018). That the majority of Aotearoa's freshwater degradation has been attributed to changes in land use, towards exotic agriculture, is consistent with her inherent vulnerability (Larned et al. 2016; Peterson et al. 1993; Monaghan et al. 2007).

In Aotearoa, freshwater 'ecosystem health' is declining despite decades of work spent developing assessments and implementing them into policy (Weeks et al., 2015; Gluckman,

2017; Knight, 2019). One reason for this may be that human health measures for water quality are routinely disassociated from ecosystem health assessments and unrecognised as representative of ecosystem degradation. It is common knowledge that degraded environments, including rivers, are a precursor to infectious disease (re)emergence (Daszak et al., 2020). However, how or if the measures we use to quantify the risk of contracting a waterborne disease (e.g., faecal indicator bacteria such as *E. coli* concentrations) relate to environmental health and ecosystem functioning is less clear.

Therefore, the aim of this study was to investigate three domains (archaea, bacteria, and microbial eukaryotes) of aquatic microorganisms through a multidisciplinary lens, focusing on two aquatic substrates (i.e., water and superficial benthic substrates), from a rural mixed land use catchment in Aotearoa to identify key community drivers. To achieve this, a longitudinal study along a source to sea continuum of the Waiotahe river, in Te Moana a Toi, Aotearoa, was carried out with a holistic, One Health approach (e.g., that views the health of the environment, animals, and humans as interdependent (Zinsstag et al., 2020; One Health High-Level Expert Panel (OHHLEP), 2022)). A longitudinal, vertical and horizontal study of a waterway intended to provide a 3 dimensional picture.

Due to the varied land uses present in the catchment and lotic nature of rivers, microbial inputs were expected to be composites, building in complexity from the headwaters to Te Ahiaua. That said, microbial community structure was expected to be influenced more strongly by land adjacent to the reaches sampled. Allochthonous microbial contributions were likely to be associated with: animals including, insects, domesticated and non-domesticated mammals (e.g., rats, mice, stoats, possum, cats, dogs, pastoral livestock, and deer), native and introduced avifauna, and humans; soil communities; and riparian vegetation communities.

6.3 Materials and Methods

6.3.1 Background

See 5.3.1 for background details.

6.3.2 Study Location

See 5.3.2 for study location, site map, and site details (Figure 5.1, Table 5.1).

6.3.3 Sample Collection

See 5.3.3 for sample collection details.

6.3.4 Sample Processing for Bacterial Culturing

See 5.3.4 for sample processing and bacterial culturing methods.

6.3.5 Bacterial Culturing and Colony Identification

See 5.3.5 for bacterial culturing and colony identification methods.

6.3.6 Sample Processing for Molecular Testing

See 5.3.6 for sample processing for molecular testing methods.

6.3.7 Metagenomic Preparation and Sequencing

See 5.3.7 for metagenomic preparation and sequencing methods.

6.3.8 Quality Control and Analysis of Sequences

See 5.3.8 for quality control and sequence analysis methods.

6.3.9 Physical Characteristics

See 5.3.9 and CH 5 Appendix 1Table 1 for physical characteristics.

6.3.10 Molecular Testing for Target Genes

See 5.3.10 for molecular testing for target genes methods and Table 5.2 for details on the primers used.

6.3.11 Data Analyses

All archaea, bacteria, and microbial eukaryote names were based on Kaiju's outputs, not necessarily the latest nomenclature. All statistical analyses were performed in R using Bray-Curtis distances (R Core Team, 2013). Because microbial communities are not normally distributed in either the water column or sediment, non-parametric tests were used for all analyses. *E. coli* concentrations were visualised using the ggplot2 package (Wickham, 2016). A permutational multivariate analysis of variance (PERMANOVA) using adonis2() in vegan (Oksanen *et al.*, 2007) identified significant explanatory variables for the observed differences in the GC content of the eDNA.

Metadata were merged by season across three taxonomic levels (e.g., class, order, and genus) for three microbial domains (e.g., archaea, bacteria, and microbial eukaryotes). Rarefactions

were plotted for each domain at the three taxonomic levels to ensure taxonomic richness was not a result of sampling effort. To correct for any bias introduced by the variation in total read numbers for each sample, (a problem inherent to metagenomic methodologies), relative abundances (i.e., the proportion of the total sample each taxa comprised) were calculated and used in place of read numbers for all taxonomic metagenomic analyses. In addition to using relative abundances, any taxa representing less than 0.0001 (i.e., 0.01%) of the total abundance were removed from the metadata prior to statistical analysis. Due to the high numbers of taxa and low relative abundances data was restricted to taxa with relative abundances $\geq$1% of the total for taxonomic richness heatmaps.

Relationships between faecal indicator bacteria (e.g., *E. coli* water column concentrations from cultures, and *Enterococcus*, *Escherichia*, and *Enterobacter* relative abundances from metagenomics), *Campylobacter* relative abundances, and spatio-temporal variables were explored with PERMANOVA and generalised linear models (GLM; Poisson response). PCR results for the presence/absence of STEC genes was compared to metagenomic results by querying the metadata for any STEC strains identified by Kaiju from all of the 160 eDNA results. Beta-diversity (e.g., Simpson's Diversity Index) and α-diversity were compared between domains, sites, and taxonomic levels. Heatmaps illustrating key compositional differences in microbial community structure for the three phylogenetic levels were plotted with the gplots package (Warnes et al., 2015). Dendrograms were projected onto the opposite/parallel axis of each heatmap (e.g., samples on the y-axis and taxa on the x-axis). Spatio-temporal drivers of community structure at each taxonomic level were identified using PERMANOVAs. Community dissimilarities and their relationships to the 48 cultivated variables were illustrated using Principal Coordinates Analysis (PCoA), for both annual and seasonal patterns, using the vegan package (Oksanen *et al.*, 2007) and ggplot2 (Wickham, 2016). Variables with significant (p-value <0.05) explanatory strength ($R^2 \geq 0.6$) were projected onto the PCoA plots.

Core biomes, all taxa present in every sample for a particular sample type, were constructed for each of the three taxonomic levels. Core biomes were defined by limiting the habitats in a stepwise manner (e.g., all freshwater samples, freshwater sediment samples, freshwater sediment samples in spring, freshwater sediment samples in spring from dairy sites) through the spatio-temporal parameters (e.g., land use, substrate, season, and water type). Because there were fewer estuarine samples, seasonal patterns were not investigated for Te Ahiaua. Core biomes were assessed individually (e.g., by domain) and treated as an interdependent to

capture variation that may have been lost as a result of low relative abundances. Core biomes were not constructed for site as differentiation by land use provided sufficient resolution. PERMANOVAs were also used to identify significant explanatory factors for core biome community variation (e.g., water type, season, substrate, percent of the whole biome, and core biome richness). Heatmaps using core biome Z-Scores, to highlight each taxa's relationship to the average relative abundance and key core biome differences, were plotted in gplots (Warnes et al., 2015). Dendrograms were projected onto the opposite/parallel axis of each heatmap for core biome types (y-axis) and taxa (x-axis). Core biome heatmaps contained all taxa $\geq$1% of the total relative abundance.

6.4 Results

6.4.1 Bacterial Culturing and Colony Identification

See 5.4.1 for bacterial culturing and colony identification results.

6.4.2 eDNA Characteristics and Quality Control

See 5.4.2, Figure 5.2, and CH 5 Appendix 1 Table 2 for details on the eDNA characteristics and quality control.

The guanine-cysteine (GC) content of the eDNA was consistently higher in sediment than in water (PERMANOVA: Substrate $F_{(1)} = 2.244$, $R^2 = 0.538$, p-value = 0.001); across seasons and sites (Figure 6.1).

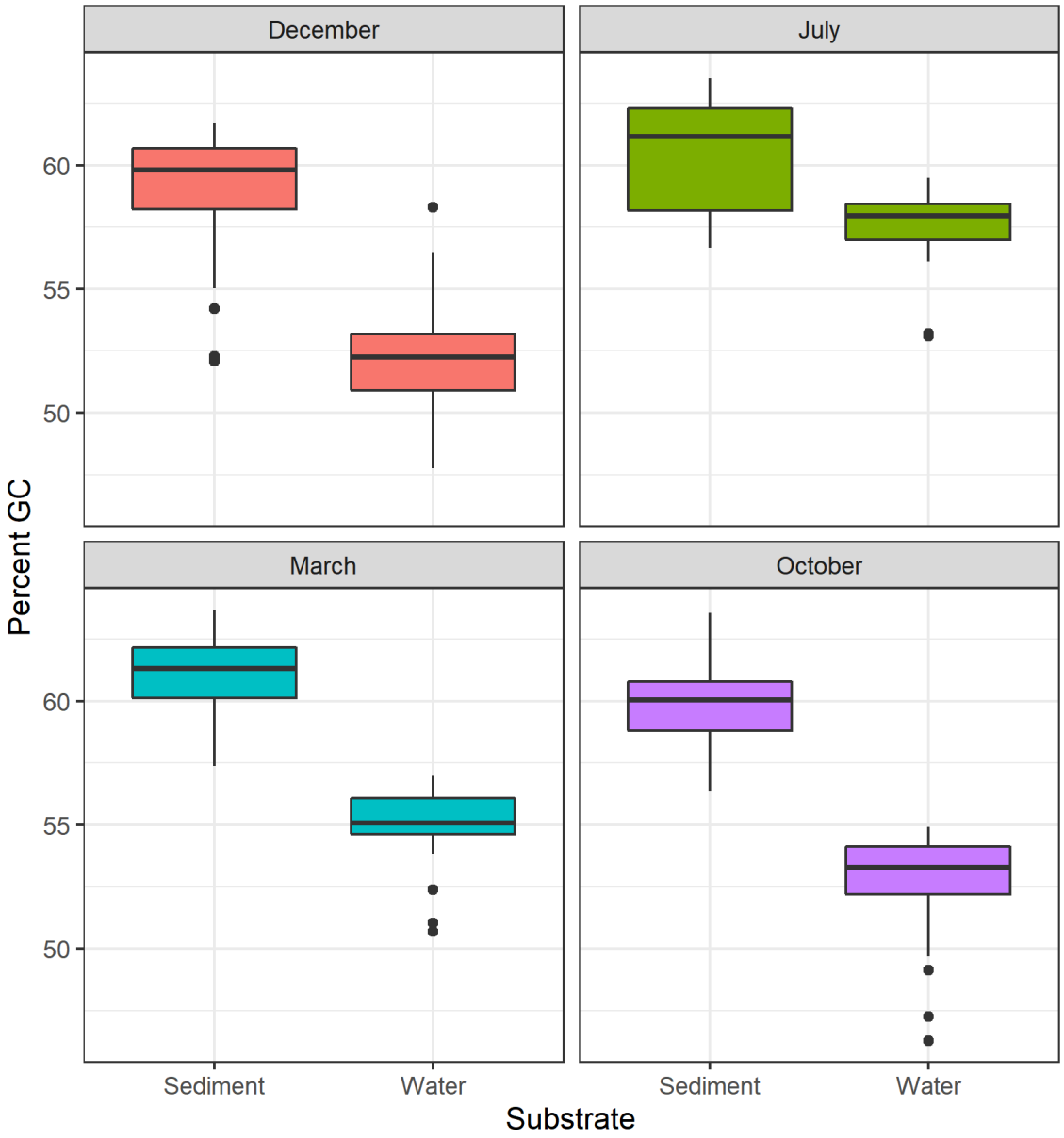


Figure 6.1 Differences in the eDNA GC content between substrates by season. Differences in the eDNA GC content of the samples by substrate from each of the eight sites along the Waiotahe river mainstem, one tributary site, and one site in Te Ahiaua, seasonally (e.g., autumn, winter, spring, and summer) in 2018.

Rarefaction curves for each domain indicated that all taxa present in a sample for bacteria were identified within the first ~200,000 reads and archaea within 10,000 reads; within the minimum read number range both domains achieved in every sample (CH 6 Appendix 1 Figure 1). In contrast, microbial eukaryotes were sensitive to sample size and increased read numbers were associated with increased taxonomic richness.

The 160 samples provided 708,850,416 demultiplexed sequences and ranged from 599,838 sequences (summer's water at site 10) to 13,561,532 sequences (spring's water at site 7), with a mean of 4,419,877 sequences per sample (CH 5 Appendix 1 Table 3). Between 39.8% (spring's water at site 2) and 88.7% (autumn's water at site 2) of the raw reads, with a mean of 76.7%, were identified as cellular organisms (i.e., archaea, bacteria, or microbial eukaryotes). A total of 545,464,573 reads were identified from the samples, ranging from 488,940 (summer's water from site 10) to 10,885,587 (spring's water at site 7) with a mean of 3,409,153 OTUs. From these, there were 38,047 unique operational taxonomic units identified across the three domains (i.e., 38,047 differentiated and characterised taxa across the three taxonomic levels).

6.4.3 Faecal Indicator Bacteria relationships

E. coli/*E. cloacae* water column concentrations were best explained by season followed by land use (PERMANOVA: Season $F_{(4)} = 95.48$, $R^2 = 0.282$, p-value = 0.001; PERMANOVA: Land use $F_{(6)} = 30.15$, $R^2 = 0.178$, p-value = 0.001) (Table 6.1). *Campylobacter*, *Enterococcus*, and *Enterobacter* were significantly associated with water column *E. coli*/*E. cloacae* group concentrations; however, neither *Campylobacter*, *Enterococcus*, nor *Enterobacter* explained any of the variation in water column *E. coli*/*E. cloacae* group concentration. Additionally, neither *Enterobacter* or *Enterococcus* relative abundances in Te Ahiaua related to each other, to *Campylobacter* relative abundances, or water column *E. coli*/*E. cloacae* group concentration (CH 6 Appendix 1 Table 1).

Table 6.1 PERMANOVA results for explanatory variables associated with the *E. coli*/*E. cloacae* water column concentrations of the 160 eDNA samples from each of the eight sites along the Waiotahe river mainstem, one tributary site, and one site in Te Ahiaua, seasonally (e.g., autumn, winter, spring, and summer) in 2018. **Bold** denotes p-value <0.05%. Variables that best represent the variance in community structure have a red R^2.

Water Column *E. coli*/*E. cloacae* concentration	**Df**	***F*-Statistic**	**R^2**	***p*-val**
Campylobacter	1	21.38	0.021	**0.001**
Enterococcus	1	8.91	0.009	**0.001**
Enterobacter	1	47.58	0.047	**0.001**
Land use	6	30.15	0.178	**0.031**
Substrate	1	0.21	0.000	0.946
Season	3	95.48	0.282	**0.001**

6.4.4 Microbial Community Structure and Metrics

Most identified cellular organisms were bacteria (81 – 98%, mean=93.9%), with eukaryotes the next most abundant domain (0.7 – 12, mean=2.4%), and archaea the least abundant (0.2 – 5%, mean=0.8%) (CH 5 Appendix 1 Table 3). Beta-diversity was consistently highest in eukaryotes except in spring classes where bacteria were higher (Figure 6.2). Beta-diversity was best explained by domain and phylogenetic level (CH 6 Appendix 1 Table 2). Alpha-diversity increased in importance to community structure as phylogenetic level decreased and was highest in bacteria followed by eukaryotes then archaea at the class and genus level; however, α-diversity for bacteria and eukaryote order communities was comparable (CH 6 Appendix 1 Figure 2 and Figure 3). Alpha-diversity was not associated with any single substrate, site or land use across the phylogenetic levels or seasons.

Class α-diversity was highest in bacteria (mean=92, range: 88-95), eukaryotes had the widest range (mean=76.13, range: 63-91), and archaea had the lowest diversity and smallest range (mean=16.46, range: 13-17) (CH 6 Appendix 1 Figure 2). Bacteria orders were tightly distributed (mean=198, range: 191-199) and eukaryote orders were similar in abundance to bacteria, except at sites 6-10 in spring where α-diversity of eukaryotes was higher than of bacteria, but wide ranging (mean=206.7, range: 153-281). Archaea orders had the narrowest range and richness (mean=27, range: 24-28). Alpha-diversity for genera was highest in bacteria (range: 2,341-2,647, mean=2601), most varied in eukaryotes (range: 515-1,055, mean=724), and lowest and least variable in archaea (range:24-28, mean=27.81).

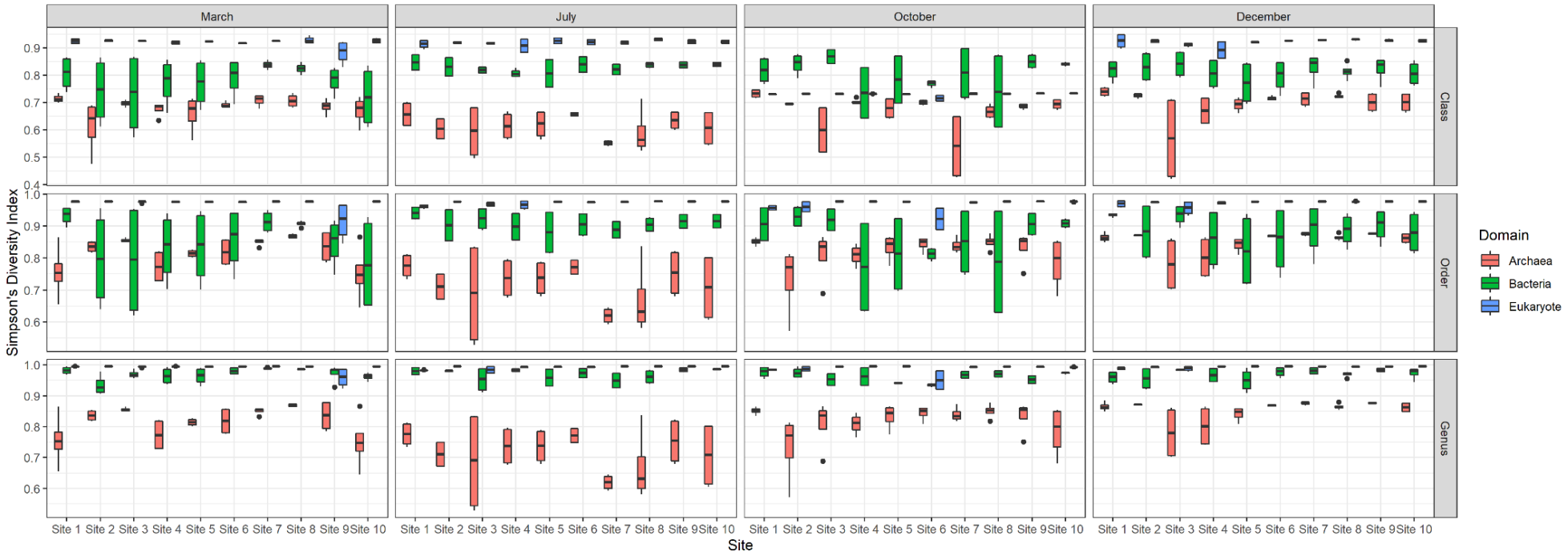


Figure 6.2 Seasonal Simpson's Diversity Index for the three microbial domains.

Simpson's Diversity Index for the three microbial domains at class, order, and genus for from each of the eight sites along the Waiotahe river mainstem, one tributary site, and one site in Te Ahiaua, seasonally (e.g., autumn, winter, spring, and summer) in 2018.

Class community composition was best explained by substrate (PERMANOVA: Substrate $F_{(2)} = 1125.38$, $R^2 = 0.265$, p-value = 0.001) (CH 6 Appendix 1 Table 3). The most abundant classes were Actinobacteria along with A--, B-, and Gammaproteobacteria. Key taxa, those that showed atypical localised or sustained, concentrated peaks in relative abundance, included Clostridia, Flavobacteriia, Deltaproteobacteria, and Cytophagia (Figure 6.3). Flavobacteriia and Cytophagia were abundant in autumn and summer water with additional localised peaks in summer estuarine water. Additional abundance peaks for Cytophagia occurred in water from Te Ahiaua in spring and again in dairy water in summer. Alphaproteobacteria prevalence was highest in freshwater sediments and estuarine water. Overall, Actinobacteria had higher abundances in sediment with localised peaks from intensive dairy and dairy (site 4) sediments in winter and effluent station water in spring. The highest abundances of Gammaproteobacteria were indicative of saline environments, across the year with water having higher abundances than sediment, and intensive dairy and dairy water in winter. Freshwater samples had higher abundances of Betaproteobacteria, differentiating them from sediment and saline samples.

The typically low abundances of Clostridia were slightly raised in exotic forest sediments in winter, effluent station water in spring, and intensive dairy sediments in autumn. Though Clostridia's abundance peaks were smaller than the other key taxa, the pattern was worth noting. In sediment, summer abundances were typical at site 6 (beef and sheep) but with the advance of each season abundances increased to peak in spring. In the spring, sediment from sites 3 (intensive dairy) and 9 (native forest) had a moderate increase in Clostridia abundance; however, water levels at these sites reached the highest abundances observed. A slightly muted, inversion of this pattern (e.g., sediment abundance was higher than water) was observed at site 7 (exotic forest). At the same time, both substrates from the upper native forest site had a moderate increase in abundance and site 6 (beef and sheep) substrates both achieved peak Clostridia abundance.

Important environmental factors related to class community composition included conductivity, temperature, downstream slope (e.g., average and average square root), and the percent of the reach in run (CH 6 Appendix 1 Table 4). Te Ahiaua's communities were associated with higher temperatures and conductivity, except in winter. Downstream slope was a significant driver in spring, while the percent of reach in run was an additional driver for summer communities. Taxonomic richness was higher in winter.

Within site variation was typically lower than between sites within substrates (e.g., water from site 10 was more alike than water from site 3 or site 10 sediment), particularly in winter

and spring samples (Figure 6.4). In autumn and summer, freshwater samples from both substrates were more variable, particularly in water. Water and sediments from Te Ahiaua were well differentiated from the freshwater samples, except in winter when the intensive dairy sediments were more similar to estuarine samples than to other freshwater samples.

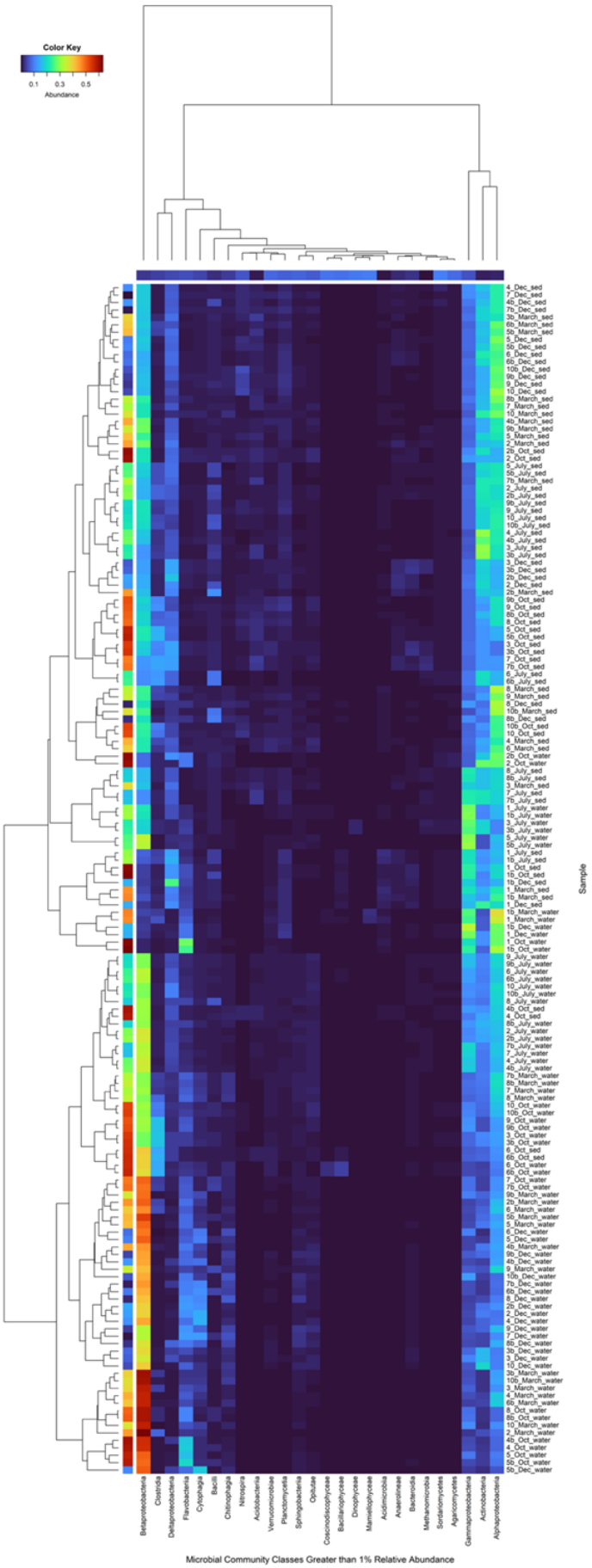
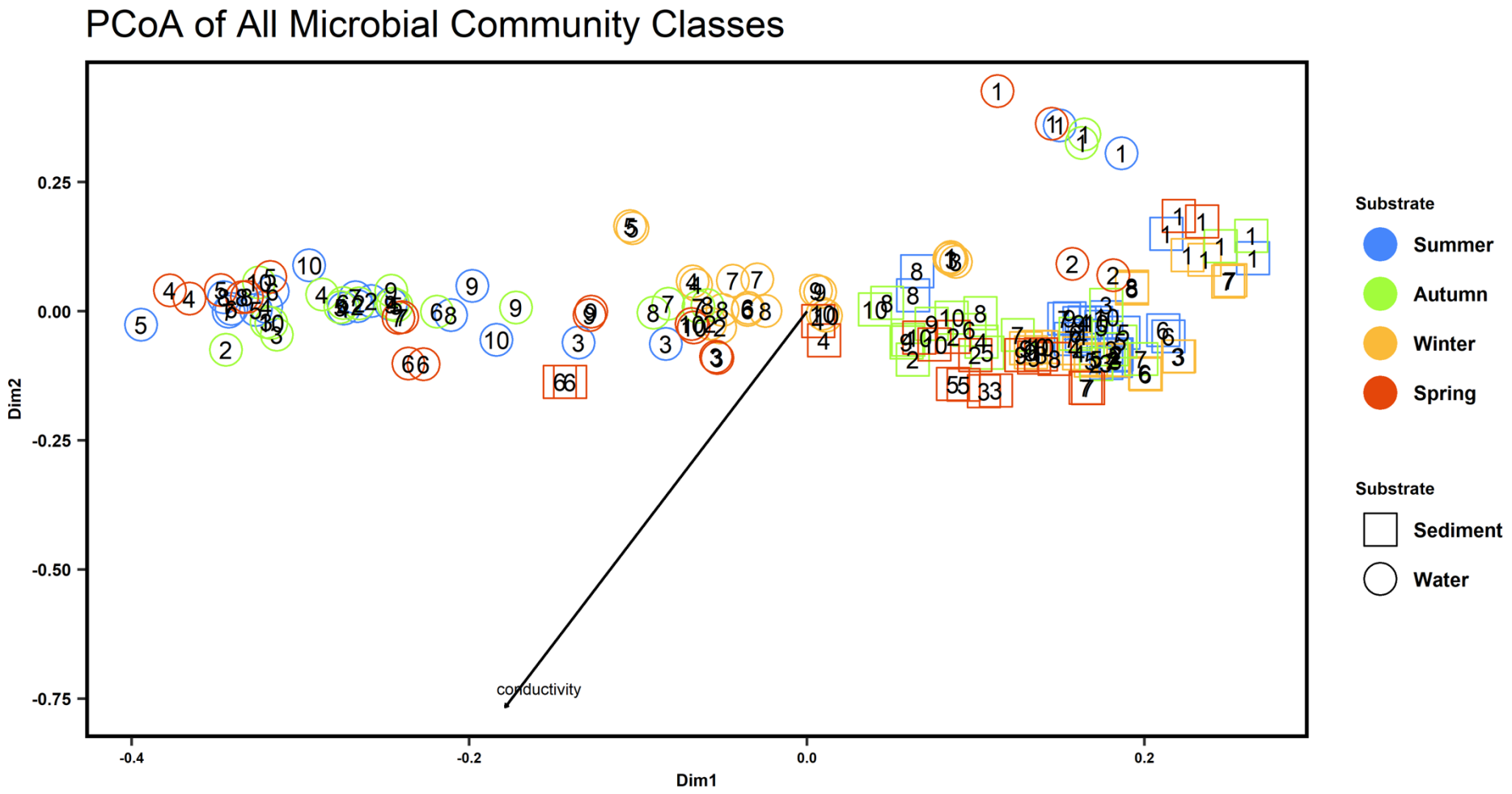


Figure 6.3 Heatmap of all microbial classes. Heatmap depicting all identified microbial classes, with relative abundances ≥1%, present in the 160 eDNA samples collected from each of the eight sites along the Waiotahe river mainstem, one tributary site, and one site in Te Ahiaua, seasonally (e.g., autumn, winter, spring, and summer) in 2018. Dendrograms, representing hierarchical clustering using Bray-Curtis dissimilarity, were projected onto the opposite/parallel axis for each sample (y-axis) and taxa (x-axis).


PCoA of All Microbial Community Classes
0.25
0.00
-0.25
-0.50
-0.75
Dim2
-0.4
-0.2
0.0
0.2
Dim1
conductivity
Substrate
Summer
Autumn
Winter
Spring
Substrate
Sediment
Water

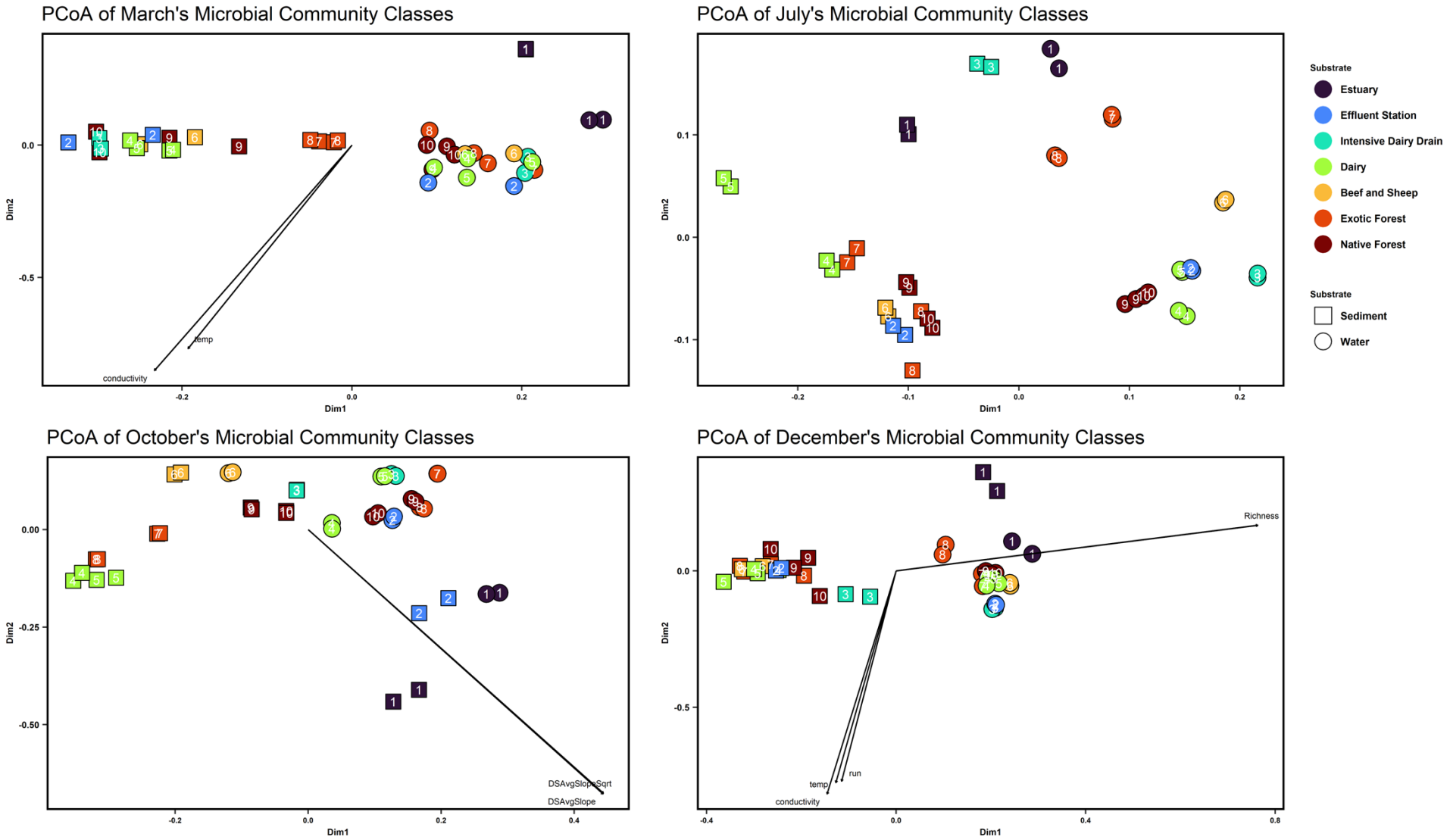


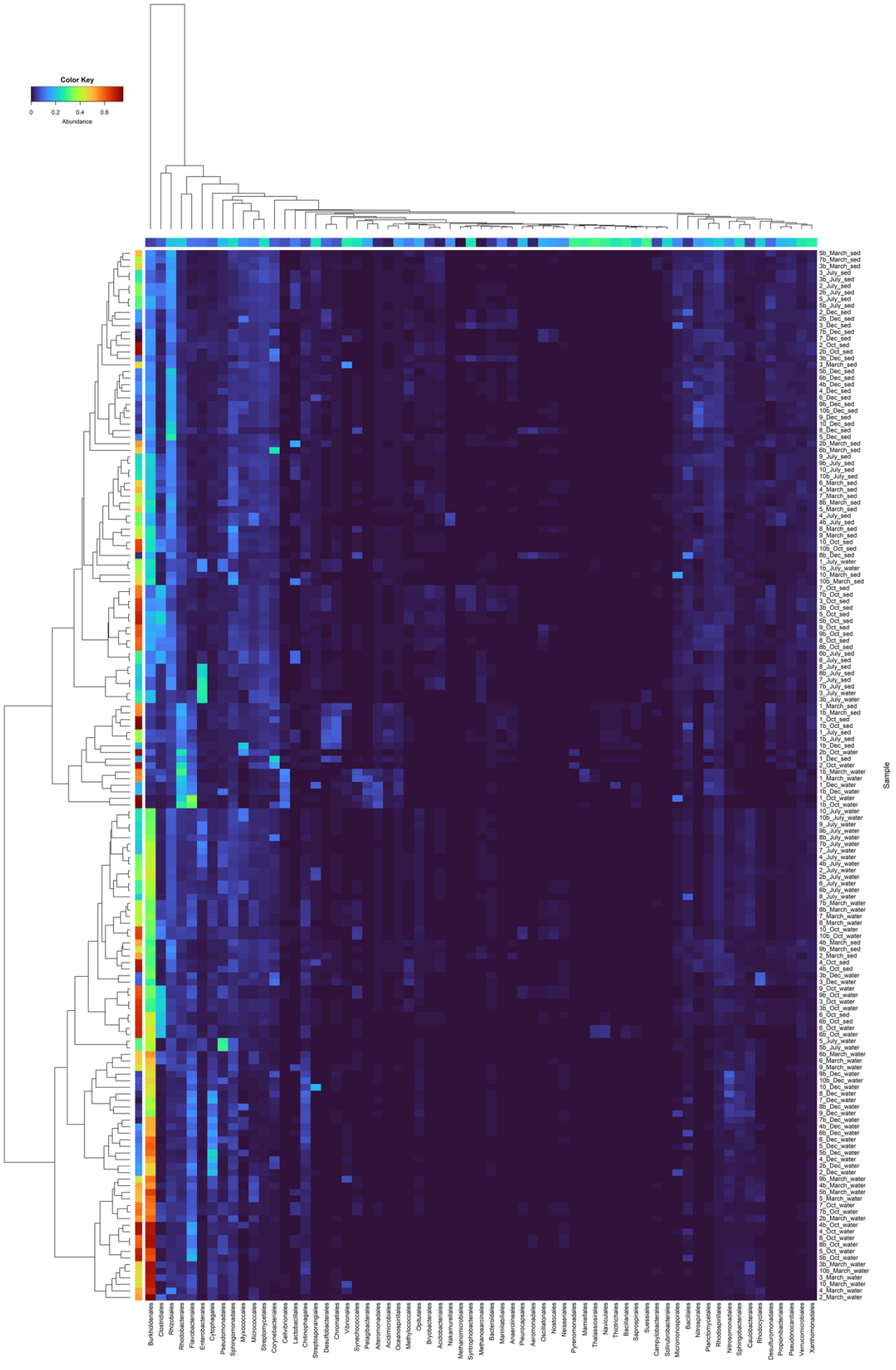
Figure 6.4 Class principal coordinates analysis, as an annual composite and by season.

Principal coordinates analysis, as an annual composite and by season (e.g., autumn, winter, spring, and summer), of microbial class communities with ≥0.1% relative abundance from the 160 eDNA samples collected from each of the eight sites along the Waiotahe river mainstem, one tributary site, and one site in Te Ahiaua, in 2018. Variables and metrics with an R^2 ≥0.6 and p-value <0.05 are projected onto the plots.

Although site, substrate, and some seasonal metrics (e.g., Simpson's in spring, *E. coli*/*E. cloacae* in autumn's waters, *E. coli*/*E. cloacae* in summer's sediments) were significant to microbial order community structure, they explained little of the variation (CH 6 Appendix 1 Table 5 and 6). Downstream slope (i.e., average and average square root) across the year and low flow in spring and summer best explained community differences for microbial orders. Though important for classes, temperature and conductivity were not strongly associated with orders.

Burkholderiales, Clostridiales, Rhodobacterales, Rhizobiales, and Flavobacteriales were the most abundant orders (Figure 6.5). Key orders included Pseudomonadales, Enterobacterales, Sphingomonadales, Corynebacteriales, Myxococcales, Streptosporangiales, Vibrionales, and Cytophagales. Samples from Te Ahiaua routinely had lower abundances of Burkholderiales with a concurrent rise in Rhodobacterales and Flavobacteriales abundance. There were only two exceptions to this, summer sediment from Te Ahiaua where increased abundances of either Corynebacteriales or Myxococcales overtook Rhodobacterales and spring water from the effluent station where Corynebacteriales or Myxococcales abundances increased but Rhodobacterales remained the dominant taxa. Enterobacterales abundances had a distinct peak in winter in intensive dairy water and exotic forest sediments. Winter water from the upper dairy site (site 5) was characterised by high Pseudomonadales abundance, and sediment from the beef and sheep site (6) in autumn had a localised increase in Corynebacteriales. Vibrionales were the dominant taxa from one intensive dairy sediment in autumn and had localised autumn peaks in native forest and one estuarine sample.

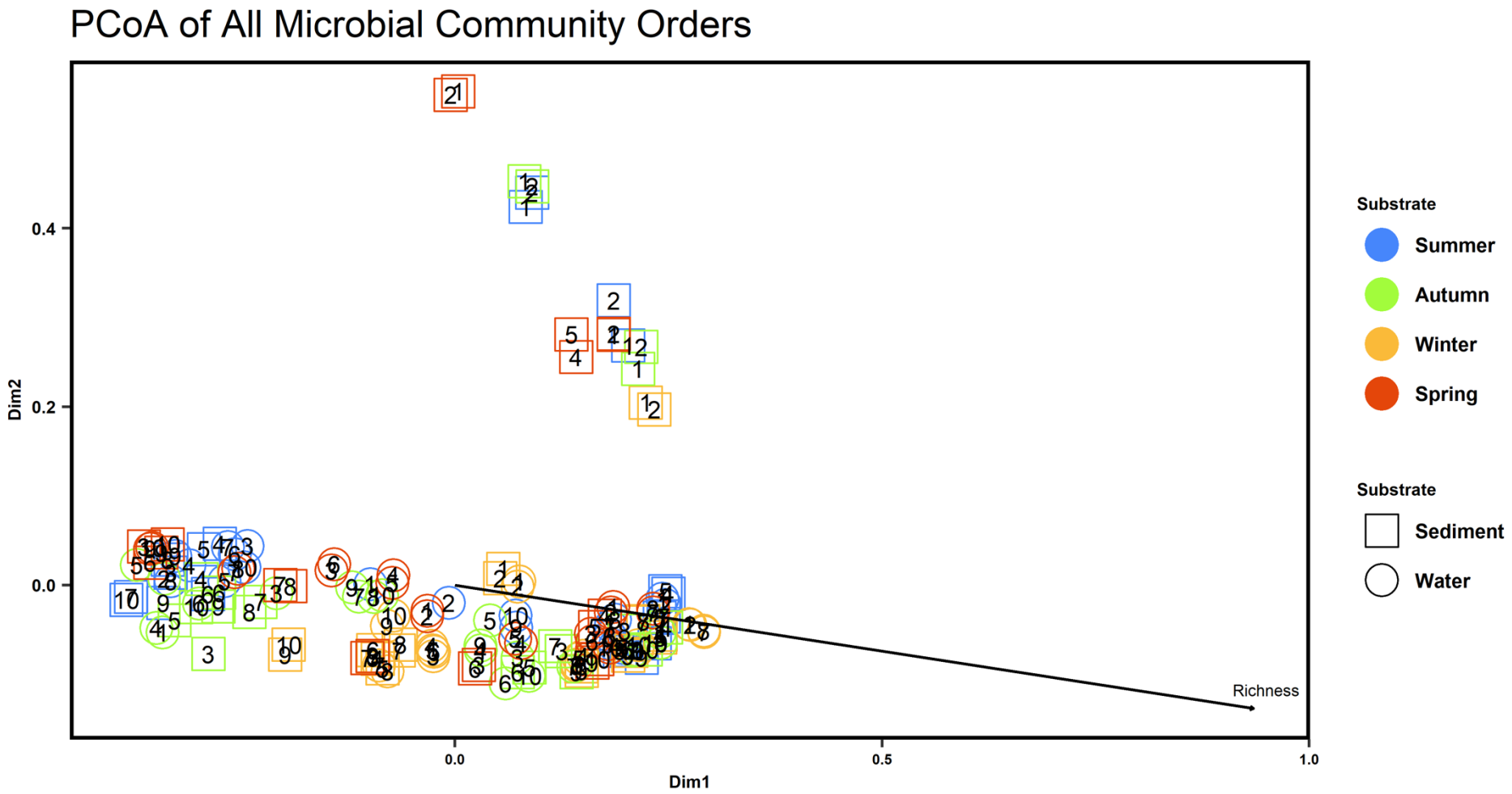
Variation within and between sites increased slightly for microbial orders, however land use/site grouping was still observable in winter and spring (Figure 6.6). Additionally, Te Ahiaua and the effluent station samples were more similar for orders than classes.


Color Key
0
0.2
0.4
0.6
Abundance
Sample
Microbial Community Orders Greater than 1% Relative Abundance

1

Figure 6.5 Heatmap of all microbial orders.

Heatmap depicting all identified microbial orders, with relative abundances ≥1%, present in the 160 eDNA samples collected from each of the eight sites along the Waiotahe river mainstem, one tributary site, and one site in Te Ahiaua, seasonally (e.g., autumn, winter, spring, and summer) in 2018. Dendrograms, representing hierarchical clustering using Bray-Curtis dissimilarity, were projected onto the opposite/parallel axis for each sample (y-axis) and taxa (x-axis).


PCoA of All Microbial Community Orders
Dim2
0.4
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1.0
Dim1
Richness
Substrate
Summer
Autumn
Winter
Spring
Substrate
Sediment
Water

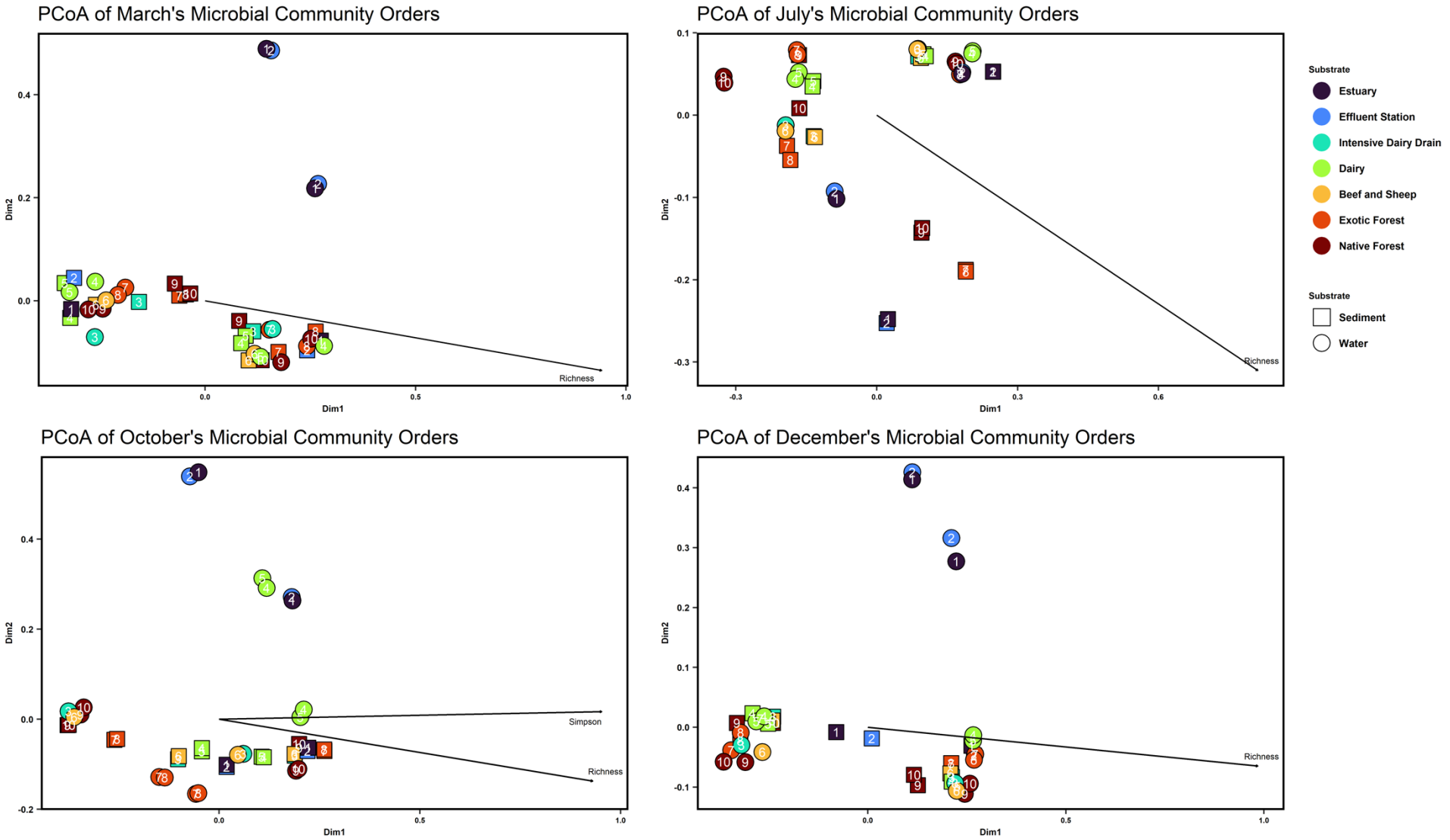


Figure 6.6 Order Principal coordinates analysis, as an annual composite and by season.

Principal coordinates analysis, as an annual composite and by season (e.g., autumn, winter, spring, and summer), of microbial order communities with ≥0.1% relative abundance from the 160 eDNA samples collected from each of the eight sites along the Waiotahe river mainstem, one tributary site, and one site in Te Ahiaua, in 2018. Variables and metrics with an R^2 ≥0.6 and p-value <0.05 are projected onto the plots.

Similar to order, spatio-temporal variables poorly explained the variation in community composition for microbial genera (CH 6 Appendix 1 Table 7). Seasonal metrics (e.g., Simpson's diversity in spring, *E. coli*/*E. cloacae* in autumn's waters, *E. coli*/*E. cloacae* in summer's sediments) were inconsistent (CH 6 Appendix 1 Table 8). However, the influence of downstream slope (i.e., average, and average square root) continued to increase as the importance of conductivity and temperature decreased. The single exception to this was summer, where conductivity and temperature were stronger associated with microbial order communities.

Genera communities had high abundances of *Clostridioides*, *Limnohabitans*, *Salmonella*, *Flavobacterium*, *Curvibacter*, *Rhodoferax*, *Nocardia*, *Pseudomonas*, *Streptomyces*, and *Bradyrhizobium* (Figure 6.7). *Ralstonia*, *Corallococcus*, *Lactobacillus*, *Nonomuraea*, *Jishengella*, *Agrobacterium*, *Winogradskyella*, *Staphlococcus*, *Acinetobacter*, *Photobacterium*, and *Mycolicibacterium* comprised key taxa for microbial genera. Of these, *Salmonella* was indicative of winter samples from both exotic forest substrates and water from site 3 (intensive dairy), site 4 (dairy), and site 9 (native forest).

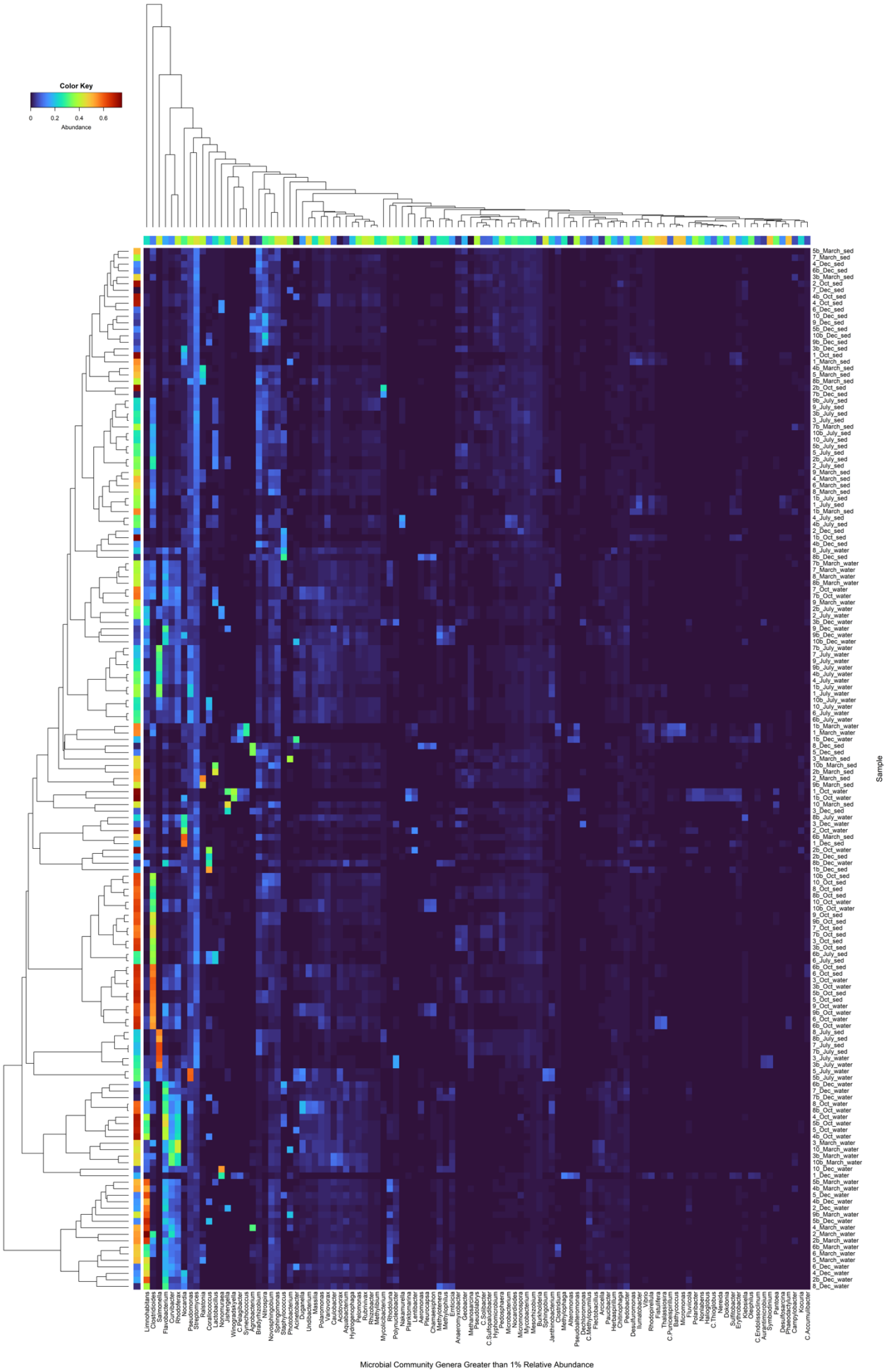
Many of the key genera had very specific sample, site, substrate, and/or seasonal relative abundance peaks (CH 6 Appendix 1 Table 9). *Limnohabitans* was ubiquitous in freshwater samples across the year. It had the highest abundances in the mid to lower catchment in summer and autumn samples where mammalian effluent was likely (e.g., dairy, intensive dairy, beef and sheep, and the effluent station). *Limnohabitans, Flavobacterium*, *Curvibacter*, and *Rhodoferax* followed similar patterns in where they were most abundant, though individual relative abundance fluctuated. All three were preferentially found in water and *Flavobacterium* abundances were highest in spring from dairy and forested sites.

Nocardia had a localised peaks in abundance in water samples from site 8 (exotic forest) in winter and summer, the effluent station in spring and summer, and intensive dairy, site 4 (dairy), and site 6 (beef and sheep) in summer. Sediment samples with high *Nocardia* abundances occurred at site 6 (beef and sheep) in autumn and Te Ahiaua in summer.

Clostridioides was the principal genus for freshwater sediment and water at site 6 (beef and sheep), site 3 (intensive dairy), and in native forest in spring. *Jishengella* and *Winogradskyella* together or *Winogradskyella* alone, were the most abundant taxa in spring estuarine water. *Jishengella*, was the most abundant genus in sediment at site 10 (native forest) in the autumn and site 3 (intensive dairy) in the summer. *Corallococcus* was the dominant genera in one water sample in spring and one sediment sample in summer from the

effluent station, water from site 8 (exotic forest) in summer, and estuarine sediment in summer.

Similarity within substrate and sites was lower for genera than order communities (Figure 6.8). Estuarine and effluent station waters generally retained their uniqueness, but their sediments were more similar to freshwater communities in spring, summer, and autumn than in order and class communities. The most noticeable difference was the increased similarity between spring's beef and sheep site (6) to the dairy sediments from sites 4 and 5.


Color Key
0
0.2
0.4
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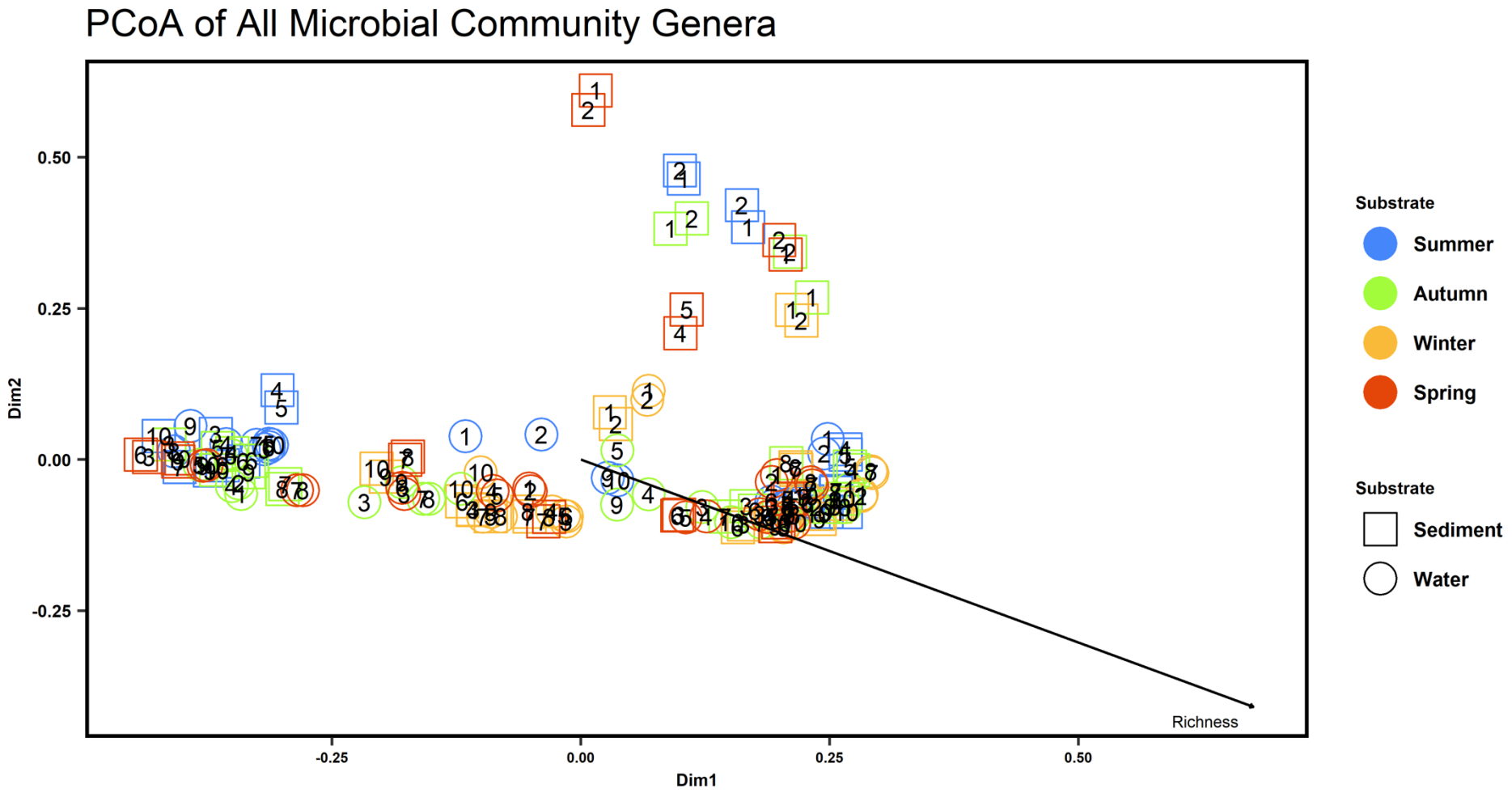
Abundance
Sample
Microbial Community Genera Greater than 1% Relative Abundance

6.7 Heatmap of all microbial genera.

Heatmap depicting all identified microbial genera, with relative abundances ≥1%, present in the 160 eDNA samples collected from each of the eight sites along the Waiotahe river mainstem, one tributary site, and one site in Te Ahiaua, seasonally (e.g., autumn, winter, spring, and summer) in 2018. Dendrograms, representing hierarchical clustering using Bray-Curtis dissimilarity, were projected onto the opposite/parallel axis for each sample (y-axis) and taxa (x-axis).


PCoA of All Microbial Community Genera
0.50
0.25
0.00
-0.25
Dim2
-0.25
0.00
0.25
0.50
Dim1
Richness
Substrate
Summer
Autumn
Winter
Spring
Substrate
Sediment
Water

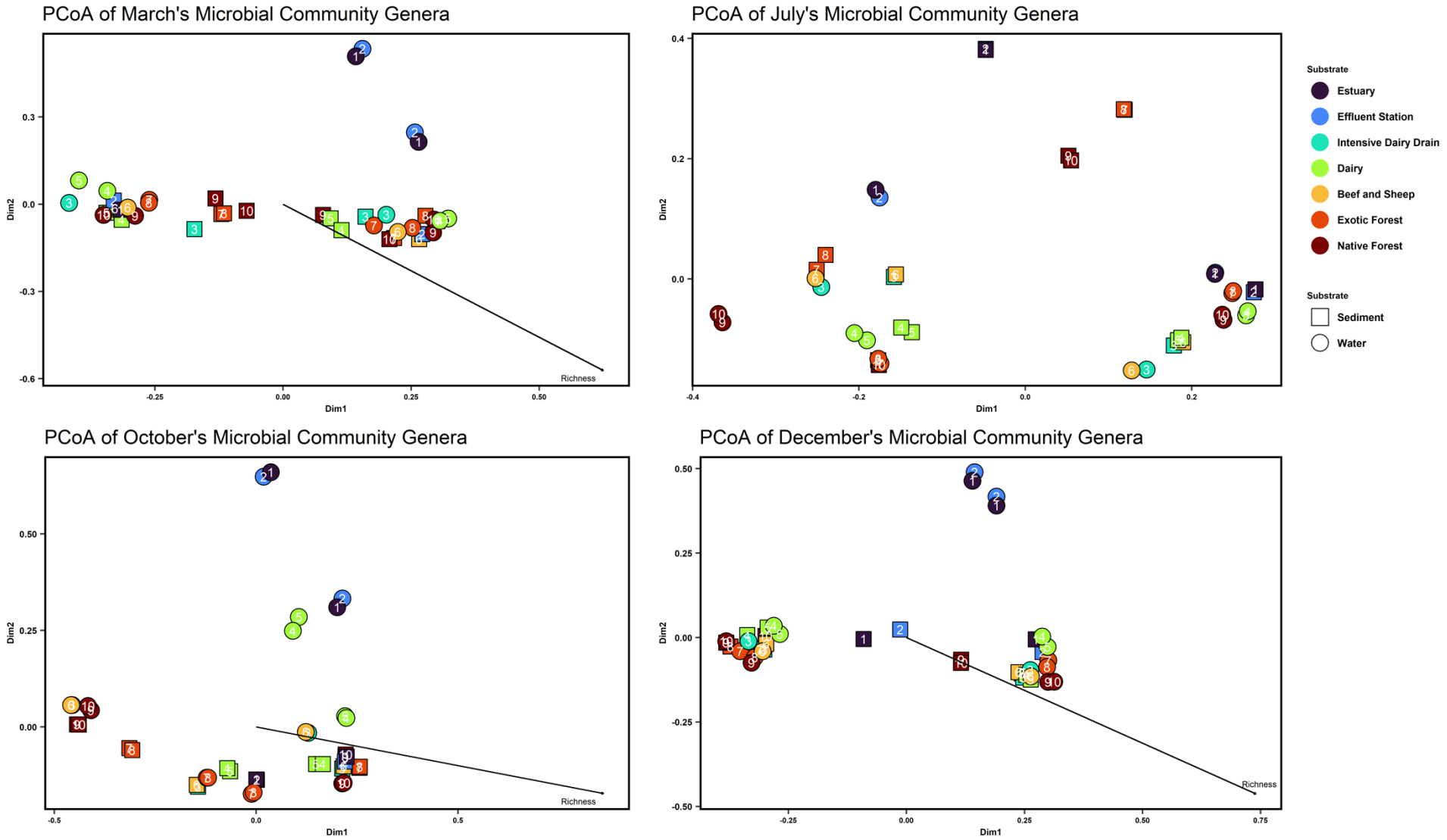


Figure 6.8 Genera Principal coordinates analysis, as an annual composite and by season.

Principal coordinates analysis, as an annual composite and by season (e.g., autumn, winter, spring, and summer), of microbial genera communities with ≥0.1% relative abundance from the 160 eDNA samples collected from each of the eight sites along the Waiotahe river mainstem, one tributary site, and one site in Te Ahiaua, in 2018. Variables and metrics with an R^2 ≥0.6 and p-value <0.05 are projected onto the plots.

Few archaea were retained in the restriction of taxa to 1% or greater in relative abundance across the taxonomic levels. However, even with the extreme differences in domain abundance, archaea were represented at each taxonomic level. Retained archaea were all methanogens (e.g., class – Methanomicrobia, order – Methanomicrobiales and Methanosarcinales, and genus– *Methanosarcina*), responsible for the final stage of transforming organic matter into CH_4 (Lambie et al., 2015).

Eukaryote abundance remained low but stable across taxonomic levels (e.g., six classes and orders and five genera); however, the types of eukaryotes with the highest relative abundances at each taxonomic level differed slightly. Class communities were comprised of fungi, algae, dinoflagellates, and diatoms. Order and genera communities did not have any fungi, instead the most abundant taxa were all primary producers including green algae, dinoflagellates, and diatoms.

Bacterial communities were the most functionally diverse microbial domain and were overwhelmingly identified as key taxa. They were also the most abundant domain present in every microbial community (e.g., 72% of classes, 87.7% of orders, and 95.1% of genera). A pattern that began to emerge in abundant and key genera, was the presence of potential plant, animal, or human pathogens (CH 6 Appendix 1 Table 10). Just under 60% of abundant and 44% of key genera had potentially harmful species/strains associated with them. Some genera, such as *Salmonella* and *Clostridioides*, are rarely associated with nonvirulent species/strains, while others (e.g., *Nocardia* and *Flavobacterium*) are more commonly opportunistic pathogens.

6.4.5 Microbial Core Biome Structure and Metrics

Core biome composition was best explained by land use for classes (PERMANOVA: Land use $F_{(8)} = 3.68$, $R^2 = 0.310$, p-value = 0.001), substrate for orders (PERMANOVA: Substrate $F_{(2)} = 25.14$, $R^2 = 0.322$, p-value = 0.001), and season for genera (PERMANOVA: Season $F_{(4)} = 28.50$, $R^2 = 0.458$, p-value = 0.001) (CH 6 Appendix 1 Table 11). Core biome size, distribution, and patterns differed by domain and taxonomic level but core biome richness and the percent of the total biome, while significant, explained little variation in community structure. The largest core biomes across the taxonomic levels were from each substrate, individually from the effluent station, intensive dairy, and both native and exotic forest. Class core biome Z-scores were closely distributed around the average, with a distinct peak to either side. The negative distribution peak, representing lower than average relative abundances, was approximately twice the size of the positive peak (e.g., representing higher

than average relative abundances). The number of taxa with below average abundances was approximately double that of taxa with higher abundances. However, as taxonomic level decreased the distribution of high abundance taxa flattened and extended away from the mean abundance (e.g., Z-scores of up to ~5) to become a long, low tail while the distribution of low abundance taxa remained a distinct peak. Domain dominance changed with taxonomic level. Bacteria had few classes (10) and genera (11) with relative abundances greater than 1% but had 34 differentiated orders. Archaea core biomes grew as taxonomic level decreased (e.g., 11 classes, 17 orders, and 20 genera) and eukaryote core biomes shrank (e.g., 38 classes, 32 orders, and 20 genera). Many abundant classes and orders were also present in the core biomes, but few genera or key taxa were retained.

The classes with the highest abundances were eukaryotes: Florideophyceae, Eustigmatophycaea, Exobaidomycetes, and Haptophyceae (Figure 6.9). They were associated with: the effluent station sediment (Florideophyceae); autumn water (Eustigmatophyceae and Cytophagia); freshwater sediments or water likely to contain suspended sediments (Exobasidiomycetes); and salt water (Haptophyceae). In addition to high abundances of Haptophyceae, estuarine samples had low abundances of Kinetoplastida, Xyariales, Glomerellales, and Pernosporales. The beef and sheep site had the most unique microbial class communities, characterised by few taxa with average abundances.

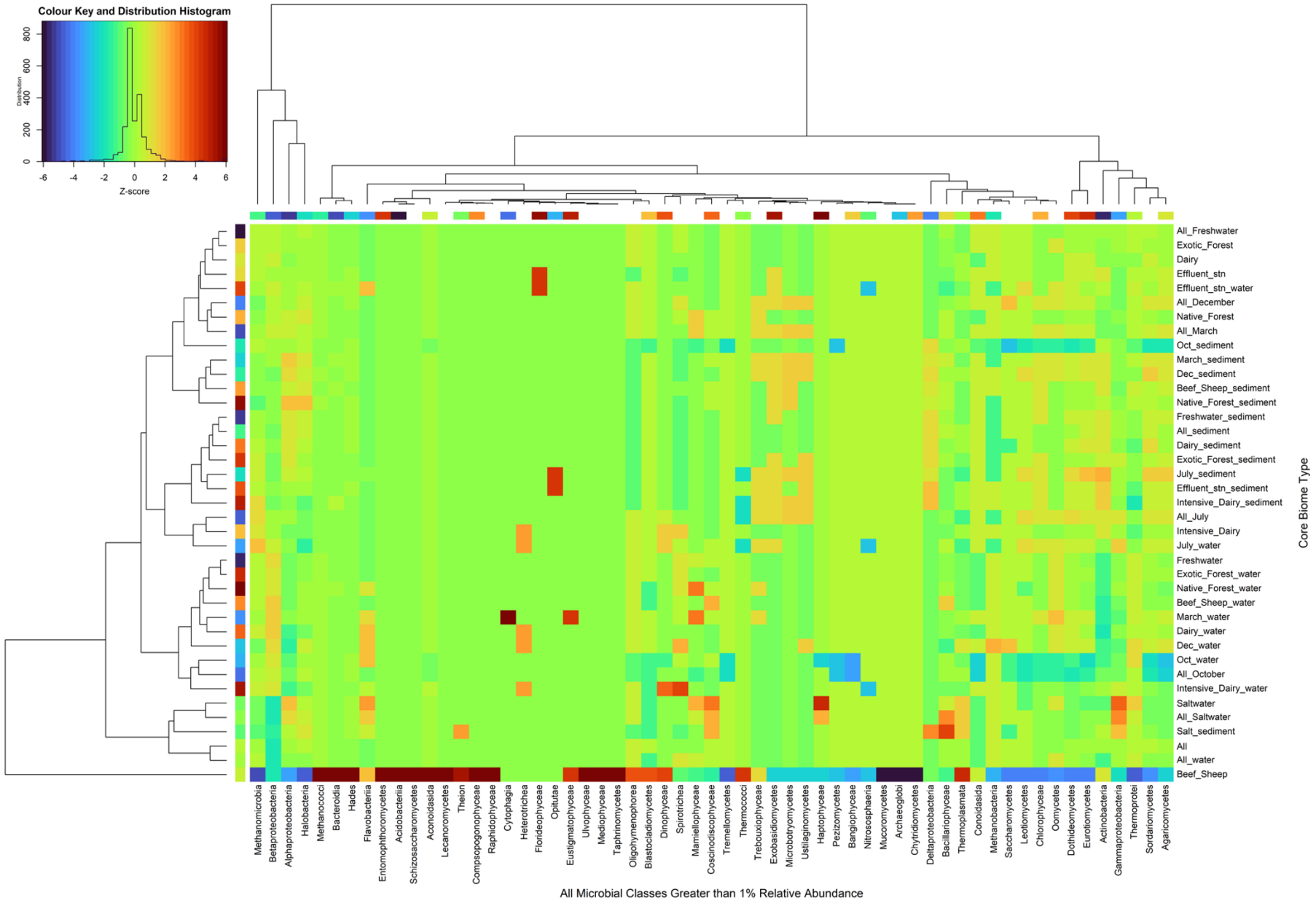

Colour Key and Distribution Histogram
Distribution
0
200
400
600
800
-6
-4
-2
0
2
4
6
Z-score
All_Freshwater
Exotic_Forest
Dairy
Effluent_stn
Effluent_stn_water
All_December
Native_Forest
All_March
Oct_sediment
March_sediment
Dec_sediment
Beef_Sheep_sediment
Native_Forest_sediment
Freshwater_sediment
All_sediment
Dairy_sediment
Exotic_Forest_sediment
July_sediment
Effluent_stn_sediment
Intensive_Dairy_sediment
All_July
Intensive_Dairy
July_water
Freshwater
Exotic_Forest_water
Native_Forest_water
Beef_Sheep_water
March_water
Dairy_water
Dec_water
Oct_water
All_October
Intensive_Dairy_water
Saltwater
All_Saltwater
Salt_sediment
All
All_water
Beef_Sheep
Core Biome Type
Methanomicrobia
Betaproteobacteria
Alphaproteobacteria
Halobacteria
Methanococci
Bacteroidia
Hades
Flavobacteriia
Entomophthoromycetes
Acidobacteriia
Schizosaccharomycetes
Aconoidasida
Lecanoromycetes
Theion
Compsopogonophyceae
Raphidophyceae
Cytophagia
Heterotrichea
Florideophyceae
Opitutae
Eustigmatophyceae
Ulvophyceae
Mediophyceae
Taphrinomycetes
Oligohymenophorea
Blastocladiomycetes
Dinophyceae
Spirotrichea
Mamiellophyceae
Coscinodiscophyceae
Tremellomycetes
Thermococci
Trebouxiophyceae
Exobasidiomycetes
Microbotryomycetes
Ustilaginomycetes
Haptophyceae
Pezizomycetes
Bangiophyceae
Nitrososphaeria
Mucoromycetes
Archaeoglobi
Chytridiomycetes
Deltaproteobacteria
Bacillariophyceae
Thermoplasmata
Conoidasida
Methanobacteria
Saccharomycetes
Leotiomycetes
Chlorophyceae
Oomycetes
Dothideomycetes
Eurotiomycetes
Actinobacteria
Gammaproteobacteria
Thermoprotei
Sordariomycetes
Agaricomycetes
All Microbial Classes Greater than 1% Relative Abundance

Figure 6.9 Heatmap of the core biome Z-scores for all identified microbial classes with ≥1% relative abundance.
Heatmap depicting the core biome Z-scores for all identified microbial classes with relative abundances ≥1%, present in the 160 eDNA samples collected from each of the eight sites along the Waiotahe river mainstem, one tributary site, and one site in Te Ahiaua, seasonally (e.g., autumn, winter, spring, and summer) in 2018. Dendrograms, representing hierarchical clustering using Bray-Curtis dissimilarity, were projected onto the opposite/parallel axis for each sample (y-axis) and taxa (x-axis).

Nitrosomonadales, Planctomycetales, Nitropirales, Propionibacterales, and Myxococcales were the most abundant orders (Figure 6.10). The importance of substrate on order core biome structure was clear as core biomes composed of both substrates (e.g., seasonal, land use, and all freshwater) contained the least taxa. The most abundant taxa in order sediment core biomes were generally functional groups important in carbon and nutrient cycling. These included taxa associated with breaking down organic matter (e.g., Micromonosporales, Propionibacteriales, Tremellales, and Polypoirales), nitrate and sulfate-reducing bacteria (e.g., Desulfobacterales and Desulfuromonadales), and methanogens (e.g., Methanomassillicoccales, Methanosarcinales, and Methanomicrobiales). Other orders important in carbon and nutrient cycling were more ubiquitously distributed between substrates (e.g., Chitinophagales, Caulobacterales, Nitrosomonadales). Water core biomes were more often characterised by localised peaks in specific taxa (e.g., Nitrosopumilales, Mamiellales, and Prymnesiales in saltwater or Chlorellales, Desulfurococcales, and Pyrenomonadales in the effluent station's water) and Te Ahiaua continued to be characterised by lower abundances of groups of taxa (e.g., Chlamydomonadales, Kinetoplastida, Eucoccidioridia, Xylariales, Glomerellales, and Peronoporales). There were also orders containing potential human, animal, and plant pathogens (e.g., Eucoccidiorida, Vibrionales, Xanthomodales, and Bacillales). These orders, when present high abundances, were commonly associated with sediment core biomes.

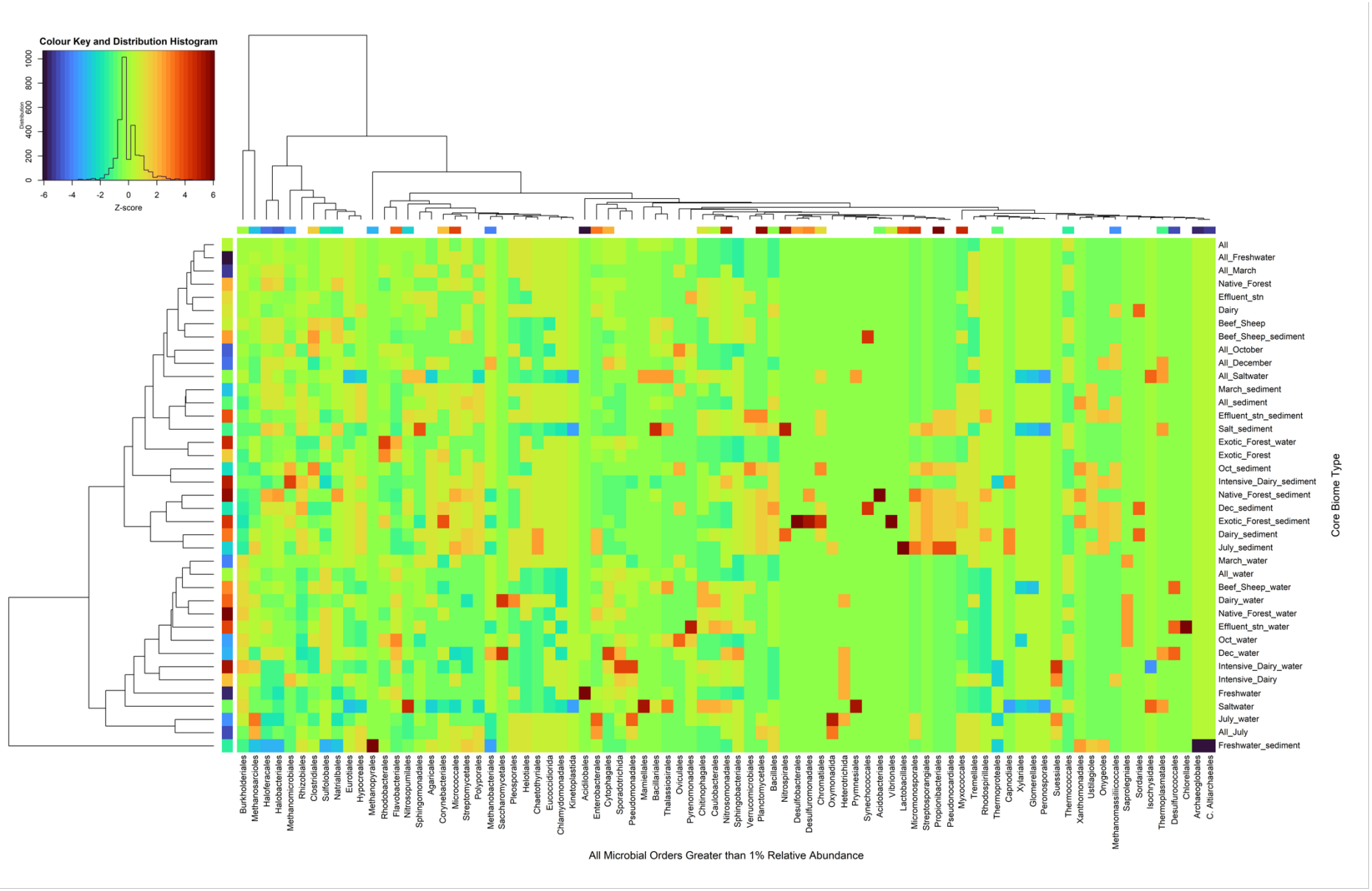

Colour Key and Distribution Histogram
Distribution
0
200
400
600
800
1000
-6
-4
-2
0
2
4
6
Z-score
All
All_Freshwater
All_March
Native_Forest
Effluent_stn
Dairy
Beef_Sheep
Beef_Sheep_sediment
All_October
All_December
All_Saltwater
March_sediment
All_sediment
Effluent_stn_sediment
Salt_sediment
Exotic_Forest_water
Exotic_Forest
Oct_sediment
Intensive_Dairy_sediment
Native_Forest_sediment
Dec_sediment
Exotic_Forest_sediment
Dairy_sediment
July_sediment
March_water
All_water
Beef_Sheep_water
Dairy_water
Native_Forest_water
Effluent_stn_water
Oct_water
Dec_water
Intensive_Dairy_water
Intensive_Dairy
Freshwater
Saltwater
July_water
All_July
Freshwater_sediment
Core Biome Type
Burkholderiales
Methanosarcinales
Haloferacales
Halobacteriales
Methanomicrobiales
Rhizobiales
Clostridiales
Sulfolobales
Natrialbales
Eurotiales
Hypocreales
Methanopyrales
Rhodobacterales
Flavobacteriales
Nitrosopumilales
Sphingomonadales
Agaricales
Corynebacteriales
Micrococcales
Streptomycetales
Polyporales
Methanobacteriales
Saccharomycetales
Pleosporales
Helotiales
Chaetothyriales
Eucoccidiorida
Chlamydomonadales
Kinetoplastida
Acidilobales
Enterobacteriales
Cytophagales
Sporadotrichida
Pseudomonadales
Mamiellales
Bacillariales
Thalassiosirales
Oviculales
Pyrenomonadales
Chitinophagales
Caulobacterales
Nitrosomonadales
Sphingobacteriales
Verrucomicrobiales
Planctomycetales
Bacillales
Nitrospirales
Desulfobacterales
Desulfuromonadales
Chromatiales
Oxymonadida
Heterotrichida
Prymnesiales
Synechococcales
Acidobacteriales
Vibrionales
Lactobacillales
Micromonosporales
Streptosporangiales
Propionibacteriales
Pseudonocardiales
Myxococcales
Tremellales
Rhodospirillales
Thermoproteales
Capnodiales
Xylariales
Glomerellales
Peronosporales
Suessiales
Thermococcales
Xanthomonadales
Ustilaginales
Onygenales
Methanomassiliicoccales
Saprolegniales
Sordariales
Isochrysidales
Thermoplasmatales
Desulfurococcales
Chlorellales
Archaeoglobales
C. Altiarchaeales
All Microbial Orders Greater than 1% Relative Abundance

Figure 6.10 Heatmap of the core biome Z-scores for all identified microbial orders with ≥1% relative abundance.

Heatmap depicting the core biome Z-scores for all identified microbial orders with relative abundances ≥1%, present in the 160 eDNA samples collected from each of the eight sites along the Waiotahe river mainstem, one tributary site, and one site in Te Ahiaua, seasonally (e.g., autumn, winter, spring, and summer) in 2018. Dendrograms, representing hierarchical clustering using Bray-Curtis dissimilarity, were projected onto the opposite/parallel axis for each sample (y-axis) and taxa (x-axis).

The most abundant taxa in genera core biomes included *Stylonychia* (in water), *Pseudonitzschia* and *Thalassiosira* (in Te Ahiaua), and *Symbiodinium* (at the intensive dairy site) were the most abundant taxa in genera core biome (Figure 6.11). Higher abundances of core biome taxa were associated with native and exotic forest, intensive diary, and the effluent station. Freshwater, effluent station sediment, and intensive dairy sediment core biomes shared *Colletotrichum*, *Trypanosoma*, *Chlamydomonas*, and *Penicillium*. Additional commonalities between effluent station and intensive dairy sediments included the taxa *Halorussus*, *Methanocella*, and *Methanospirilum*. However, high *Methanospirillum*, *Methanoregula*, *Methanocella* and *Sulfolobus* abundances only in intensive dairy sediments and *Halococcus* only in effluent station sediments differentiated the two land uses. Beef and sheep core biomes were most similar to those from estuarine water and sediment. Localised peak abundances of taxa associated with core biome type included: *Clostridium* in winter freshwater sediments; *Oleiphilus* and *Dechloromonas* in autumn's freshwater; and *Staphylococcus* in native forest sediments.

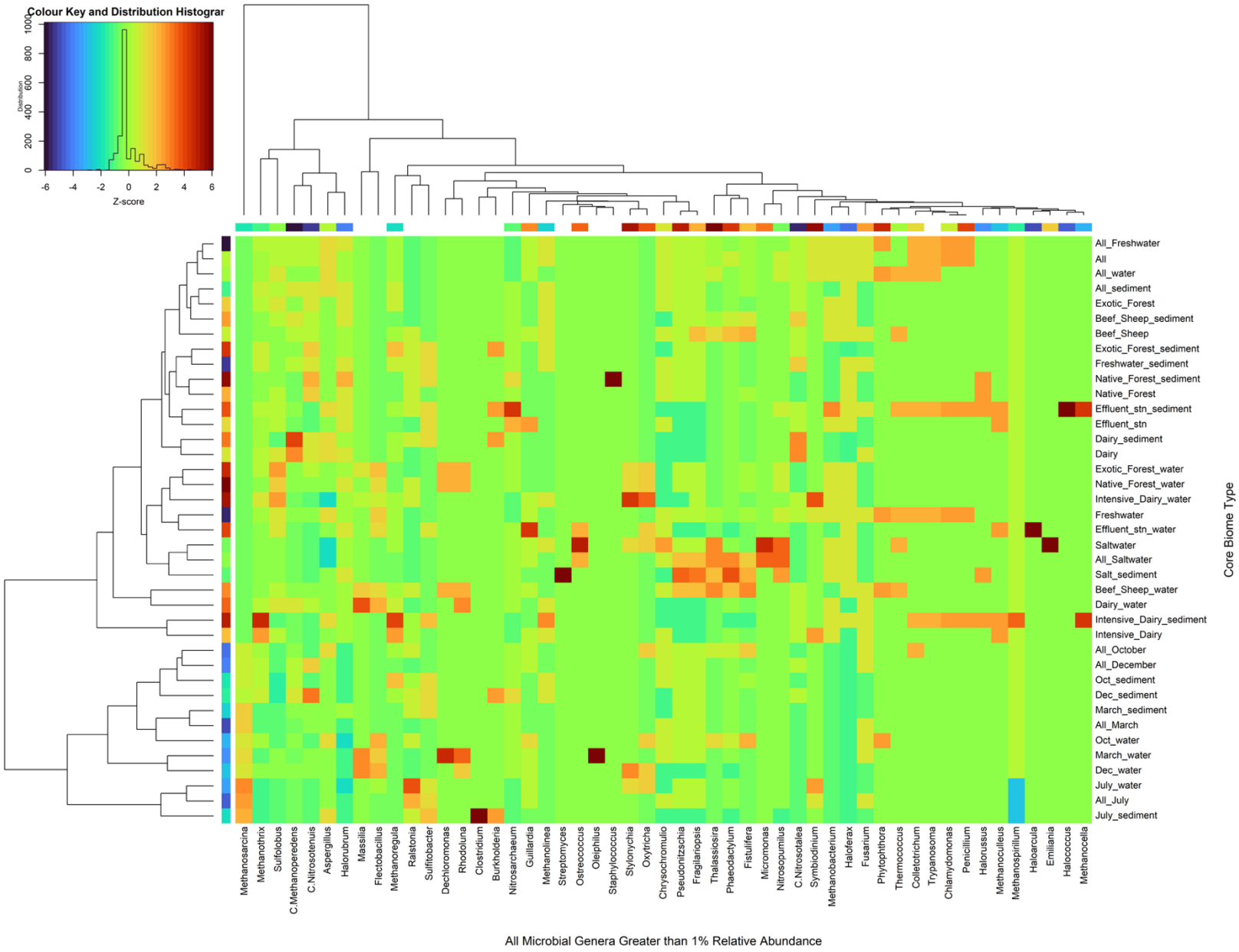

Colour Key and Distribution Histogram
Distribution
Z-score
All_Freshwater
All
All_water
All_sediment
Exotic_Forest
Beef_Sheep_sediment
Beef_Sheep
Exotic_Forest_sediment
Freshwater_sediment
Native_Forest_sediment
Native_Forest
Effluent_stn_sediment
Effluent_stn
Dairy_sediment
Dairy
Exotic_Forest_water
Native_Forest_water
Intensive_Dairy_water
Freshwater
Effluent_stn_water
Saltwater
All_Saltwater
Salt_sediment
Beef_Sheep_water
Dairy_water
Intensive_Dairy_sediment
Intensive_Dairy
All_October
All_December
Oct_sediment
Dec_sediment
March_sediment
All_March
Oct_water
March_water
Dec_water
July_water
All_July
July_sediment
Core Biome Type
Methanosarcina
Methanothrix
Sulfolobus
C.Methanoperedens
C.Nitrosotenuis
Aspergillus
Halorubrum
Massilia
Flectobacillus
Methanoregula
Ralstonia
Sulfitobacter
Dechloromonas
Rhodoluna
Clostridium
Burkholderia
Nitrosarchaeum
Guillardia
Methanolinea
Streptomyces
Ostreococcus
Oleiphilus
Staphylococcus
Stylonychia
Oxytricha
Chrysochromulio
Pseudonitzschia
Fragilariopsis
Thalassiosira
Phaeodactylum
Fistulifera
Micromonas
Nitrosopumilus
C.Nitrosotalea
Symbiodinium
Methanobacterium
Haloferax
Fusarium
Phytophthora
Thermococcus
Colletotrichum
Trypanosoma
Chlamydomonas
Penicillium
Halorussus
Methanoculleus
Methanospirillum
Haloarcula
Emiliania
Halococcus
Methanocella
All Microbial Genera Greater than 1% Relative Abundance

Figure 6.11 Heatmap of the core biome Z-scores for all identified microbial genera with ≥1% relative abundance.

Heatmap depicting the core biome Z-scores for all identified microbial genera with relative abundances ≥1%, present in the 160 eDNA samples collected from each of the eight sites along the Waiotahe river mainstem, one tributary site, and one site in Te Ahiaua, seasonally (e.g., autumn, winter, spring, and summer) in 2018. Dendrograms, representing hierarchical clustering using Bray-Curtis dissimilarity, were projected onto the opposite/parallel axis for each sample (y-axis) and taxa (x-axis).

6.4.6 Molecular Testing for Target Genes

See 5.4.6 for the results of molecular testing for target genes.

6.5 Discussion

GC content was consistently higher in sediment than in water samples but the organisms identified from each substrate were similar in many instances. GC content is an important aspect when using molecular techniques due to its effects on primer specificity for both PCR and metabarcoding methodologies, eDNA longevity, and genome size (Creer et al., 2016; Laursen, Dalgaard & Bahl, 2017; Fonseca, 2018). However, understanding what drives organisms to have GC heavy genomes and how that affects their function is only hypothesised; mutation bias, the energy expended to retain GC content, organism complexity, and increased stability/thermotolerance of GC bonds when compared to adenine-thymine (Miseta & Csutora, 2000; Hildebrand, Meyer & Eyre-Walker, 2010; Hu et al., 2022). Importantly, similar observations of environments influencing GC content have been made around the world (Foerstner et al., 2005; Moura, Savageau & Alves, 2013; Chuckran et al., 2023). Studies have documented GC content in prokaryotes is higher in sediments/soils, decreased in unison with optimal growth temperature, was lower in pathogens isolated from hosts, affected the structure of proteins microbes made, and was associated with higher rates of DNA repair (Gu, Zhou & Wilke, 2010; Weissman, Fagan & Johnson, 2019; Barceló-Antemate et al., 2023). Given this, it is likely that some of the more dynamic aspects associated with benthic life in lotic systems (e.g., have higher optimal growth temperatures, protein production/metabolisms that rely on higher GC content, or a greater need for DNA repair) select for the retention of GC content in microbial genomes over those same organisms present in the water column.

The adaptability of microbial communities was further reinforced as no singular spatio-temporal factor explained the entire microbial community structure or core biome composition along the Waiotahe river well. On the surface this appears to contradict most other studies from Aotearoa, which have asserted that land use and season, typically a result of precipitation, are the primary drivers of poor water quality in rivers (Phiri et al. 2020; French et al. 2022; Muirhead 2019). However, those studies focused on studying select groups or individual microorganisms from within a single domain and taxonomic level (e.g., family, genus, or species), that are associated with the risk of disease in humans (Phiri et al. 2020; French et al. 2022; Muirhead 2019). Importantly class communities, which were dominated by bacteria, did share drivers such as conductivity and temperature with other studies (Adams, Crump & Kling, 2010; Galfi et al., 2016).

Substrate was best at explaining the variation observed in microbial community classes and order core biomes. However, it did not differentiate microbial community orders and genera or class or genera core biomes well, indicating that many organisms found in the water column are also present in superficial freshwater benthic substrates. This may be related to benthic substrate suspension in the water column, interface crossover, or a lack of substrate preference (Wilkinson et al., 1995; Guo et al., 2010; Erbilgin et al., 2017). In winter, the GC content of the water column was closest to that in sediment and the *E. coli*/*E. cloacae* group concentrations were more similar. This was related to high levels of suspended sediment in the water column as a result of higher flows and resuspension of benthic substrates and overland loss of soil into the river.

Annually, downstream slope and the distance to the coast increased in strength as phylogenetic level decreased and these factors were less important to community structuring during winter, when flow is typically highest due to precipitation. Rivers are lotic systems and microorganisms, as well as other allochthonous and autochthonous inputs, accumulate in a downstream direction. Additionally, stream habitants are influenced by in-stream geomorphology, water flow, habitat suitability, and their morphological characteristics (Johnston, Bridgham & Schubauer-Berigan, 2001; Deiner et al., 2016). Many microorganisms are spread by passive dispersal in rivers; however, short term colonisation or long persistence are determined by the stability and availability of energy sources and habitat (de Oliveira & Margis, 2015). Furthermore, bacterial α-diversity in benthic substrates may be an indicator of heterotrophic production in waterways (Schmidt et al., 2020). Although not the strongest driver, adjacent land use was important to microbial class community structure which was reflected in both α- and β-diversity.

It is clear that microbial inputs from both terrestrial and connected aquatic habitats are capable of migrating significant distances in rivers by the distributions in rarer community taxa observed. Enterobacterales, the order that contains both *E. coli* and *Enterococcus* (e.g., the two taxa used as faecal bacteria indicators in recreational water in Aotearoa), was only a key taxa for order communities in sediment at intensive dairy and exotic forest sites in winter. Additionally, water column *E. coli*/*E. cloacae* group concentrations were only weakly associated with microbial order and genera communities in autumn, were best explained by season followed by land use, and while significant, were not strongly reflective of *Campylobacter* relative abundances. Importantly, neither *Enterobacter* nor *Enterococcus* relative abundances were associated with either *E. coli*/*E. cloacae* group concentrations or *Campylobacter* relative abundances. Microbiological water testing in Aotearoa is restricted to bathing season (i.e., late spring to early autumn) and to culturing either *Enterococcus* in saline water or *E. coli* in freshwater as a faecal indicator associated with contracting campylobacteriosis and other enteric diseases. Given that understanding, it was important to highlight the weak association. This is not a novel finding, but one that is consistently, and stubbornly advocated against by Aotearoa's government bodies. Globally, numerous studies have discussed the significant limitations of using *E. coli*, *Enterococcus*, and *Enterobacter* use as an indicator of bathing water quality and suggested using more updated technology (e.g., molecular methods or a community approach). I strongly support their call to develop and implement alternative and updated methods to monitor recreational waters (Dufour, 1984; Kinzelman et al., 2004; Ishii & Sadowsky, 2008). We have the technology to identify disease causing organisms directly. It is time to put aside the centuries old methodology of using proxies and start working on developing robust methods of direct detection.

High microbial biodiversity is commonly associated with increased nutrient and carbon cycling, does not imply a healthy environment, or remain stable (Cardinale, 2012; Thaler, 2021). Edging effects, interfaces or transitional zones from one habitat type to another, are often linked to changes in microbial diversity (Malmivaara-Lämsä et al., 2008). In this study, heavily degraded sites (e.g., intensive dairy, the effluent station, and Te Ahiaua) often had high biodiversity.

Although imperfect, patterns in the similarity of the microbial communities was observable. This was most obvious in winter and spring communities. However, it was the most abundant and key taxa that provided insight to the localised drivers of the dissimilarities observed in the communities. Rough scale observations of the effects of substrate on microbial classes were similar to other studies with Proteobacteria, Actinobacter, and Flavobacteriia

abundances indicative of salinity, organic matter availability, and/or eutrophic water (Zhao et al., 2016; Sajjad et al., 2021; Zhang & Wang, 2022). Primary production and carbon cycling were key functions of abundant and key orders, with a number of potential plant and human pathogens represented (Zhang et al., 2021; Courcoul et al., 2022; Moghadam et al., 2022). The high representation of potential pathogens continued for abundant and key genera but functional groups shifted to include nitrogen fixing, nitrate-reducing, iron-oxidising, and toxin or antibiotic producing taxa (Arantes et al., 2020; Dong et al., 2021; Nishimura et al., 2021). For example, the high relative abundance of *Methanosarcina* found across the catchment, a methyltrophic genera linked to effluents (both human and ruminant (Lambie et al., 2015; Tiirik et al., 2021)) and aquatic (fresh and saline) sediments, and responsible for the majority of global methane production (Guss et al., 2008), in riverine and estuarine samples has been found in many other studies (Whitman et al., 2001; Wang et al., 2019). Similar drivers highlighted in those studies (e.g., the effects of effluents and nitrate enrichment on archaeal communities) were present and significantly associated with community structure in the Waiotahe catchment (Li et al., 2021; Tiirik et al., 2021). Additionally, wastewater and effluents entering waterways are also associated with increased abundances of human pathogenic microorganisms and outbreaks of waterborne disease (Huffman, Quinter-Betancourt & Rose, 2003; Gilpin et al., 2020; Plowright et al., 2021). The presence and persistence of antimicrobial resistance genes in the lower catchment, may simply reflect the presence of organisms capable of producing antibiotics (e.g., *Streptomyces* or *Nonomuraea*). However, the concurrent presence of potential human pathogens with these mobile genetic elements is an area in need of further work (Fuchsman et al., 2017; Guan et al., 2022).

6.6 Conclusion

Lotic systems contain adaptable microbial communities. Within domain drivers differ significantly, reducing the explanatory strength of historical drivers associate with singular domains (e.g., bacteria with pH, salinity, temperature, and precipitation). Aquatic microbial community structure and α-diversity may be best understood through in-stream conditions as non-pathogenic organisms have different drivers than current ‘water quality’ proxies. Importantly stable drivers associated with substrate and microbial mobility, such as downstream slope and distance to the coast, were best at explaining microbial community composition. Water chemistry, carbon, and nutrient species entering rivers and estuaries are reflected in the functional aspects of the microbial communities present. Their metabolism supports more complex species shaping Aotearoa’s riverine ecosystems in the process. They

are the driving force behind primary production, without which complex life on earth could not exist.

Chapter 6 Appendix 1

Tables

Table 1. GLMs of the effect faecal indicator bacteria (e.g. *Enterococcus* or *Enterobacter*) relative abundances, *Campylobacter*, and water column *E. coli*/*E. cloacae* concentration had on the relative abundances of *Enterococcus* and *Enterobacter* in Te Ahiaua, seasonally (e.g., March, July, October, and December) in 2018. **Bold** denotes a significance >0.05.

GLM (poisson)		Std. Error	Z-value	*p*-val
***Enterobacter* in salt water**	Intercept	4.082e+00	1.766	0.077
	Campylobacter	1.33e+03	0.017	0.986
	Enterococcus	3.327e+03	0.092	0.927
	Water Column *E. coli*/*E. cloacae* group concentration	7.034e-04	0.021	0.983
***Enterococcus* in salt water**	Intercept	1.647e+01	-0.441	0.659
	Campylobacter	2.469e+03	0.005	0.996
	Enterobacter	1.522e+04	0.011	0.991
	Water Column *E. coli*/*E. cloacae* group concentration	3.628e-02	-0.004	0.996

Table 2. PERMANOVA results for Simpson's Diversity Index explanatory variables across the three domains at all taxonomic levels for the 160 samples collected from each of the eight sites along the Waiotahe river mainstem, one tributary site, and one site in Te Ahiaua, seasonally (e.g., March, July, October, and December) in 2018. **Bold** denotes p-value <0.05%. Variables that best represent the variance in community structure have a red R^2.

Simpson's Diversity Index	**Df**	***F*-Statistic**	**R^2**	***p*-val**
Site	9	17.4	0.006	**0.001**
Substrate	1	227.4	0.008	**0.001**
Taxonomic level	2	3,179.8	0.222	**0.001**
Season	3	164.3	0.017	**0.001**
Domain	2	5,814.3	0.406	**0.001**
Site : Substrate	9	8.4	0.003	**0.001**
Site : Taxonomic level	18	2.7	0.002	**0.002**
Substrate : Taxonomic level	2	28.2	0. 002	**0.001**
Site : Season	27	13.1	0.012	**0.001**
Substrate : Season	3	17.7	0.002	**0.001**
Taxonomic level : Season	6	61.0	0.013	**0.001**
Site : Domain	18	17.1	0.010	**0.001**
Substrate : Domain	2	239.7	0.017	**0.001**
Taxonomic level : Domain	4	189.5	0.026	**0.001**
Season : Domain	6	284.5	0.06	**0.001**
Site : Substrate : Taxonomic level	18	3.6	0.002	**0.001**
Site : Substrate : Season	27	17.0	0.016	**0.001**

Simpson's Diversity Index	**Df**	***F*-Statistic**	**R^2**	***p*-val**
Site : Taxonomic level : Season	54	3.1	0.006	**0.001**
Substrate : Taxonomic level : Season	6	5.4	0.001	**0.001**
Site : Substrate : Domain	18	11.5	0.007	**0.001**
Site : Taxonomic level : Domain	36	3.7	0.005	**0.001**
Substrate : Taxonomic level : Domain	4	57.5	0.008	**0.001**
Site : Season : Domain	54	13.3	0.025	**0.001**
Substrate : Season : Domain	6	64.8	0.014	**0.001**
Taxonomic level : Season : Domain	12	55.9	0.023	**0.001**
Site : Substrate : Taxonomic level : Season	54	3.3	0.006	**0.002**
Site : Substrate : Taxonomic level : Domain	36	4.2	0.005	**0.001**
Site : Substrate : Season : Domain	54	14.2	0.027	**0.001**
Site : Taxonomic level : Season : Domain	108	3.1	0.012	**0.001**
Substrate : Taxonomic level : Season : Domain	12	4.8	0.002	**0.001**
Site : Substrate : Taxonomic level : Season : Domain	108	3.1	0.012	**0.003**

Table 3. PERMANOVA results for explanatory variables associated with community composition for the microbial classes from each of the 160 samples collected from the eight sites along the Waiotahe river mainstem, one tributary site, and one site in Te Ahiaua, seasonally (e.g., March, July, October, and December) in 2018. Bold denotes p-value >0.05%. Variables that best represent the variance in community structure have a red R^2.

Class	Df	*F*-Statistic	R^2	*p*-val
Land use	6	78.20	0.146	**0.001**
Site	1	157.13	0.049	**0.001**
Substrate	1	1125.38	0.351	**0.001**
Season	3	105.33	0.098	**0.001**
Land use : Site	2	7.10	0.004	**0.001**
Land use : Substrate	6	17.84	0.033	**0.001**
Site : Substrate	1	97.17	0.030	**0.001**
Land use : Season	18	15.17	0.085	**0.001**
Site : Season	3	17.84	0.017	**0.001**
Substrate : Season	3	54.80	0.051	**0.001**
Land use : Site : Substrate	2	7.84	0.005	**0.001**
Land use : Site : Season	6	4.34	0.008	**0.001**
Land use : Substrate : Season	18	12.60	0.071	**0.001**
Land use : Site : Substrate : Season	6	4.23	0.008	**0.001**

Table 4. PCoA results, including distances, R^2, and p-values for the microbial classes present in each of the 160 samples collected from each of the eight sites along the Waiotahe river mainstem, one tributary site, and one site in Te Ahiaua, seasonally (e.g., March, July, October, and December) in 2018. **Bold** denotes a p-value <0.05%. The metric that best represented the variance in community structure is red.

Microbial classes																				
Sample Time	**Annual**				**March / autumn**				**July / winter**				**October / spring**				**December / summer**			
	Dim 1	**Dim 2**	**R^2**	**p-val**	**Dim 1**	**Dim 2**	**R^2**	**p-val**	**Dim 1**	**Dim 2**	**R^2**	**p-val**	**Dim 1**	**Dim 2**	**R^2**	**p-val**	**Dim 1**	**Dim 2**	**R^2**	**p-val**
Metrics																				
Site	-0.327	-0.945	0.122	**0.001**	-0.323	-0.946	0.096	0.144	-0.057	-0.998	0.160	0.039	-0.376	0.927	0.291	**0.002**	-0.598	-0.802	0.057	0.355
Richness	0.985	-0.173	0.396	**0.001**	0.911	-0.411	0.522	**0.001**	-0.789	0.614	0.385	**0.001**	0.693	0.721	0.467	**0.001**	0.977	0.214	0.610	**0.001**
Simpson's Diversity Index	0.994	-0.111	0.301	**0.001**	1.000	-0.004	0.421	**0.001**	0.558	-0.830	0.159	**0.039**	0.968	-0.253	0.319	**0.002**	0.913	-0.407	0.428	**0.001**
Water column *E. coli*/*E. cloacae*	0.502	-0.865	0.010	0.436	-0.294	-0.956	0.235	**0.006**	0.500	0.866	0.099	0.127	-0.362	-0.932	0.040	0.457	-0.062	-0.998	0.257	**0.004**
Sediment *E. coli*/*E. cloacae*	0.593	0.805	0.031	0.098	-0.403	-0.915	0.125	0.101	0.494	0.869	0.114	0.093	-0.704	0.710	0.069	0.283	0.291	0.957	0.267	**0.005**
Environmental Variables																				
Low Flow	-0.050	0.999	0.069	**0.006**	0.331	0.944	0.041	0.453	-0.726	-0.688	0.063	0.301	-0.115	-0.993	0.193	**0.023**	-0.035	0.999	0.160	**0.040**
Jan Air Temp.	0.316	0.949	0.010	0.435	0.339	0.941	0.041	0.464	-0.721	-0.693	0.066	0.278	-0.120	-0.993	0.189	**0.024**	-0.039	0.999	0.164	**0.035**
Flow	-0.047	0.999	0.069	**0.006**	0.076	0.997	0.006	0.889	0.204	0.979	0.046	0.410	0.066	-0.998	0.054	0.333	0.611	-0.792	0.021	0.697

Microbial classes																				
Sample Time	**Annual**				**March / autumn**				**July / winter**				**October / spring**				**December / summer**			
	Dim 1	**Dim 2**	**R^2**	**p-val**	**Dim 1**	**Dim 2**	**R^2**	**p-val**	**Dim 1**	**Dim 2**	**R^2**	**p-val**	**Dim 1**	**Dim 2**	**R^2**	**p-val**	**Dim 1**	**Dim 2**	**R^2**	**p-val**
Flow4th	-0.064	0.998	0.066	**0.007**	0.345	0.939	0.038	0.489	-0.694	-0.720	0.072	0.239	-0.138	-0.990	0.178	**0.031**	-0.048	0.999	0.166	**0.033**
Flow Variability	-0.335	0.942	0.017	0.297	0.682	0.732	0.009	0.863	-0.491	-0.871	0.104	0.130	-0.604	-0.797	0.021	0.680	-0.176	0.984	0.132	0.071
Slope	-0.254	0.967	0.019	0.201	-0.241	-0.971	0.002	0.973	-0.974	-0.225	0.020	0.661	-0.251	-0.968	0.155	**0.046**	-0.111	0.994	0.002	0.985
Slope Sqrt	0.052	0.999	0.028	0.111	-0.603	0.797	0.006	0.899	-0.278	-0.960	0.071	0.257	0.373	-0.928	0.138	0.063	0.112	0.994	0.036	0.524
Riparian Shade	-0.239	-0.971	0.041	**0.036**	-0.361	-0.932	0.063	0.285	-0.024	-1.000	0.081	0.235	-0.058	0.998	0.102	0.135	-0.332	-0.943	0.022	0.688
Historic Shade	-0.184	0.983	0.038	0.060	0.338	0.941	0.017	0.728	-0.479	-0.878	0.120	0.078	-0.271	-0.963	0.080	0.212	-0.101	0.995	0.165	**0.033**
Riparian Native	-0.407	-0.913	0.032	0.069	-0.332	-0.943	0.070	0.241	-0.165	-0.986	0.107	0.126	-0.329	0.944	0.047	0.414	-0.543	-0.839	0.016	0.780
Clues N	0.224	-0.974	0.034	0.076	-0.340	-0.940	0.014	0.769	0.464	0.886	0.124	0.071	0.345	0.939	0.072	0.256	0.110	-0.994	0.158	**0.044**
Distance to Coast	-0.369	-0.929	0.078	**0.004**	-0.302	-0.953	0.051	0.367	-0.102	-0.995	0.131	0.060	-0.403	0.915	0.233	**0.007**	-0.940	-0.341	0.037	0.516
Downstream Average Slope	0.347	0.938	0.238	**0.001**	0.271	0.963	0.172	**0.034**	0.049	0.999	0.104	0.121	0.548	-0.837	0.656	**0.001**	0.369	0.929	0.134	**0.077**
Downstream Average Slope Sqrt	0.349	0.937	0.236	**0.001**	0.272	0.962	0.169	**0.035**	0.050	0.999	0.105	0.117	0.549	-0.836	0.654	**0.001**	0.375	0.927	0.131	**0.080**
Downstream Max Local Slope	-0.316	-0.949	0.010	0.435	-0.076	-0.997	0.006	0.889	-0.204	-0.979	0.046	0.410	-0.066	0.998	0.054	0.333	-0.611	0.792	0.021	0.697

Microbial classes																				
Sample Time	**Annual**				**March / autumn**				**July / winter**				**October / spring**				**December / summer**			
	Dim 1	**Dim 2**	**R^2**	**p-val**	**Dim 1**	**Dim 2**	**R^2**	**p-val**	**Dim 1**	**Dim 2**	**R^2**	**p-val**	**Dim 1**	**Dim 2**	**R^2**	**p-val**	**Dim 1**	**Dim 2**	**R^2**	**p-val**
Upstream Days Rain	-0.436	0.900	0.019	0.240	0.374	0.928	0.003	0.944	-0.381	-0.925	0.146	**0.038**	-0.656	-0.755	0.030	0.570	-0.160	0.987	0.131	0.069
Upstream Average Slope	-0.380	0.925	0.023	0.181	0.354	0.935	0.005	0.912	-0.402	-0.915	0.142	**0.043**	-0.572	-0.820	0.043	0.441	-0.147	0.989	0.138	0.059
Upstream Calcium	-0.449	-0.894	0.005	0.685	-0.301	-0.954	0.003	0.950	-0.169	-0.986	0.075	0.258	0.046	0.999	0.031	0.544	-0.362	0.932	0.026	0.642
Upstream Hardness	-0.100	0.995	0.008	0.533	0.300	0.954	0.003	0.941	-0.128	0.992	0.015	0.753	-0.402	-0.916	0.073	0.240	0.893	0.451	0.002	0.971
Upstream Phosphorus	-0.348	0.937	0.014	0.352	0.343	0.939	0.004	0.937	-0.356	-0.934	0.135	0.060	-0.450	-0.893	0.012	0.830	-0.151	0.988	0.123	0.080
Upstream Indigenous Forrest	-0.507	0.862	0.016	0.311	0.274	0.962	0.002	0.971	-0.343	-0.939	0.154	**0.036**	-0.737	-0.675	0.021	0.705	-0.167	0.986	0.120	0.090
Upstream Native Forest	-0.504	0.864	0.017	0.296	0.263	0.965	0.002	0.970	-0.344	-0.939	0.155	**0.035**	-0.726	-0.687	0.022	0.683	-0.166	0.986	0.121	0.089
Upstream Pasture	0.398	-0.917	0.024	0.171	-0.366	-0.931	0.005	0.907	0.409	0.913	0.141	**0.043**	0.605	0.796	0.048	0.407	0.150	-0.989	0.137	0.061
Reach Sediment	-0.264	0.965	0.034	0.080	0.352	0.936	0.009	0.847	-0.469	-0.883	0.127	0.068	-0.387	-0.922	0.081	0.201	-0.119	0.993	0.143	0.063
Reach Habitat	-0.113	0.994	0.042	**0.032**	0.637	0.771	0.044	0.472	-0.984	-0.176	0.057	0.334	-0.361	-0.933	0.117	0.094	-0.126	0.992	0.138	0.051
Width in m	-0.227	-0.974	0.128	**0.001**	-0.186	-0.983	0.237	**0.005**	-0.127	-0.992	0.200	**0.018**	-0.432	0.902	0.018	0.733	-0.291	-0.957	0.136	0.082
Depth in cm	0.125	-0.992	0.049	**0.027**	-0.296	-0.955	0.085	0.199	0.672	-0.741	0.039	0.503	0.983	-0.183	0.095	0.158	0.015	-1.000	0.144	0.055
Velocity m/s	-0.403	-0.915	0.234	**0.001**	-0.343	-0.939	0.289	**0.002**	-0.223	-0.975	0.141	**0.050**	-0.695	0.719	0.355	**0.001**	-0.467	-0.884	0.142	0.068

Microbial classes																				
Sample Time	**Annual**				**March / autumn**				**July / winter**				**October / spring**				**December / summer**			
	Dim 1	**Dim 2**	**R^2**	**p-val**	**Dim 1**	**Dim 2**	**R^2**	**p-val**	**Dim 1**	**Dim 2**	**R^2**	**p-val**	**Dim 1**	**Dim 2**	**R^2**	**p-val**	**Dim 1**	**Dim 2**	**R^2**	**p-val**
Temperature	-0.232	-0.973	0.344	**0.001**	-0.244	-0.970	0.627	**0.001**	0.187	-0.982	0.244	**0.004**	-0.123	0.992	0.212	0.014	-0.162	-0.987	0.617	**0.001**
Conductivity	-0.227	-0.974	0.619	**0.001**	-0.264	-0.965	0.778	**0.001**	0.118	-0.993	0.272	**0.003**	-0.406	0.914	0.544	0.001	-0.175	-0.985	0.689	**0.001**
% Fine Sediment	-0.113	-0.994	0.077	**0.002**	-0.309	-0.951	0.128	0.071	0.543	-0.840	0.052	0.385	-0.521	0.853	0.000	0.990	-0.041	-0.999	0.192	**0.027**
% Riffle	-0.272	-0.962	0.020	0.221	-0.239	-0.971	0.015	0.743	-0.107	-0.994	0.072	0.242	-0.079	0.997	0.096	0.169	-0.713	0.701	0.014	0.785
% Run	-0.224	-0.974	0.465	**0.001**	-0.269	-0.963	0.591	**0.001**	0.183	-0.983	0.151	**0.050**	-0.482	0.876	0.353	0.002	-0.148	-0.989	0.606	**0.001**
% Macrophyte Cover	0.173	-0.985	0.042	0.046	-0.278	-0.961	0.019	0.696	0.461	0.887	0.123	0.075	0.245	0.970	0.095	0.150	0.089	-0.996	0.169	**0.032**
% Debris Jam	-0.135	-0.991	0.003	0.776	-0.450	-0.893	0.007	0.871	0.002	-1.000	0.046	0.433	0.308	0.951	0.027	0.646	-0.071	0.997	0.002	0.975
Periphyton	0.064	-0.998	0.051	0.018	-0.338	-0.941	0.019	0.688	0.532	0.847	0.114	0.095	0.066	0.998	0.123	0.079	0.047	-0.999	0.169	**0.037**
Substrate size	-0.331	-0.944	0.036	0.069	-0.405	-0.914	0.009	0.820	-0.040	-0.999	0.090	0.172	-0.276	0.961	0.156	0.039	-0.733	0.681	0.024	0.688
Embeddedness	-0.333	-0.943	0.077	**0.001**	-0.328	-0.945	0.091	0.158	-0.081	-0.997	0.232	**0.011**	-0.272	0.962	0.105	0.113	-0.811	-0.586	0.040	0.493
Stream Shade	-0.526	-0.850	0.090	**0.001**	-0.496	-0.868	0.116	0.079	-0.066	-0.998	0.166	**0.031**	-0.854	0.520	0.169	0.027	-0.328	-0.945	0.036	0.503

Table 5. PERMANOVA results for explanatory variables associated with community composition for the orders from each of the 160 samples collected from the eight sites along the Waiotahe river mainstem, one tributary site, and one site in Te Ahiaua, seasonally (e.g., March, July, October, and December) in 2018. Bold denotes p-value >0.05%. Variables that best represent the variance in community structure have a red R^2.

Order	Df	*F*-Statistic	R^2	*p*-val
Site	1	6.77	0.048	**0.001**
Land use	6	1.30	0.055	0.157
Substrate	1	2.86	0.020	**0.037**
Season	3	4.64	0.098	**0.001**
Land use : Site	2	0.06	0.001	1.000
Site : Substrate	1	5.06	0.036	**0.004**
Land use : Substrate	6	1.11	0.047	0.328
Site : Season	3	0.08	0.017	0.630
Land use : Season	18	0.27	0.034	1.000
Substrate : Season	3	0.99	0.021	0.444
Land use : Site : Substrate	2	0.02	0.000	1.000
Land use : Site : Season	6	0.05	0.002	1.000
Site : Substrate : Season	3	0.98	0.021	0.424
Land use : Substrate : Season	18	0.26	0.033	1.000
Land use : Site : Substrate : Season	6	0.05	0.002	1.000

Table 6. PCoA results, including distances, R^2, and p-values for the microbial orders present in each of the 160 samples collected from each of the eight sites along the Waiotahe river mainstem, one tributary site, and one site in Te Ahiaua, seasonally (e.g., March, July, October, and December) in 2018. **Bold** denotes R^2 >0.5 and/or p-value <0.05%. The metric that best represented the variance in community structure is red.

	Microbial orders																			
	Annual				**March / autumn**				**July / winter**				**October / spring**				**December / summer**			
	Dim 1	**Dim 2**	**R^2**	**p-val**	**Dim 1**	**Dim 2**	**R^2**	**p-val**	**Dim 1**	**Dim 2**	**R^2**	**p-val**	**Dim 1**	**Dim 2**	**R^2**	**p-val**	**Dim 1**	**Dim 2**	**R^2**	**p-val**
Metrics																				
Site	-0.273	-0.962	0.199	**0.001**	-0.078	-0.997	0.166	**0.029**	-0.852	0.524	0.056	0.337	-0.852	0.524	0.056	0.337	-0.233	-0.973	0.301	**0.002**
Richness	0.989	-0.146	0.894	**0.001**	0.990	-0.142	0.905	**0.001**	0.934	-0.357	0.754	**0.001**	0.934	-0.357	0.754	**0.001**	0.998	-0.066	0.973	**0.001**
Simpson's Diversity Index	0.849	0.528	0.105	**0.001**	0.246	0.969	0.065	0.287	-0.036	0.999	0.017	0.729	-0.036	0.999	0.017	0.729	0.110	0.994	0.057	0.336
Water column *E. coli*/*E. cloacae*	0.284	-0.959	0.010	0.461	-0.256	-0.967	0.212	**0.010**	-0.256	0.967	0.029	0.600	-0.256	0.967	0.029	0.600	0.667	-0.745	0.001	0.979
Sediment *E. coli*/*E. cloacae*	0.888	0.460	0.004	0.772	0.132	0.991	0.018	0.712	-0.351	0.936	0.018	0.738	-0.351	0.936	0.018	0.738	0.221	0.975	0.298	**0.004**
Environmental Variables																				
Low Flow	0.297	0.955	0.082	**0.006**	0.111	0.994	0.063	0.288	0.715	-0.699	0.032	0.575	0.715	-0.699	0.032	0.575	0.218	0.976	0.105	0.118
Jan Air Temp.	0.264	0.965	0.072	**0.002**	0.123	0.992	0.060	0.306	0.690	-0.723	0.032	0.576	0.690	-0.723	0.032	0.576	0.217	0.976	0.100	0.130
Flow	0.295	0.956	0.086	**0.004**	-0.232	0.973	0.054	0.364	0.553	0.833	0.029	0.591	0.553	0.833	0.029	0.591	0.267	0.964	0.120	0.097
Flow4th	0.299	0.954	0.073	**0.008**	0.133	0.991	0.054	0.335	0.668	-0.744	0.030	0.598	0.668	-0.744	0.030	0.598	0.214	0.977	0.087	0.177

Microbial orders																				
	Annual				March / autumn				July / winter				October / spring				December / summer			
	Dim 1	Dim 2	R^2	p-val	Dim 1	Dim 2	R^2	p-val	Dim 1	Dim 2	R^2	p-val	Dim 1	Dim 2	R^2	p-val	Dim 1	Dim 2	R^2	p-val
Flow Variability	0.082	-0.997	0.001	0.960	0.963	-0.268	0.009	0.852	0.038	-0.999	0.020	0.688	0.038	-0.999	0.020	0.688	-0.436	-0.900	0.004	0.943
Slope	0.347	0.938	0.038	0.052	-0.475	0.880	0.024	0.657	0.341	0.940	0.045	0.432	0.341	0.940	0.045	0.432	0.231	0.973	0.047	0.431
Slope Sqrt	0.216	0.976	0.031	0.099	0.052	0.999	0.022	0.689	0.904	0.427	0.001	0.987	0.904	0.427	0.001	0.987	0.146	0.989	0.024	0.642
Riparian Shade	-0.291	-0.957	0.117	**0.001**	-0.053	-0.999	0.092	0.163	-0.871	0.492	0.044	0.415	-0.871	0.492	0.044	0.415	-0.253	-0.968	0.192	**0.027**
Historic Shade	0.313	0.950	0.016	0.294	0.344	0.939	0.014	0.809	0.339	-0.941	0.015	0.774	0.339	-0.941	0.015	0.774	0.159	0.987	0.013	0.878
Riparian Native	-0.290	-0.957	0.068	**0.002**	-0.190	-0.982	0.068	0.289	-0.565	0.825	0.040	0.474	-0.565	0.825	0.040	0.474	-0.254	-0.967	0.118	0.104
Clues N	-0.320	-0.948	0.011	0.421	-0.356	-0.934	0.010	0.888	-0.333	0.943	0.012	0.811	-0.333	0.943	0.012	0.811	-0.153	-0.988	0.009	0.934
Distance to Coast	-0.271	-0.962	0.180	**0.001**	-0.056	-0.998	0.148	**0.039**	-0.910	0.414	0.047	0.412	-0.910	0.414	0.047	0.412	-0.237	-0.971	0.275	**0.006**
Downstream Average Slope	0.257	0.966	0.336	**0.001**	0.211	0.977	0.352	**0.002**	0.450	-0.893	0.145	0.057	0.450	-0.893	0.145	0.057	0.215	0.977	0.459	**0.001**
Downstream Average Slope Sqrt	0.257	0.966	0.333	**0.001**	0.211	0.977	0.349	**0.002**	0.451	-0.893	0.144	0.059	0.451	-0.893	0.144	0.059	0.215	0.977	0.455	**0.001**
Downstream Max Local Slope	-0.264	-0.965	0.072	**0.002**	0.232	-0.973	0.054	0.364	-0.553	-0.833	0.029	0.591	-0.553	-0.833	0.029	0.591	-0.267	-0.964	0.120	0.097

Microbial orders																				
	Annual				March / autumn				July / winter				October / spring				December / summer			
	Dim 1	Dim 2	R^2	p-val	Dim 1	Dim 2	R^2	p-val	Dim 1	Dim 2	R^2	p-val	Dim 1	Dim 2	R^2	p-val	Dim 1	Dim 2	R^2	p-val
Upstream Days Rain	0.149	-0.989	0.000	0.993	0.944	-0.329	0.003	0.963	-0.002	-1.000	0.008	0.871	-0.002	-1.000	0.008	0.871	-0.430	-0.903	0.003	0.974
Upstream Average Slope	0.444	0.896	0.001	0.935	0.880	0.475	0.002	0.979	0.124	-0.992	0.007	0.894	0.124	-0.992	0.007	0.894	-0.986	0.169	0.000	0.999
Upstream Calcium	-0.280	-0.960	0.051	**0.015**	0.294	-0.956	0.040	0.470	-0.516	-0.857	0.029	0.566	-0.516	-0.857	0.029	0.566	-0.275	-0.961	0.094	0.165
Upstream Hardness	0.292	0.956	0.054	**0.017**	-0.358	0.934	0.040	0.448	0.497	0.868	0.035	0.502	0.497	0.868	0.035	0.502	0.276	0.961	0.091	0.177
Upstream Phosphorus	-0.197	-0.980	0.001	0.938	0.928	-0.373	0.008	0.876	-0.079	-0.997	0.017	0.722	-0.079	-0.997	0.017	0.722	-0.394	-0.919	0.007	0.891
Upstream Indigenous Forrest	-0.206	-0.979	0.001	0.915	0.711	-0.704	0.004	0.953	-0.122	-0.993	0.008	0.874	-0.122	-0.993	0.008	0.874	-0.352	-0.936	0.007	0.910
Upstream Native	-0.197	-0.980	0.001	0.925	0.721	-0.693	0.004	0.953	-0.115	-0.993	0.008	0.881	-0.115	-0.993	0.008	0.881	-0.356	-0.934	0.007	0.913
Upstream Pasture	-0.443	-0.897	0.001	0.919	-0.837	-0.548	0.002	0.986	-0.162	0.987	0.006	0.907	-0.162	0.987	0.006	0.907	0.715	-0.699	0.000	0.999
Reach Sediment	0.326	0.945	0.011	0.426	0.243	0.970	0.007	0.930	0.473	-0.881	0.008	0.881	0.473	-0.881	0.008	0.881	0.160	0.987	0.009	0.930
Reach Habitat	0.383	0.924	0.032	0.086	0.286	0.958	0.026	0.628	0.539	-0.843	0.036	0.516	0.539	-0.843	0.036	0.516	0.209	0.978	0.040	0.461
Width in m	0.323	0.946	0.010	0.478	0.100	0.995	0.025	0.637	0.610	-0.793	0.003	0.954	0.610	-0.793	0.003	0.954	0.236	0.972	0.006	0.901
Depth in cm	0.247	0.969	0.103	**0.002**	0.193	0.981	0.118	0.081	0.434	-0.901	0.039	0.489	0.434	-0.901	0.039	0.489	0.236	0.972	0.154	**0.045**
Velocity m/s	-0.263	-0.965	0.073	**0.004**	-0.302	-0.953	0.097	0.156	-0.303	0.953	0.041	0.462	-0.303	0.953	0.041	0.462	-0.216	-0.977	0.147	0.054

Microbial orders																				
	Annual				March / autumn				July / winter				October / spring				December / summer			
	Dim 1	Dim 2	R^2	p-val	Dim 1	Dim 2	R^2	p-val	Dim 1	Dim 2	R^2	p-val	Dim 1	Dim 2	R^2	p-val	Dim 1	Dim 2	R^2	p-val
Temperature	-0.346	-0.938	0.086	**0.001**	-0.193	-0.981	0.155	0.046	-0.527	0.850	0.020	0.677	-0.527	0.850	0.020	0.677	-0.203	-0.979	0.185	0.026
Conductivity	-0.248	-0.969	0.118	**0.001**	-0.277	-0.961	0.134	0.063	-0.333	0.943	0.078	0.211	-0.333	0.943	0.078	0.211	-0.195	-0.981	0.153	0.075
% Fine Sediment	0.339	0.941	0.016	0.272	0.043	0.999	0.010	0.840	0.660	0.751	0.006	0.874	0.660	0.751	0.006	0.874	0.252	0.968	0.032	0.534
% Riffle	-0.307	-0.952	0.086	**0.002**	0.043	-0.999	0.054	0.349	-0.960	-0.278	0.028	0.574	-0.960	-0.278	0.028	0.574	-0.252	-0.968	0.143	0.072
% Run	-0.210	-0.978	0.046	**0.022**	-0.378	-0.926	0.074	0.235	-0.178	0.984	0.058	0.328	-0.178	0.984	0.058	0.328	-0.154	-0.988	0.048	0.421
% Macrophyte Cover	-0.302	-0.953	0.022	0.188	-0.276	-0.961	0.018	0.742	-0.402	0.916	0.015	0.786	-0.402	0.916	0.015	0.786	-0.177	-0.984	0.019	0.776
% Debris Jam	-0.421	-0.907	0.019	0.214	-0.153	-0.988	0.008	0.920	-0.860	0.511	0.014	0.777	-0.860	0.511	0.014	0.777	-0.264	-0.964	0.037	0.524
Periphyton	-0.310	-0.951	0.034	0.076	-0.274	-0.962	0.028	0.592	-0.415	0.910	0.020	0.717	-0.415	0.910	0.020	0.717	-0.149	-0.989	0.044	0.462
Substrate size	-0.284	-0.959	0.113	**0.001**	0.079	-0.997	0.082	0.203	-0.853	-0.521	0.034	0.497	-0.853	-0.521	0.034	0.497	-0.239	-0.971	0.174	**0.028**
Embeddedness	-0.283	-0.959	0.079	**0.001**	0.044	-0.999	0.059	0.317	-0.988	-0.157	0.025	0.616	-0.988	-0.157	0.025	0.616	-0.252	-0.968	0.136	0.080
Stream Shade	-0.263	-0.965	0.061	**0.010**	-0.393	-0.920	0.125	0.091	-0.240	0.971	0.119	0.099	-0.240	0.971	0.119	0.099	-0.208	-0.978	0.095	0.165

Table 7. PERMANOVA results for explanatory variables associated with community composition for the genera from each of the 160 samples collected from the eight sites along the Waiotahe river mainstem, one tributary site, and one site in Te Ahiaua, seasonally (e.g., March, July, October, and December) in 2018. Bold denotes p-value >0.05%. Variables that best represent the variance in community structure have a red R^2.

Genus	**Df**	***F*-Statistic**	**R^2**	***p*-val**
Site	1	5.18	0.037	**0.001**
Land use	6	1.07	0.046	0.358
Substrate	1	2.41	0.017	**0.045**
Season	3	4.45	0.097	**0.001**
Land use : Site	2	0.14	0.002	1.000
Site : Substrate	1	4.14	0.030	**0.004**
Land use : Substrate	6	1.07	0.046	0.369
Site : Season	3	0.98	0.021	0.432
Land use : Season	18	0.32	0.04	1.000
Substrate : Season	3	0.81	0.018	0.654
Land use : Site : Substrate	2	0.04	0.001	1.000
Land use : Site : Season	6	0.12	0.005	1.000
Site : Substrate : Season	3	0.85	0.018	0.591
Land use : Substrate : Season	18	0.30	0.039	1.000
Land use : Site : Substrate : Season	6	0.06	0.003	1.000

Table 8. PCoA results, including distances, R^2, and p-values for the microbial classes present in each of the 160 samples collected from each of the eight sites along the Waiotahe river mainstem, one tributary site, and one site in Te Ahiaua, seasonally (e.g., March, July, October, and December) in 2018. **Bold** denotes R^2 >0.5 and/or p-value <0.05%. The metric that best represented the variance in community structure is red.

Microbial genera																				
	Annual				March / autumn				July / winter				October / spring				December / summer			
	Dim 1	Dim 2	R^2	p-val	Dim 1	Dim 2	R^2	p-val	Dim 1	Dim 2	R^2	p-val	Dim 1	Dim 2	R^2	p-val	Dim 1	Dim 2	R^2	p-val
Metrics																				
Site	-0.230	-0.973	0.229	**0.001**	-0.057	-0.998	0.193	**0.021**	-0.597	-0.802	0.066	0.278	-0.534	-0.845	0.243	**0.008**	-0.224	-0.975	0.318	**0.001**
Richness	0.855	-0.518	0.623	**0.001**	0.738	-0.675	0.717	**0.001**	0.661	-0.750	0.544	**0.001**	0.981	-0.196	0.769	**0.001**	0.848	-0.530	0.761	**0.001**
Simpson's Diversity Index	0.064	0.998	0.026	0.114	0.237	0.971	0.021	0.687	-0.591	0.807	0.002	0.960	0.093	0.996	0.198	**0.015**	-0.774	-0.633	0.015	0.748
Water column *E. coli*/*E. cloacae*	0.337	-0.942	0.006	0.609	-0.172	-0.985	0.188	**0.023**	-0.061	-0.998	0.046	0.436	0.658	0.753	0.146	0.052	0.912	-0.409	0.001	0.975
Sediment *E. coli*/*E. cloacae*	0.597	0.803	0.006	0.635	0.216	0.976	0.018	0.732	-0.116	-0.993	0.030	0.611	0.539	0.842	0.025	0.623	0.207	0.978	0.330	**0.001**
Environmental Variables																				
Low Flow	0.253	0.967	0.098	**0.002**	0.156	0.988	0.065	0.271	0.394	0.919	0.044	0.475	0.609	0.793	0.112	0.099	0.203	0.979	0.119	0.104
Jan Air Temp.	0.237	0.971	0.091	**0.001**	0.164	0.986	0.062	0.293	0.377	0.926	0.045	0.464	0.614	0.789	0.104	0.118	0.202	0.979	0.112	0.117
Flow	0.252	0.968	0.103	**0.002**	-0.077	0.997	0.074	0.244	0.861	-0.509	0.026	0.603	0.558	0.830	0.174	**0.038**	0.244	0.970	0.137	0.063
Flow4th	0.255	0.967	0.087	**0.002**	0.176	0.984	0.054	0.337	0.358	0.934	0.043	0.486	0.621	0.784	0.091	0.145	0.200	0.980	0.098	0.148

Microbial genera																				
	Annual				March / autumn				July / winter				October / spring				December / summer			
	Dim 1	Dim 2	R^2	p-val	Dim 1	Dim 2	R^2	p-val	Dim 1	Dim 2	R^2	p-val	Dim 1	Dim 2	R^2	p-val	Dim 1	Dim 2	R^2	p-val
Flow Variability	-0.036	-0.999	0.001	0.914	0.576	-0.817	0.009	0.851	-0.150	0.989	0.029	0.580	-0.340	-0.941	0.014	0.773	-0.337	-0.942	0.005	0.924
Slope	0.311	0.950	0.049	**0.020**	-0.197	0.980	0.031	0.565	0.663	-0.749	0.031	0.566	0.720	0.694	0.174	**0.029**	0.218	0.976	0.061	0.335
Slope Sqrt	0.158	0.987	0.041	**0.044**	0.092	0.996	0.026	0.620	0.256	0.967	0.003	0.950	0.372	0.928	0.070	0.262	0.096	0.995	0.049	0.395
Riparian Shade	-0.256	-0.967	0.136	**0.001**	-0.064	-0.998	0.110	0.111	-0.660	-0.751	0.047	0.416	-0.605	-0.797	0.140	0.060	-0.255	-0.967	0.186	**0.014**
Historic Shade	0.257	0.966	0.018	0.221	0.449	0.894	0.012	0.863	0.101	0.995	0.026	0.650	0.650	0.760	0.012	0.822	0.138	0.990	0.016	0.828
Riparian Native	-0.247	-0.969	0.076	**0.002**	-0.123	-0.992	0.074	0.238	-0.440	-0.898	0.043	0.457	-0.555	-0.832	0.043	0.443	-0.270	-0.963	0.103	0.125
Clues N	-0.265	-0.964	0.014	0.340	-0.509	-0.861	0.009	0.918	-0.090	-0.996	0.022	0.710	-0.680	-0.733	0.009	0.867	-0.129	-0.992	0.011	0.905
Distance to Coast	-0.229	-0.973	0.209	**0.001**	-0.045	-0.999	0.179	**0.030**	-0.669	-0.744	0.053	0.350	-0.530	-0.848	0.234	**0.009**	-0.228	-0.974	0.294	**0.001**
Downstream Average Slope	0.204	0.979	0.381	**0.001**	0.143	0.990	0.349	**0.001**	0.270	0.963	0.193	**0.015**	0.403	0.915	0.265	**0.003**	0.201	0.980	0.501	**0.001**
Downstream Average Slope Sqrt	0.204	0.979	0.377	**0.001**	0.142	0.990	0.346	**0.001**	0.271	0.963	0.191	**0.018**	0.404	0.915	0.263	**0.003**	0.201	0.980	0.497	**0.001**
Downstream Max Local Slope	-0.237	-0.971	0.091	**0.001**	0.077	-0.997	0.074	0.244	-0.861	0.509	0.026	0.603	-0.558	-0.830	0.174	**0.038**	-0.244	-0.970	0.137	0.063

Microbial genera																				
	Annual				March / autumn				July / winter				October / spring				December / summer			
	Dim 1	Dim 2	R^2	p-val	Dim 1	Dim 2	R^2	p-val	Dim 1	Dim 2	R^2	p-val	Dim 1	Dim 2	R^2	p-val	Dim 1	Dim 2	R^2	p-val
Upstream Days Rain	0.017	-1.000	0.000	0.998	0.597	-0.802	0.006	0.931	-0.196	0.981	0.015	0.785	-0.382	-0.924	0.003	0.956	-0.490	-0.872	0.002	0.981
Upstream Average Slope	0.346	0.938	0.001	0.944	0.931	-0.364	0.004	0.964	-0.088	0.996	0.014	0.801	0.743	-0.669	0.000	0.999	-0.651	0.759	0.000	1.000
Upstream Calcium	-0.259	-0.966	0.064	**0.003**	0.118	-0.993	0.054	0.358	-0.800	0.601	0.028	0.587	-0.610	-0.792	0.135	0.073	-0.264	-0.965	0.101	0.150
Upstream Hardness	0.274	0.962	0.070	**0.002**	-0.115	0.993	0.048	0.407	0.808	-0.589	0.029	0.556	0.642	0.767	0.160	**0.037**	0.258	0.966	0.101	0.153
Upstream Phosphorus	-0.268	-0.964	0.001	0.897	0.557	-0.831	0.010	0.858	-0.265	0.964	0.025	0.633	-0.590	-0.807	0.013	0.781	-0.412	-0.911	0.006	0.908
Upstream Indigenous Forrest	-0.226	-0.974	0.001	0.897	0.429	-0.904	0.008	0.892	-0.311	0.951	0.014	0.785	-0.536	-0.844	0.007	0.890	-0.394	-0.919	0.006	0.928
Upstream Native	-0.221	-0.975	0.001	0.913	0.438	-0.899	0.008	0.898	-0.304	0.953	0.013	0.788	-0.528	-0.849	0.006	0.904	-0.401	-0.916	0.006	0.938
Upstream Pasture	-0.360	-0.933	0.001	0.923	-0.947	0.322	0.003	0.970	0.062	-0.998	0.012	0.826	-0.980	0.199	0.000	0.994	0.417	-0.909	0.000	0.999
Reach Sediment	0.277	0.961	0.014	0.332	0.433	0.901	0.007	0.930	0.158	0.987	0.014	0.809	0.704	0.710	0.015	0.781	0.135	0.991	0.012	0.880
Reach Habitat	0.344	0.939	0.034	0.067	0.289	0.957	0.020	0.701	0.298	0.954	0.047	0.441	0.910	0.414	0.029	0.581	0.251	0.968	0.033	0.524
Width in m	0.305	0.952	0.010	0.428	0.087	0.996	0.027	0.621	0.234	0.972	0.005	0.898	0.973	0.232	0.005	0.910	0.246	0.969	0.009	0.849
Depth in cm	0.192	0.981	0.114	**0.001**	0.113	0.994	0.119	0.104	0.286	0.958	0.049	0.390	0.328	0.945	0.074	0.204	0.221	0.975	0.169	**0.028**
Velocity m/s	-0.175	-0.984	0.098	**0.001**	-0.170	-0.985	0.087	0.181	-0.172	-0.985	0.052	0.358	-0.270	-0.963	0.104	0.128	-0.200	-0.980	0.153	**0.040**

Microbial genera																			
	Annual				March / autumn				July / winter				October / spring				December / summer			
	Dim 1	Dim 2	R^2	p-val	Dim 1	Dim 2	R^2	p-val	Dim 1	Dim 2	R^2	p-val	Dim 1	Dim 2	R^2	p-val	Dim 1	Dim 2	R^2	p-val
Temperature	-0.347	-0.938	0.080	**0.002**	-0.169	-0.986	0.161	**0.047**	-0.297	-0.955	0.027	0.618	-0.431	-0.902	0.014	0.752	-0.181	-0.984	0.214	**0.017**
Conductivity	-0.193	-0.981	0.137	**0.001**	-0.214	-0.977	0.124	0.076	-0.166	-0.986	0.104	0.118	-0.307	-0.952	0.072	0.233	-0.175	-0.984	0.167	**0.032**
% Fine Sediment	0.282	0.959	0.016	0.259	-0.086	0.996	0.014	0.784	0.906	-0.423	0.007	0.877	0.661	0.750	0.030	0.577	0.247	0.969	0.031	0.559
% Riffle	-0.268	-0.963	0.101	**0.001**	0.005	-1.000	0.073	0.240	-0.984	-0.180	0.028	0.586	-0.627	-0.779	0.153	**0.045**	-0.248	-0.969	0.149	**0.046**
% Run	-0.148	-0.989	0.053	**0.011**	-0.307	-0.952	0.059	0.310	-0.017	-1.000	0.083	0.198	0.050	-0.999	0.019	0.684	-0.127	-0.992	0.055	0.356
% Macrophyte Cover	-0.248	-0.969	0.026	0.118	-0.360	-0.933	0.016	0.795	-0.145	-0.989	0.025	0.665	-0.604	-0.797	0.021	0.707	-0.151	-0.989	0.024	0.689
% Debris Jam	-0.382	-0.924	0.021	0.185	-0.096	-0.995	0.009	0.912	-0.855	-0.518	0.012	0.811	-0.860	-0.509	0.027	0.620	-0.308	-0.951	0.032	0.560
Periphyton	-0.259	-0.966	0.040	**0.041**	-0.304	-0.953	0.024	0.662	-0.154	-0.988	0.035	0.546	-0.600	-0.800	0.031	0.579	-0.137	-0.991	0.054	0.364
Substrate size	-0.247	-0.969	0.135	**0.001**	0.034	-0.999	0.109	0.112	-0.990	-0.139	0.031	0.530	-0.598	-0.802	0.220	**0.012**	-0.230	-0.973	0.193	**0.015**
Embeddedness	-0.251	-0.968	0.093	**0.001**	0.011	-1.000	0.079	0.209	-0.942	-0.335	0.025	0.610	-0.595	-0.803	0.124	0.093	-0.253	-0.967	0.135	0.054
Stream Shade	-0.202	-0.979	0.060	**0.011**	-0.251	-0.968	0.089	0.172	-0.102	-0.995	0.141	0.053	0.118	-0.993	0.003	0.932	-0.237	-0.971	0.076	0.215

Table 9. Specific key relative abundance associations for microbial genera communities from the 160 eDNA samples collected from each of the eight sites along the Waiotahe river mainstem, one tributary site, and one site in Te Ahiaua, seasonally (e.g., March, July, October, and December) in 2018.

Key genera	Site(s)	Substrate	Season(s)	Land use(s)
Limnohabitans	2, 3, 4, 5, 6, 7, 8, 9, 10	Freshwater	Annually	All except estuary
Flavobacterium	2, 3, 4, 5, 6, 7, 8, 9, 10	Freshwater	Annually	All except estuary
Curvibacter	2, 3, 4, 5, 6, 7, 8, 9, 10	Freshwater	Annually	All except estuary
Rhodoferax	2, 3, 4, 5, 6, 7, 8, 9, 10	Freshwater	Annually	All except estuary
Agrobacterium	4	Freshwater	Autumn	Dairy
	5 and 8	Sediment	Summer	Dairy and Exotic Forest
Acinetobacter	1	Saline water	Summer	Estuary
Photobacterium	3	Sediment	Autumn	Intensive Dairy
Nonomuraea	1, 9, 10	Fresh and saline water	Summer	Estuary and Native Forest
Pseudomonas	5	Freshwater	Winter	Dairy
Mycolicibacterium	2	Sediment	Spring	Effluent Station
Nocardia	1, 2, 3, 6	Saline sediment	Summer	Estuary
	2	Freshwater	Spring	Effluent Station
	3	Freshwater	Summer	Intensive Dairy
	6	Freshwater sediment	Autumn	Beef and Sheep
Staphylococcus	1	Saline sediment	Spring	Estuary
	2, 4, 8	Freshwater sediment	Summer	Effluent Station, Dairy, Exotic Forest
	8	Freshwater	Winter	Exotic Forest

Key genera	Site(s)	Substrate	Season(s)	Land use(s)
Lactobacillus	2 and 10	Freshwater sediment	Spring	Effluent Station and Native Forest
Jishengella	1	Saline water	Spring	Estuary
	10	Freshwater sediment	Autumn	Native Forest
	3	Freshwater sediment	Summer	Intensive Dairy
Rhodoferax	3 and 10	Freshwater	Autumn	Intensive Dairy and Native Forest
Curvibacter	3 and 10	Freshwater	Autumn	Intensive Dairy and Native Forest
Ralstonia	2, 4, 5, 8, 9	Freshwater sediment	Autumn	Effluent Station, Dairy, Exotic and Native Forest
Corallococcus	2	Freshwater	Spring	Effluent Station
	1 and 2	Freshwater sediment	Summer	Estuary and Effluent Station
	5 and 8	Freshwater	Summer	Dairy and Exotic Forest
	6	Freshwater sediment	Winter	Beef and Sheep
Winogradskyella	1	Saline water	Spring	Estuary

Table 10. The most abundant and key genera identified for microbial communities from the 160 eDNA samples collected from each of the eight sites along the Waiotahe river mainstem, one tributary site, and one site in Te Ahiaua, seasonally (e.g., autumn, winter, spring, and summer) in 2018. Some of the important ecological and biogeochemical traits along with the potential for pathogenicity are noted. **Bold** taxa names identifies those with plant, animal, and/or human pathogenic potential.

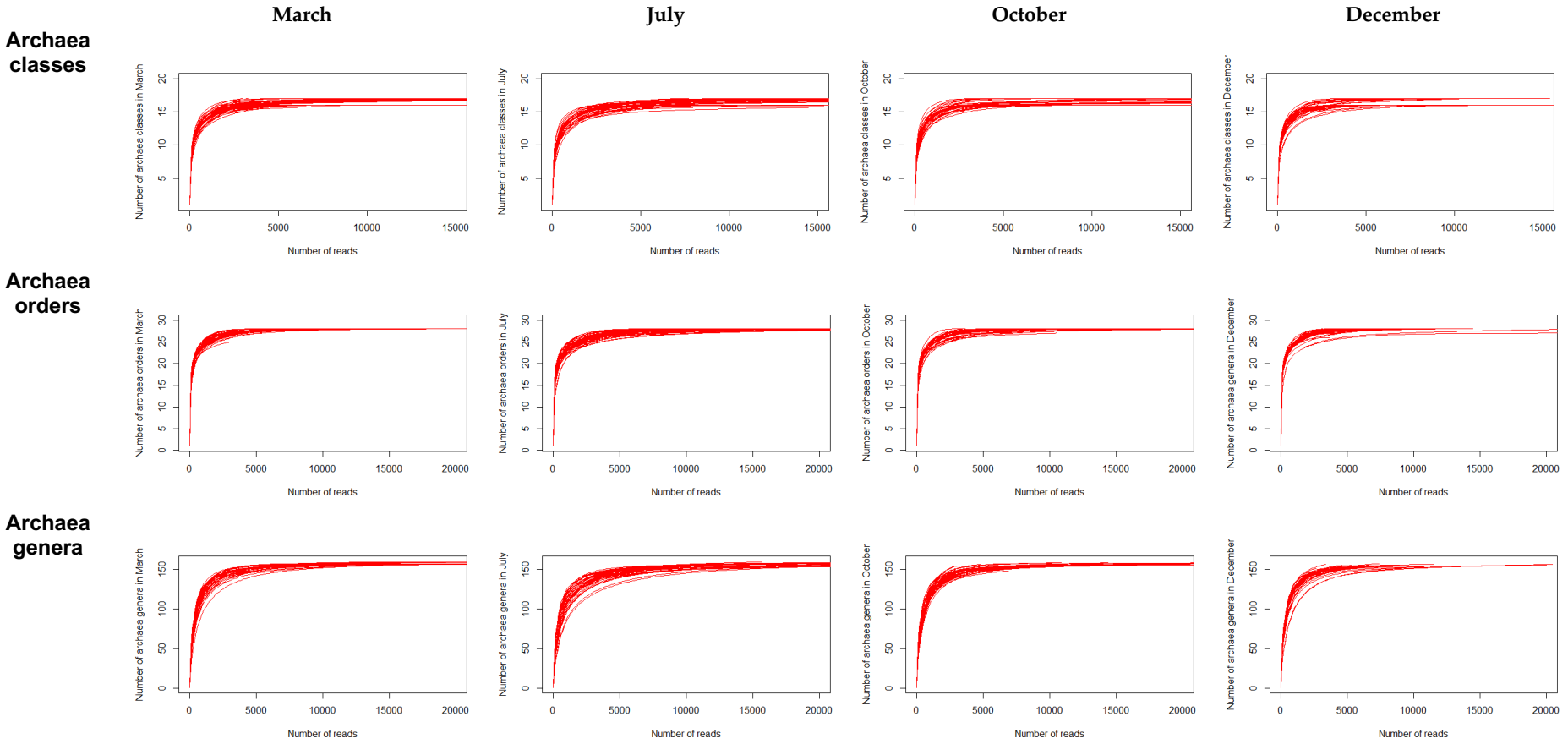
Taxa	Domain and Type	Taxonomic Level	Type of taxa	Importance	Reference
Winogradskyella	Bacteria	Genus	Abundant	Algicidal properties and some species cause skin ulcers in fish	(Begum et al., 2013)
Staphylococcus	Bacteria	Genus	Abundant	Contains human pathogenic species	(Chambers, 2001)
Limnohabitans	Bacteria Plankton	Genus	Abundant	Planktonic bacteria	(Kasalický et al., 2013)
Clostridioides	Bacteria	Genus	Abundant	Contains human pathogenic (toxin-producing) and arsenic cycling species	(Suhadolnik et al., 2017)
Salmonella	Bacteria	Genus	Abundant	Most species are pathogenic to humans and/or animals	(Groisman & Ochman, 1997)
Flavobacterium	Bacteria	Genus	Abundant	Contains fish pathogenic species	(Bernardet & Bowman, 2006)
Curvibacter	Bacteria	Genus	Abundant	Nitrate-reducing, iron-oxidising	(Gülay et al., 2018)
Rhodoferax	Bacteria Purple non-sulfur	Genus	Abundant	Iron oxidising and fermenting,	(Kaden et al., 2014)
Nocardia	Bacteria	Genus	Abundant	Contains some opportunistic human pathogen species	(Conville et al., 2018)
Pseudomonas	Bacteria	Genus	Abundant	Contains opportunistic human pathogenic species	(Spiers, Buckling & Rainey, 2000)
Streptomyces	Bacteria	Genus	Abundant	Antibiotic production, contains infrequent plant and human pathogenic species	(Procópio et al., 2012)
Bradyrhizobium	Bacteria	Genus	Abundant	Nitrogen fixing symbiote in legumes	(Ormeño-Orrillo &

Taxa	Domain and Type	Taxonomic Level	Type of taxa	Importance	Reference
					Martínez-Romero, 2019)
Ralstonia	Bacteria	Genus	Key	Contains plant and opportunistic human pathogenic species and found in deer and goat milk	(Genin & Denny, 2012)
Corallococcus	Bacteria Predatory Myxobacteria	Genus	Key	Antimicrobial production, mobile, synthesise secondary metabolites, typically lives in soil habitats but can occur in freshwater (never marine)	(Li et al., 2017)
Lactobacillus	Bacteria	Genus	Key	Mutualistic in mammals	(Hammes & Vogel, 1995)
Nonomuraea	Eukaryote 'Rare' Actinobacter	Genus	Key	Some species produce glycopeptide antibiotics (last line of defence), others degrade biopolymers	(Sungthong & Nakaew, 2015)
Jishengella	Bacteria	Genus	Key	Mangrove root mutualist	(Thawai, He & Tadtong, 2018)
Agrobacterium	Bacteria	Genus	Key	Uses horizontal gene transfer to cause tumours on plants, phytopathogen	(Gelvin, 2009)
Acinetobacter	Bacteria	Genus	Key	Contains human pathogenic species (labelled a 'superbug')	(Towner, 2009)
Photobacterium	Bacteria	Genus	Key	Some species are bioluminescent which is density dependant, salt used for growth	(Urbanczyk, Ast & Dunlap, 2011)
Mycolicibacterium	Bacteria	Genus	Key	Contains human pathogenic species, nitrate oxidising, organic matter decomposition	(Moi et al., 2017)

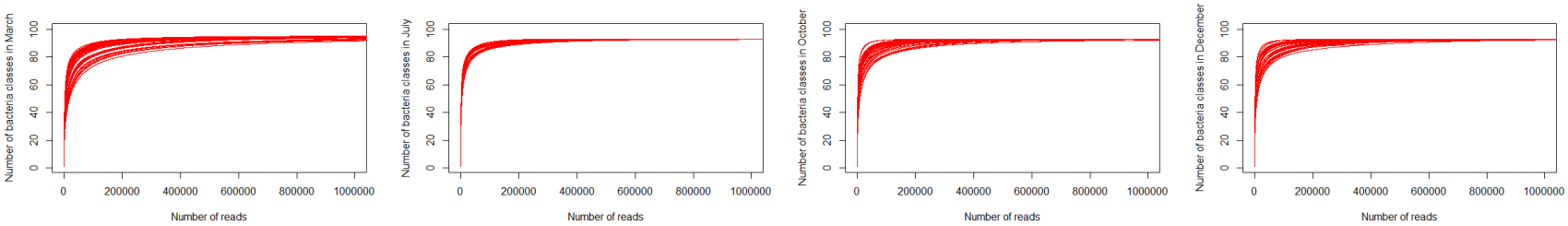
Table 11. PERMANOVA results for class, order, and genera spatio-temporal variables (e.g., season – annual, autumn, winter, spring, and summer, substrate – both, sediment, and water, land use, and the percent of the total biome) associated with the core biome communities at each of the eight sites along the Waiotahe river mainstem, one tributary site, and one site in Te Ahiaua, in 2018. **Bold** denotes p-value <0.05%. The factor that best represent the variance in community structure have a red R^2.

Variable	Df	*F*-statistic	R^2	*p*-val
Class				
Season	4	2.63	0.111	**0.016**
Substrate	2	7.25	0.153	**0.001**
Land use	8	3.68	0.310	**0.001**
% of the Total Biome	1	17.50	0.184	**0.001**
Order				
Season	4	8.98	0.230	**0.001**
Substrate	2	25.14	0.322	**0.001**
Land use	8	4.42	0.227	**0.001**
% of the Total Biome	1	9.25	0.059	**0.001**
Genera				
Season	4	28.50	0.458	**0.001**
Substrate	2	23.02	0.185	**0.001**
Land use	8	8.13	0.261	**0.001**
% of the Total Biome	1	0.80	0.003	0.556

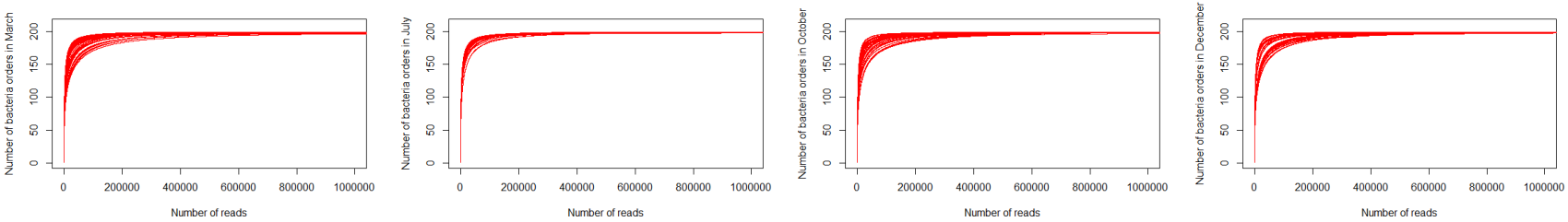
Figures


March
July
October
December
Archaea classes
Archaea orders
Archaea genera
Number of archaea classes in March
Number of archaea classes in July
Number of archaea classes in October
Number of archaea classes in December
Number of archaea orders in March
Number of archaea orders in July
Number of archaea orders in October
Number of archaea genera in December
Number of archaea genera in March
Number of archaea genera in July
Number of archaea genera in October
Number of reads

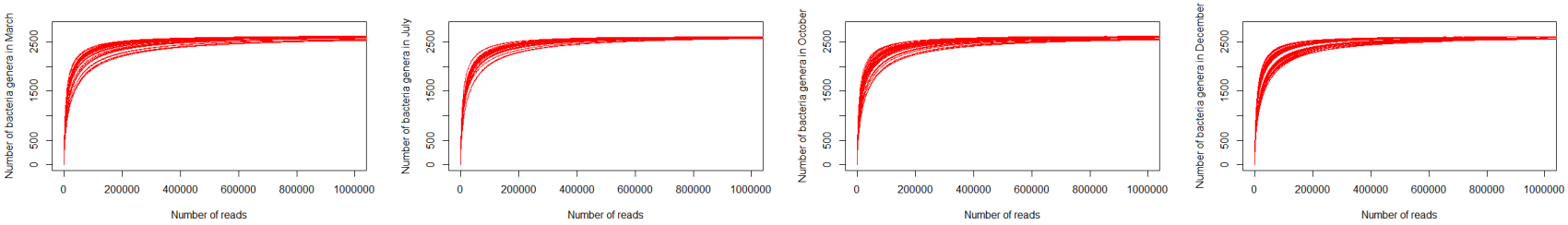
Bacteria classes



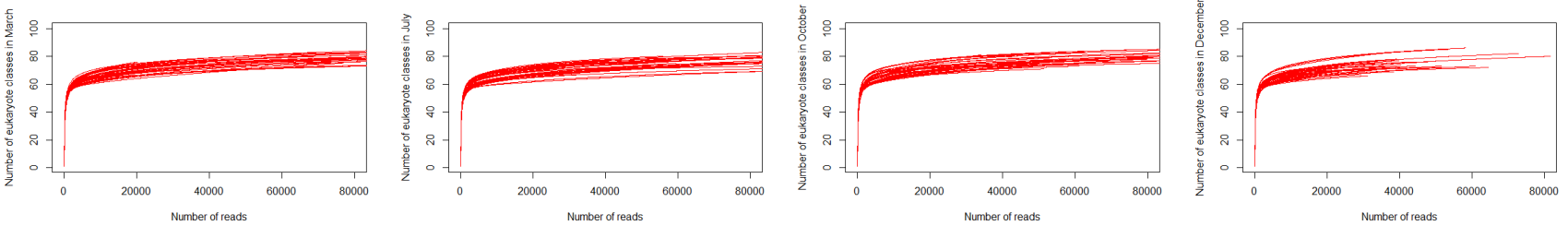
Bacteria orders



Bacteria genera



Eukaryote classes



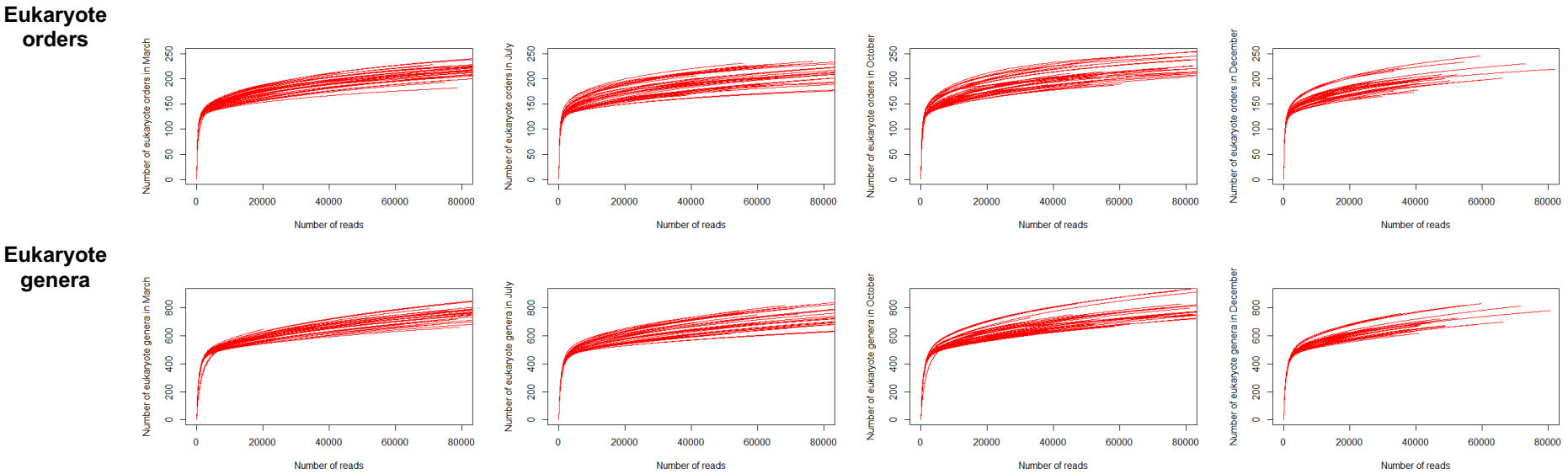


Figure 1. Class, order, and genus, seasonal rarefaction curves for the three microbial domains (i.e., archaea, bacteria, and eukaryotes) from each of the eight sites along the Waiotahe river mainstem, one tributary site, and one site in Te Ahiaua, seasonally (e.g., March, July, October, and December) in 2018.

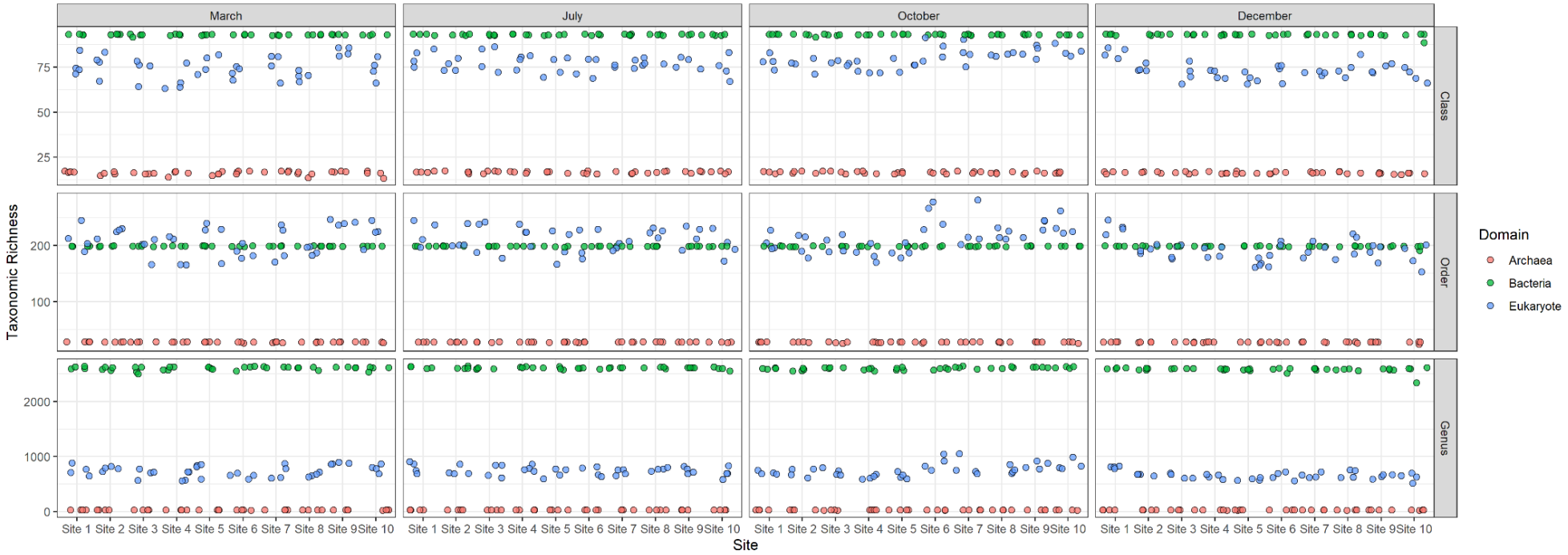


Figure 2. Taxonomic richness for the three microbial domains at class, order, and genus for from each of the eight sites along the Waiotahe river mainstem, one tributary site, and one site in Te Ahiaua, seasonally (e.g., March, July, October, and December) in 2018.

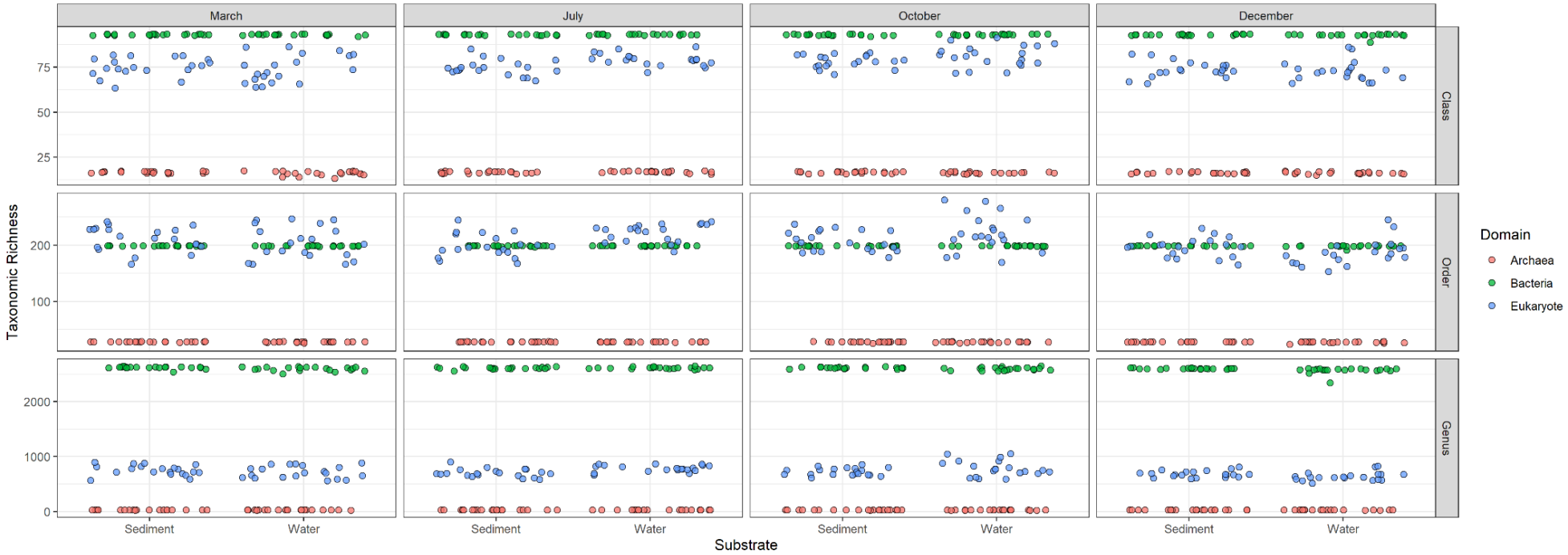


Figure 3. Taxonomic richness in water and sediment for the three microbial domains at the class, order, and genus taxonomic levels across from each of the eight sites along the Waiotahe river mainstem, one tributary site, and one site in Te Ahiaua, seasonally (e.g., March, July, October, and December) in 2018.

Preface to Chapter 7

“I was still water, held by my surroundings. I am now a river, carving my own path.”

– Scott Stabile

7 Synthesis

Implications for monitoring water quality in Aotearoa

Water, the hypothesised environment where life on earth originated, is a victim of poor prioritisation. That water covers ~70% of the planet and is essential to all known life only serves to highlight our dearth of knowledge regarding aquatic microbial communities. The goal of this thesis was to better understand how microbial communities were structured and explore the knowledge gaps in monitoring recreational water for public health in Aotearoa. I wanted to explore the riverscape holistically and determine whether current recreational water quality monitoring (e.g., water samples from rivers and estuaries are assessed for faecal indicator bacteria) was sufficient (Wright, 2015; Gluckman, 2017). This holistic approach was adhered to in each experimental chapter by monitoring both water and benthic substrate samples. Riverine and estuarine beach sands are well documented refuges for faecal bacteria and zoonotic organisms (McDonald, Kay & Jenkins, 1982; Hartz et al., 2008; Byappanahalli & Ishii, 2011). I hypothesised that sediments in Aotearoa's waterways were likely unaccounted stores of human pathogens and that by monitoring water column samples only, current water quality monitoring ignored how people used waterways (NEMS Steering Group, 2019). When entering a river or beach, people dislodged substrates with their feet, resuspending benthic sediments within the water column. Importantly, this resuspension of sediments into the water column is not accounted for in Aotearoa's risk analysis for recreational waterway users. The prevalence and abundance of potentially human pathogenic microbes identified from benthic substrates in the Waiotahe catchment (see chapter 6) and genetic loci associated with human pathogen virulence identified from the Canterbury rivers (see chapter 3) was similar to those in water. Additionally, human enteropathogenic strains of *Escherichia coli* (*E. coli*) (e.g., Shiga-toxin-producing *E. coli* – STEC) settled out of the water column (see chapter 4) within 24 hours in microcosms. These findings should be enough to motivate further investigation into how Aotearoa monitors recreational waterways, with particularly relevance at estuarine beaches where wave action and tidal flows are present, where benthic dredging occurs, during and after heavy precipitation events, and wherever benthic substrates may be disturbed by recreational users.

Next, I wanted to determine whether *E. coli* concentrations, as they were currently being monitored (e.g., water column concentrations) or from benthic sediments, were related to the presence of *Campylobacter*, other faecal bacteria (e.g., *Enterococcus* and *Enterobacter*), or

human pathogen virulence and/or antimicrobial genes. *E. coli* concentrations formed no repeatable relationship to *Campylobacter* relative abundances (see chapter 6), human pathogen virulence or antimicrobial resistance genes (see chapter 3), or to the presence or abundance of enteropathogenic *E. coli* (see chapter 4). Globally, *E. coli*'s use as a proxy for faecal contamination has been scrutinised and called into question (Odonkor & Ampofo, 2013; Sharp et al., 2021). Its use continues, in my opinion, because it is inexpensive and easy. Continuing to test for *E. coli* also gives the appearance of government oversight and care for public health and water quality, even when experts are aware that samples and the water monitoring strategy is woefully unfit for its purpose.

The reality is, by using these cheap and uninformative monitoring techniques, we cannot be certain what is cultured is even *E. coli*. This was confirmed in every experimental chapter of this thesis (see chapters 3-6) where *E. coli* cultures consistently contained *Enterobacter cloacae* (*E. cloacae*) colonies. To critically assess the validity of the methods currently being used in Aotearoa and globally for water quality monitoring, all of the colonies in the *E. coli*/*E. cloacae* group were counted as *E. coli*. This was done as typical water quality testing does not routinely investigate colonies that appear to be *E. coli* for further confirmation. Meaning the *E. cloacae* colonies mixed in with the *E. coli* colonies would never have been identified as 'not *E. coli*'. Despite the evidence that *E. coli* and other faecal bacteria are not good indicators of the presence of waterborne human pathogens, Aotearoa continues to myopically hunt for ways to resurrect *E. coli* as a suitable measure of water quality (Yakirevich et al., 2013; Devane et al., 2020; Holcomb & Stewart, 2020).

In addition to this, *E. coli* (in freshwater) and *Enterococcus* (in saline water) concentrations from recreational water in Aotearoa are used as a direct proxy for the risk of contracting campylobacteriosis (Ministry for the Environment, 2020). None of the *E. coli*/*E. cloacae* concentrations had any strong relationship to *Campylobacter* relative abundances, or sediment *E. coli*/*E. cloacae* concentrations, or even *Escherichia* relative abundances, in the Waiotahe catchment. Nor did *E. coli*/*E. cloacae* concentrations correlate to the presence of Shiga-toxin-producing *E. coli* or antimicrobial resistance genetic elements in the three Canterbury rivers. It is possible that this is a result of using relative abundances, not total read numbers or direct culture counts, or that there is a tipping point (i.e., a point at which the risk of contracting a specific disease increases); however, quantifying this in a scientifically repeatable and valid way has not been undertaken for recreational waters in Aotearoa. Importantly, environmentally sourced *E. coli*/*E. cloacae* concentrations did persist for longer in microcosms when water nitrate-nitrogen levels were at 1 mg/l or 3 mg/l than they did at 0

mg/l (see chapter 4). Why is this important? Well, eutrophication is a global issue and we have a significant nitrate problem in Aotearoa (Stewart & Aitchison-Earl, 2020; McDowell et al., 2021; Joy et al., 2022). If that nitrate problem means that environmental *E. coli*/*E. cloacae* persist for longer we need to understand how that will affect the accuracy of *E. coli* as a predictor of campylobacteriosis. It will likely make it even worse than it is now.

To replace *E. coli* (and all proxies) as the primary water quality measure for human health, alternatives need to be explored. I explored the use of both shotgun metagenomics and targeted PCRs as alternatives. Studies and methodologies have been developed on Metabarcoding using environmental DNA (eDNA) (Fonseca 2018, David et al. 2021). However, I wanted a method that allowed me to use all the DNA present in the environmental samples I collected and I did not want to be restricted by primers. Additionally, I wanted an alternative that was: sensitive, but not so sensitive as to artificially inflate presence; economic, most molecular methods will be more expensive than culturing *E. coli* but it needed to be reasonable; and specific, to reduce our reliance on proxies. While it was outside of the scope of this thesis to propose or build a working methodology to monitor human pathogens in waterways, understanding what could and could not be identified from aquatic samples was a necessary first step on that journey.

Metagenomics was a successful methodology for studying benthic substrates and water column microbial communities. While computer intensive, open source software like Kaiju (Menzel, Ng & Krogh, 2016) made interpretation and visualisation quick and easy. I successfully identified antimicrobial resistance and human pathogen virulence genes, as well as bacteria, archaea, and eukaryotes from river and estuarine water and sediment samples using similar collection techniques to those currently used for water quality monitoring. While the sampling method I employed offered sufficient resolution to study aquatic microorganisms locally, it is unlikely to be sufficient to study mobile, terrestrial, or sparsely disbursed organisms. The sheer volume and speed of water moving through lotic systems, and the temporary nature of it, limits the information and therefore the conclusions we can draw from it. However, benthic sediments are more stable presenting an opportunity to extend the timeline of our findings. Benthic substrate core biomes were routinely larger than the water column but importantly, contained all the same base taxa in similar relative abundances as the water column. This could be inferred to mean that if we had to choose a single substrate to inform our understanding of microbiological water quality from river and estuarine systems, it may be better to sample benthic sediments than the water column.

The final aspect of my thesis was to survey the microbial communities present in Aotearoa's river and estuarine ecosystems and determine what, if any, spatio-temporal and environmental variables related to their structure. The lack of spatio-temporal drivers in microbial communities was not entirely unexpected. Because microbial community members are capable of similar functions under different conditions, function can be retained while member relative abundances fluctuate (Begon, Townsend & Harper, 2005; Diao et al., 2023). This allows for functional replication and preservation of services. However, it results in no singular driver for entire community composition. Many studies have identified the drivers of bacterial communities (e.g., pH, conductivity, temperature, and precipitation (Phiri et al. 2020); therefore, I chose to focus on archaea and the entire microbial community. Archaea are interesting in their seasonality, gene sharing, and replication of bacterial functions. They also deserve further investigation regarding their potential ties to antimicrobial resistance development. While I wish I were able to spend time and energy exploring archaeal communities more, it was beyond the scope of this thesis. There are very few studies on archaeal community structure, even fewer on archaeal communities along the river to sea continuum (Auguet, Barberan & Casamayor, 2010). Suffice to say, archaea do respond to land use and anthropogenic pollutants and are underappreciated microorganisms.

The presence of bacteria and archaea methanogenic, nitrifying, and denitrifying functional groups where livestock were present along the Waiotahe river and in Te Ahiaua suggests that ruminants, their effluents, and the nutrients their farming relies on, are influencing and affecting carbon cycling and greenhouse gas production in the catchment that is currently unaccounted for in greenhouse gas emissions schemes (Whitman et al., 2001; Auguet, Barberan & Casamayor, 2010; Sakadevan & Nguyen, 2017). This is further bolstered by the explanatory power of land use and upstream nutrient inputs to archaeal community structure, and to some extent other microbial organisms documented in this study. Further work on these neglected microbes and their role in carbon and nitrate cycling and their effects on climate change is vital.

Finally, antimicrobial resistance was most common where the intensive farming of cattle was present. The three Canterbury rivers, the lower reaches of the Waiotahe river, and Te Ahiaua all drain water from land used for intensive dairy farming (see chapters 3 & 6). In contrast, the mid-reaches of the Waiotahe, where less intensive, once-a-day milking was the normal farming practice and riparian planting was more likely to be present, antimicrobial resistance genes were not found once. The link between livestock, environmental degradation, and human disease is well documented and habitat and species interfaces (e.g., estuaries) are

prime places for disease spill over (Daszak, Cunningham & Hyatt, 2000, Daszak et al., 2020). Te Ahiaua is a culturally significant area with year-round food harvesting and seasonal recreational swimming. Taonga (highly treasured) species and places are often subjected to high levels of pollution because of their placement, often at the mouths of rivers and in estuaries. Knowing this and in light of these findings, we should be more careful and protective of our vulnerable estuarine ecosystems and prioritise conservation and restoration of these areas to prevent and reduce negative public and environmental health consequences. Future studies could focus on further elucidation of eDNA trade-offs (e.g., result reliability, reproducibility, and genetic indicators) associated with various methodological approaches.

References

Abatenh E, Gizaw B, Tsegaye Z, Wassie M. 2017. The role of microorganisms in bioremediation-A review. Open Journal of Environmental Biology 2:38–46.

Abbott BW, Bishop K, Zarnetske JP, Hannah DM, Frei RJ, Minaudo C, Chapin III FS, Krause S, Conner L, Ellison D. 2019a. A water cycle for the Anthropocene. Hydrological Processes 33:3046–3052.

Abbott BW, Bishop K, Zarnetske JP, Minaudo C, Chapin FS, Krause S, Hannah DM, Conner L, Ellison D, Godsey SE. 2019b. Human domination of the global water cycle absent from depictions and perceptions. Nature Geoscience 12:533–540.

Acinas SG, Rodríguez-Valera F, Pedrós-Alió C. 1997. Spatial and temporal variation in marine bacterioplankton diversity as shown by RFLP fingerprinting of PCR amplified 16S rDNA. FEMS Microbiology Ecology 24:27–40.

Adams HE, Crump BC, Kling GW. 2010. Temperature controls on aquatic bacterial production and community dynamics in arctic lakes and streams. Environmental Microbiology 12:1319–1333.

Aguirre AA, Catherina R, Frye H, Shelley L. 2020. Illicit wildlife trade, wet markets, and COVID-19: preventing future pandemics. World Medical & Health Policy 12:256–265.

Ahmed SAKS, Ajisola M, Azeem K, Bakibinga P, Chen Y-F, Choudhury NN, Fayehun O, Griffiths F, Harris B, Kibe P. 2020. Impact of the societal response to COVID-19 on access to healthcare for non-COVID-19 health issues in slum communities of Bangladesh, Kenya, Nigeria and Pakistan: results of pre-COVID and COVID-19 lockdown stakeholder engagements. BMJ Global Health 5:e003042.

Ahmed W, Gyawali P, Toze S. 2015. Quantitative PCR measurements of Escherichia coli including Shiga toxin-producing E. coli (STEC) in animal feces and environmental waters. Environmental Science & Technology 49:3084–3090.

Aktan I, Carter B, Wilking H, La Ragione RM, Wieler L, Woodward MJ, Anjum MF. 2007. Influence of geographical origin, host animal and stx gene on the virulence characteristics of Escherichia coli O26 strains. Journal of Medical Microbiology 56:1431–1439.

Albert MJ, Faruque SM, Ansaruzzaman M, Islam MM, Haider K, Alam K, Kabir I, Robins-Browne R. 1992. Sharing of virulence-associated properties at the phenotypic and genetic levels between enteropathogenic Escherichia coli and Hafnia alvei. Journal of Medical Microbiology 37:310–314.

Albright MBN, Martiny JBH. 2017. Dispersal alters bacterial diversity and composition in a natural community. ISME Journal 12:296–299. DOI: 10.1038/ismej.2017.161.

Alexander KA, Carlson CJ, Lewis BL, Getz WM, Marathe M V, Eubank SG, Sanderson CE, Blackburn JK. 2018. The ecology of pathogen spillover and disease emergence at the human-wildlife-environment interface. In: Hurst CJ ed. The connections between ecology and infectious disease. New York, USA: Springer, 267–298.

Alizon S, Hurford A, Mideo N, Van Baalen M. 2009. Virulence evolution and the trade-off hypothesis: history, current state of affairs and the future. Journal of Evolutionary Biology 22:245–259.

Alkemade R, Van Oorschot M, Miles L, Nellemann C, Bakkenes M, Ten Brink B. 2009. GLOBIO3: a framework to investigate options for reducing global terrestrial biodiversity loss. Ecosystems 12:374–390.

Allan JD, Castillo MM, Capps KA. 2021. Stream microbial ecology. In: Stream Ecology. Springer, 225–245. DOI: https://doi.org/10.1007/978-3-030-61286-3_8.

Allen DC, Vaughn CC, Kelly JF, Cooper JT, Engel MH. 2012. Bottom-up biodiversity effects increase resource subsidy flux between ecosystems. Ecology 93:2165–2174.

Almaganbetov NK, Atanassov I, Chirila E, Cojocaru A, Dimitrova A, Donkova R, Draghici

C, Dura G, Efendiev A, Girotti S, Janecka B, Javaux M, Kedziorek-Dupuy M, Kharytonov M, Khomich V, Kucharski R, Lambot S, Lokhanska V, Macaev F, Rubio R, Sargsyan V, Sas-Nowosielska A, Simeonov L, Solodoukhina D, Tchuldjian H, Terytze K, Vidal M. 2008. Soil chemical pollution, risk assessment, remediation and security. Dordrecht, The Netherlands: Springer.
Almeria S, Robertson L, Santin M. 2021. Why foodborne and waterborne parasites are important for veterinarians. Research in Veterinary Science 136:198–199.
Alonso A, Camargo JA. 2006. Toxicity of nitrite to three species of freshwater invertebrates. Environmental Toxicology: An International Journal 21:90–94.
Alotaibi GF. 2020. Occurrence of potentially pathogenic bacteria in epilithic biofilm forming bacteria isolated from Porter Brook river-stones, Sheffield, UK. Saudi Journal of Biological Sciences 27:3405.
Álvarez-Cabria M, Barquín J, Juanes JA. 2010. Spatial and seasonal variability of macroinvertebrate metrics: Do macroinvertebrate communities track river health? Ecological Indicators 10:370–379.
Amann S, Neef K, Kohl S. 2019. Antimicrobial resistance (AMR). European Journal of Hospital Pharmacy 26:175–177.
Américo-Pinheiro JHP, Bellatto LC, Mansano CFM, da Silva Vilar D, Ferreira LFR, Torres NH, Bilal M, Iqbal HMN. 2021. Monitoring microbial contamination of antibiotic resistant Escherichia coli isolated from the surface water of urban park in southeastern Brazil. Environmental Nanotechnology, Monitoring & Management 15:100438.
Anderson KL, Whitlock JE, Harwood VJ. 2005. Persistence and differential survival of fecal indicator bacteria in subtropical waters and sediments. Applied Environmental Microbiology. 71:3041–3048.
Anderson OR. 2018. Evidence for coupling of the carbon and phosphorus biogeochemical cycles in freshwater microbial communities. Frontiers in Marine Science 5:20.
Anderson RM, May RM. 1982. Coevolution of hosts and parasites. Parasitology 85:411–426.
Anderson-Carpenter LL, McLachlan JS, Jackson ST, Kuch M, Lumibao CY, Poinar HN. 2011. Ancient DNA from lake sediments: bridging the gap between paleoecology and genetics. BMC Evolutionary Biology 11:1–15.
Angelidaki I, Karakashev D, Batstone DJ, Plugge CM, Stams AJM. 2011. Methods in methane metabolism, part A. In: Rosenzweig A, Ragsdale S eds. Methods in enzymology. Cambridge Mass.: Academic Press, 327–351.
Anklam KS, Kanankege KST, Gonzales TK, Kaspar CW, Doepfer D. 2012. Rapid and reliable detection of Shiga toxin–producing Escherichia coli by real-time multiplex PCR. Journal of Food Protection 75:643–650.
Anne-Sophie M-H, Dorner SM, Sauvé S, Aboulfadl K, Galarneau M, Servais P, Prévost M. 2015. Temporal analysis of E. coli, TSS and wastewater micropollutant loads from combined sewer overflows: implications for management. Environmental Science: Processes & Impacts 17:965–974.
ANZG. 2018. Rational and background information (Ch 8), Australian and New Zealand guidelines for fresh and marine water quality. In: Aquatic ecosystems. Canberra, ACT, Australia: Australian and New Zealand governments and Australian state and territory governments, 1–313.
Arantes AL, Moreira JPC, Diender M, Parshina SN, Stams AJM, Alves MM, Alves JI, Sousa DZ. 2020. Enrichment of anaerobic syngas-converting communities and isolation of a novel carboxydotrophic Acetobacterium wieringae strain JM. Frontiers in Microbiology 11:58.
Araújo SC da S, Silva-Portela RCB, de Lima DC, da Fonsêca MMB, Araújo WJ, da Silva UB, Napp AP, Pereira E, Vainstein MH, Agnez-Lima LF. 2020. MBSP1: a biosurfactant

protein derived from a metagenomic library with activity in oil degradation. Scientific Reports 10:1–13.
Araújo SC da S, Silva-Portela RCB, de Lima DC, da Fonsêca MMB, Araújo WJ, da Silva UB, Napp AP, Pereira E, Vainstein MH, Agnez-Lima LF. 2020. MBSP1: a biosurfactant protein derived from a metagenomic library with activity in oil degradation. Scientific Reports 10:1–13.
Asakura H, Makino S, Shirahata T, Tsukamoto T, Kurazono H, Ikeda T, Takeshi K. 1998. Detection and genetical characterization of Shiga toxin-producing Escherichia coli from wild deer. Microbiology and Immunology 42:815–822.
Astorga A, Heino J, Luoto M, Muotka T. 2011. Freshwater biodiversity at regional extent: determinants of macroinvertebrate taxonomic richness in headwater streams. Ecography 34:705–713. DOI: 10.1111/j.1600-0587.2010.06427.x.
Auer MT, Niehaus SL. 1993. Modeling fecal coliform bacteria—I. Field and laboratory determination of loss kinetics. Water Research 27:693–701.
Auffret MD, Dewhurst RJ, Duthie C-A, Rooke JA, Wallace RJ, Freeman TC, Stewart R, Watson M, Roehe R. 2017. The rumen microbiome as a reservoir of antimicrobial resistance and pathogenicity genes is directly affected by diet in beef cattle. Microbiome 5:1–11.
Auguet J-C, Barberan A, Casamayor EO. 2010. Global ecological patterns in uncultured Archaea. The ISME Journal 4:182–190.
Auguet J, Casamayor EO. 2008. A hotspot for cold crenarchaeota in the neuston of high mountain lakes. Environmental Microbiology 10:1080–1086.
Avery LM, Williams AP, Killham K, Jones DL. 2008. Survival of Escherichia coli O157: H7 in waters from lakes, rivers, puddles and animal-drinking troughs. Science of The Total Environment 389:378–385.
Avery SM, Moore A, Hutchison ML. 2004. Fate of Escherichia coli originating from livestock faeces deposited directly onto pasture. Letters in Applied Microbiology 38:355–359.
Azcona G, Bhatt A, Encarnacion J, Plazaola-Castaño J, Seck P, Staab S, Turquet L. 2020. From insights to action: Gender equality in the wake of COVID-19. United Nations Entity for Gender Equality and the Empowerment of Women.
Baehren C, Pembaur A, Weil PP, Wewers N, Schult F, Wirth S, Postberg J, Aydin M. 2023. The overlookedmicrobiome—considering archaea and eukaryotes using multiplex nanopore-16S-/18S-rDNA-sequencing: A technical report focusing on nasopharyngeal microbiomes. International Journal of Molecular Sciences 24:1426.
Bahram M, Anslan S, Hildebrand F, Bork P, Tedersoo L. 2019. Newly designed 16S rRNA metabarcoding primers amplify diverse and novel archaeal taxa from the environment. Environmental Microbiology Reports 11:487–494.
Bailey RC, Norris RH, Reynoldson TB. 2001. Taxonomic resolution of benthic macroinvertebrate communities in bioassessments. Journal of the North American Benthological Society 20:280–286.
Baisero D, Visconti P, Pacifici M, Cimatti M, Rondinini C. 2020. Projected global loss of mammal habitat due to land-use and climate change. One Earth 2:578–585.
Balasingham KD, Walter RP, Heath DD. 2017. Residual eDNA detection sensitivity assessed by quantitative real-time PCR in a river ecosystem. Molecular Ecology Resources 17:523–532.
Baldursson S, Karanis P. 2011. Waterborne transmission of protozoan parasites: Review of worldwide outbreaks – An update 2004–2010. Water Research 45:6603–6614. DOI: http://dx.doi.org/10.1016/j.watres.2011.10.013.
Balière C, Rincé A, Blanco J, Dahbi G, Harel J, Vogeleer P, Giard J-C, Mariani-Kurkdjian P,

Gourmelon M. 2015. Prevalence and characterization of Shiga toxin-producing and enteropathogenic Escherichia coli in shellfish-harvesting areas and their watersheds. Frontiers in Microbiology 6:1356.
Bancroft EA. 2007. Antimicrobial resistance: it's not just for hospitals. Jama 298:1803–1804.
Banks B. 2011. The Waiotahi catchment management plan. Whakatane, New Zealand.
Barceló-Antemate D, Fontove-Herrera F, Santos W, Merino E. 2023. The effect of the genomic GC content bias of prokaryotic organisms on the secondary structures of their proteins. Plos One 18:e0285201.
Bare JC. 2002. TRACI: The tool for the reduction and assessment of chemical and other environmental impacts. Journal of Industrial Ecology 6:49–78.
Barlow ND. 1994. Bovine tuberculosis in New Zealand: epidemiology and models. Trends in Microbiology 2:119–124.
Barnett T, Fournié G. 2021. Zoonoses and wet markets: beyond technical interventions. The Lancet. Planetary Health 5:2.
Baron JS, Poff NL, Angermeier PL, Dahm CN, Gleick PH, Hairston NG, Jackson RB, Johnston CA, Richter BD, Steinman AD. 2002. Meeting ecological and societal needs for freshwater. Ecological Applications 12:1247–1260. DOI: 10.2307/3099968.
Barzilai-Nahon K. 2009. Gatekeeping: A critical review. Annual Review of Information Science and Technology 43:1–79.
Baskaran R, Cullen R, Colombo S. 2009. Estimating values of environmental impacts of dairy farming in New Zealand. New Zealand Journal of Agricultural Research 52:377–389.
Bates AE, Primack RB, Duarte CM, Group P-EW. 2021. Global COVID-19 lockdown highlights humans as both threats and custodians of the environment. Biological Conservation:109175.
Battin TJ, Besemer K, Bengtsson MM, Romani AM, Packmann AI. 2016. The ecology and biogeochemistry of stream biofilms. Nature Reviews Microbiology 14:251.
Bay of Plenty Regional Council Toi Moana. 2022.Bay of Plenty focus catchments. Available at https://www.boprc.govt.nz/environment/fresh-water/focus-catchments (accessed August 7, 2022).
Bean AGD, Baker ML, Stewart CR, Cowled C, Deffrasnes C, Wang L-F, Lowenthal JW. 2013. Studying immunity to zoonotic diseases in the natural host—keeping it real. Nature Reviews Immunology 13:851–861.
Begon M, Townsend R. C, Harper L. J. 2005. Ecology : from individuals to ecosystems. Malden, MA: Blackwell Pub.
Begum Z, Srinivas TNR, Manasa P, Sailaja B, Sunil B, Prasad S, Shivaji S. 2013. Winogradskyella psychrotolerans sp. nov., a marine bacterium of the family Flavobacteriaceae isolated from Arctic sediment. International Journal of Systematic and Evolutionary Microbiology 63:1646–1652.
Bengis RG, Kock RA, Fischer J. 2002. Infectious animal diseases: the wildlife/livestock interface. Revue scientifique et technique (International Office of Epizootics) 21:53–65.
Bento AM, Klotz R. 2014. Climate policy decisions require policy-based lifecycle analysis. Environmental Science & Technology 48:5379–5387.
Berdjeb L, Pollet T, Chardon C, Jacquet S. 2013. Spatio-temporal changes in the structure of archaeal communities in two deep freshwater lakes. FEMS Microbiology Ecology 86:215–230.
Bernardet JF, Bowman JP. 2006. The genus Flavobacterium. New York, New York, USA: Springer New York. DOI: 10.1007/0-387-30747-8_17.
Berthe T, Ratajczak M, Clermont O, Denamur E, Petit F. 2013. Evidence for coexistence of distinct Escherichia coli populations in various aquatic environments and their survival

in estuary water. Applied and Environmental Microbiology 79:4684–4693.
Bertrand KN, Gido KB, Dodds WK, Murdock JN, Whiles MR. 2009. Disturbance frequency and functional identity mediate ecosystem processes in prairie streams. Oikos 118:917–933. DOI: 10.1111/j.1600-0706.2008.16849.x.
Beville ST, Kerr GN, Hughey KFD. 2012. Valuing impacts of the invasive alga Didymosphenia geminata on recreational angling. Ecological Economics 82:1–10.
Bhakdi S, Bohl J. 2003. Prions, mad cow disease, and preventive measures: a critical appraisal. Medical Microbiology and Immunology 192:117–122.
Bidaisee S, Macpherson CNL. 2014. Zoonoses and one health: a review of the literature. Journal of Parasitology Research 2014.
Bimpitsos C, Petridou E. 2012. A transdisciplinary approach to training: preliminary research findings based on a case analysis. European Journal of Training and Development 36:911-929.
Birk S, Bonne W, Borja A, Brucet S, Courrat A, Poikane S, Solimini A, van de Bund W, Zampoukas N, Hering D. 2012. Three hundred ways to assess Europe's surface waters: an almost complete overview of biological methods to implement the water framework directive. Ecological Indicators 18:31–41. DOI: 10.1016/j.ecolind.2011.10.009.
Bižić-Ionescu M, Ionescu D, Grossart H-P. 2018. Organic particles: heterogeneous hubs for microbial interactions in aquatic ecosystems. Frontiers in Microbiology 9:2569.
Blocksom KA, Johnson BR. 2009. Development of a regional macroinvertebrate index for large river bioassessment. Ecological Indicators 9:313–328.
Bloomfield S, Wilkinson D, Rogers L, Biggs P, French N, Mohan V, Savoian M, Venter P, Midwinter A. 2020. Campylobacter novaezeelandiae sp. nov., isolated from birds and water in New Zealand. International Journal of Systematic and Evolutionary Microbiology 70:3775.
Blount ZD. 2015. The natural history of model organisms: The unexhausted potential of E. coli. Elife 4:e05826.
Boehm Jr AB, Ashbolt NJ, Colford Jr JM, Dunbar LE, Fleming LE, Gold MA, Hansel JA, Hunter PR, Ichida AM, McGee CD. 2009. A sea change ahead for recreational water quality criteria. Journal of Water and Health 7:9–20.
Boeraş I, Burcea A, Coman C, Bănăduc D, Curtean-Bănăduc A. 2021. Bacterial microbiomes in the sediments of lotic systems ecologic drivers and role: A case study from the Mureş River, Transylvania, Romania. Water 13:3518.
Boerlin P, McEwen SA, Boerlin-Petzold F, Wilson JB, Johnson RP, Gyles CL. 1999. Associations between virulence factors of Shiga toxin-producing Escherichia coli and disease in humans. Journal of Clinical Microbiology 37:497–503.
Bogard MJ, Del Giorgio PA, Boutet L, Chaves MCG, Prairie YT, Merante A, Derry AM. 2014. Oxic water column methanogenesis as a major component of aquatic CH4 fluxes. Nature Communications 5:5350.
Boithias L, Ribolzi O, Lacombe G, Thammahacksa C, Silvera N, Latsachack K, Soulileuth B, Viguier M, Auda Y, Robert E. 2021. Quantifying the effect of overland flow on Escherichia coli pulses during floods: use of a tracer-based approach in an erosion-prone tropical catchment. Journal of Hydrology 594:125935.
Bolton FJ, Coates D. 1983. A comparison of microaerobic systems for the culture of Campylobacter jejuni and Campylobacter coli. European Journal of Clinical Microbiology 2:105–110.
Bonada N, Prat N, Resh VH, Statzner B. 2006. Developments in aquatic insect biomonitoring: a comparative analysis of recent approaches. Annual Review of Entomology 51:495–523.
Bonnet R. 2004. Growing group of extended-spectrum β-lactamases: the CTX-M enzymes.

Antimicrobial Agents and Chemotherapy 48:1–14.
Borel KEM. 2019. Estimating Associated Human Health Risk from Recreational Exposures in Fresh Water Bodies Impacted by Multiple Fecal Sources. Texas A&M.
Boss R, Overesch G, Baumgartner A. 2016. Antimicrobial resistance of Escherichia coli, Enterococci, Pseudomonas aeruginosa, and Staphylococcus aureus from raw fish and seafood imported into Switzerland. Journal of Food Protection 79:1240–1246.
Boulton AJ. 1999. An overview of river health assessment: philosophies, practice, problems and prognosis. Freshwater Biology 41:469–479.
Bouwmeester MM, Goedknegt MA, Poulin R, Thieltges DW. 2021. Collateral diseases: aquaculture impacts on wildlife infections. Journal of Applied Ecology 58:453–464.
Brandl MT. 2006. Fitness of human enteric pathogens on plants and implications for food safety. Annual Review Phytopathology 44:367–392.
Bremermann HJ, Pickering J. 1983. A game-theoretical model of parasite virulence. Journal of Theoretical Biology 100:411–426.
Bricheux G, Le Moal G, Hennequin C, Coffe G, Donnadieu F, Portelli C, Bohatier J, Forestier C. 2013. Characterization and evolution of natural aquatic biofilm communities exposed in vitro to herbicides. Ecotoxicology and Environmental Safety 88:126–134.
Brockmann D, Helbing D. 2013. The hidden geometry of complex, network-driven contagion phenomena. Science 342:1337–1342.
Brooijmans RJW, Pastink MI, Siezen RJ. 2009. Hydrocarbon-degrading bacteria: the oil-spill clean-up crew. Microbial Biotechnology 2:587–594.
Brown JR, Doolittle WF. 1997. Archaea and the prokaryote-to-eukaryote transition. Microbiology and Molecular Biology Reviews 61:456–502.
Browne AS, Midwinter AC, Withers H, Cookson AL, Biggs PJ, Marshall JC, Benschop J, Hathaway S, Haack NA, Akhter RN. 2018. Molecular epidemiology of Shiga toxin-producing Escherichia coli (STEC) on New Zealand dairy farms: application of a culture-independent assay and whole-genome sequencing. Applied and Environmental Microbiology 84:e00481-18.
Brunauer M, Roch FF, Conrady B. 2021. Prevalence of worldwide neonatal calf diarrhoea caused by bovine rotavirus in combination with bovine coronavirus, Escherichia coli K99 and Cryptosporidium spp.: a meta-analysis. Animals 11:1014.
Bryan M, Hea SY. 2017. A survey of antimicrobial use in dairy cows from farms in four regions of New Zealand. New Zealand Veterinary Journal 65:93–98.
Bubb S, Jones M-A. 2020. Learning from the COVID-19 home-schooling experience: Listening to pupils, parents/carers and teachers. Improving Schools 23:209–222.
Burden A, Smeaton C, Angus S, Garbutt A, Jones L, Lewis H, Rees S. 2020. Impacts of climate change on coastal habitats, relevant to the coastal and marine environment around the UK. Lowestoft, UK. DOI: 10.14465/2020.arc11.chb.
Burgess SA, Aplin J, Biggs PJ, Breckell G, Benschop J, Fayaz A, Toombs-Ruane LJ, Midwinter AC. 2021. Characterisation of AmpC and extended-spectrum beta-lactamase producing E. coli from New Zealand dairy farms. International Dairy Journal 117:104998.
Burian A, Mauvisseau Q, Bulling M, Domisch S, Qian S, Sweet M. 2021. Improving the reliability of eDNA data interpretation. Molecular Ecology Resources 21:1422–1433.
Byappanahalli MN, Ishii S. 2011. Environmental sources of fecal bacteria. In: Sadowsky MJ, Whitman RL eds. The fecal bacteria. Washington D.C., USA: ASM Press, 93–110.
Camargo J, Alonso A, Salamanca A. 2005. Nitrate toxicity to aquatic animals: a review with new data for freshwater invertebrates. Chemosphere 58:1255–1267. DOI: http://dx.doi.org/10.1016/j.chemosphere.2004.10.044.

Camargo JA, Alonso Á. 2006. Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: a global assessment. Environment International 32:831–849. DOI: http://dx.doi.org/10.1016/j.envint.2006.05.002.
Campbell LM. 2005. Overcoming obstacles to interdisciplinary research. Conservation Biology 19:574–577.
Canning AD. 2020. Nutrients in New Zealand rivers and streams: an exploration and derivation of national nutrient criteria. Wellington, New Zealand. DOI: doi.org/10.6084/m9.figshare.12116460.
Carattoli A. 2013. Plasmids and the spread of resistance. International Journal of Medical Microbiology 303:298–304.
Cardinale B. 2012. Impacts of Biodiversity Loss. Science 336:552–553. DOI: 10.1126/science.1222102.
Carney M. 1991. European drinking water standards. Journal-American Water Works Association 83:48–55.
Carpenter SR, Caraco NF, Correll DL, Howarth RW, Sharpley AN, Smith VH. 1998. Nonpoint pollution of surface waters with phosphorus and nitrogen. Ecological Society of America 8:559-568. DOI: 10.1890/1051-0761(1998)008[0559:NPOSWW]2.0.CO;2.
Carver S, Lunn T. 2020. When are pathogen dynamics likely to reflect host population genetic structure? Molecular Ecology 29:859–861.
Casadevall A, Pirofski L-A. 2002. What is a pathogen? Annals of Medicine 34:2–4.
Cazzolla Gatti R. 2016. Freshwater biodiversity: a review of local and global threats. International Journal of Environmental Studies 73:887–904.
Centers for Disease Control and Prevention. 2017.Zoonotic diseases. Available at https://www.cdc.gov/onehealth/basics/zoonotic-diseases.html (accessed February 26, 2021).
Centers for Disease Control and Prevention. 2018.National enteric disease surveillance: Shiga toxin-producing Escherichia coli (STEC) annual report 2016. Available at https://www.cdc.gov/ecoli/surv2016/index.html (accessed 4 October 2020).
Centers for Disease Control and Prevention. 2021.One Health. Available at https://www.cdc.gov/onehealth/index.html (accessed October 1, 2021).
Cervantes FJ. 2009. Environmental technologies to treat nitrogen pollution. London, UK: IWA publishing.
Ceylan RF, Ozkan B, Mulazimogullari E. 2020. Historical evidence for economic effects of COVID-19. The European Journal of Health Economics 21:817–823.
Chambers HF. 2001. The changing epidemiology of Staphylococcus aureus? Emerging Infectious Diseases 7:178.
Chambers T, Wilson N, Hales S, Baker M. 2021. Nitrate contamination in drinking water and adverse birth outcomes: emerging evidence is concerning for NZ. Available at https://www.phcc.org.nz/briefing/nitrate-contamination-drinking-water-and-adverse-birth-outcomes-emerging-evidence (accessed February 27, 2023).
Champion PD. 2018. Knowledge to action on aquatic invasive species: Island biosecurity–the New Zealand and South Pacific story. Management of Biological Invasions 9:383.
Chandler DS, Farran I, Craven JA. 1981. Persistence and distribution of pollution indicator bacteria on land used for disposal of piggery effluent. Applied Environmental Microbiology 42:453–460.
Chapman DF, Dalley DE, Edwards GR, Cameron KC, Malcolm BJ, Clement A, Romera AJ, Pinxterhuis IB, Beukes PC, Di HJ. 2020. Production, profit and nitrogen flows in irrigated dairy systems representing different industry development pathways: the Pastoral 21 experience in Canterbury. New Zealand Journal of Agricultural Research 64:3–33.

Chen L, Yang J, Yu J, Yao Z, Sun L, Shen Y, Jin Q. 2005. VFDB: a reference database for bacterial virulence factors. Nucleic Acids Research 33:D325–D328.
Chenia HY, Jacobs A. 2017. Antimicrobial resistance, heavy metal resistance and integron content in bacteria isolated from a South African tilapia aquaculture system. Diseases of Aquatic Organisms 126:199–209.
Chiappini S, Guirguis A, John A, Corkery JM, Schifano F. 2020. COVID-19: the hidden impact on mental health and drug addiction. Frontiers in Psychiatry 11:767.
Cho KH, Han D, Park Y, Lee SW, Cha SM, Kang J-H, Kim JH. 2010. Evaluation of the relationship between two different methods for enumeration fecal indicator bacteria: Colony-forming unit and most probable number. Journal of Environmental Sciences 22:846–850.
Chowell G, Nishiura H. 2014. Transmission dynamics and control of Ebola virus disease (EVD): a review. BMC Medicine 12:196.
Christensen NL, Bartuska AM, Brown JH, Carpenter S, D'Antonio C, Francis R, Franklin JF, MacMahon JA, Noss RF, Parsons DJ, Peterson CH, Turner MG, Woodmansee RG. 1996. The report of the Ecological Society of America committee on the scientific basis of ecosystem management. Ecological Applications 6:665–691.
Chuckran PF, Flagg C, Propster J, Rutherford WA, Sieradzki ET, Blazewicz SJ, Hungate B, Pett-Ridge J, Schwartz E, Dijkstra P. 2023. Edaphic controls on genome size and GC content of bacteria in soil microbial communities. Soil Biology and Biochemistry 178:108935.
Clark DR, McKew BA, Binley A, Heppell CM, Whitby C, Trimmer M. 2022. Hydrological properties predict the composition of microbial communities cycling methane and nitrogen in rivers. ISME Communications 2:5.
Clark S. 1998. Hastings District Council Water Supply Contamination Investigation. Upper Hutt, New Zealand.
Clarke RT, Wright JF, Furse MT. 2003. RIVPACS models for predicting the expected macroinvertebrate fauna and assessing the ecological quality of rivers. Ecological Modelling 160:219–233.
Cleaveland S, Borner M, Gislason M. 2014. Ecology and conservation: contributions to One Health. Revue Scientifique et Techique (International Office of Epizootics) 33:615–627.
Cleaveland S, Laurenson MK, Taylor LH. 2001. Diseases of humans and their domestic mammals: pathogen characteristics, host range and the risk of emergence. Philosophical Transactions of the Royal Society of London B: Biological Sciences 356:991–999.
Clermont O, Olier M, Hoede C, Diancourt L, Brisse S, Keroudean M, Glodt J, Picard B, Oswald E, Denamur E. 2011. Animal and human pathogenic Escherichia coli strains share common genetic backgrounds. Infection, Genetics and Evolution 11:654–662.
Close M, Noonan M, Hector R, Bright J. 2010. Microbial transport from dairying under two spray-irrigation systems in Canterbury, New Zealand. Journal of Environmental Quality 39:824–833.
Coll C, Bier R, Li Z, Langenheder S, Gorokhova E, Sobek A. 2020. Association between aquatic micropollutant dissipation and river sediment bacterial communities. Environmental Science & Technology 54:14380–14392.
Collins R, Rutherford K. 2004. Modelling bacterial water quality in streams draining pastoral land. Water Research 38:700–712.
Coltart CEM, Lindsey B, Ghinai I, Johnson AM, Heymann DL. 2017. The Ebola outbreak, 2013–2016: old lessons for new epidemics. Philosophical Transactions of the Royal Society B: Biological Sciences 372:20160297.
Conley DJ, Paerl HW, Howarth RW, Boesch DF, Seitzinger SP, Havens KE, Lancelot C, Likens GE. 2009. Controlling eutrophication: nitrogen and phosphorus. Science

323:1014–1015. DOI: 10.1126/science.1167755.
Conville PS, Brown-Elliott BA, Smith T, Zelazny AM. 2018. The complexities of Nocardia taxonomy and identification. Journal of Clinical Microbiology 56:10–1128.
Cooley MB, Jay-Russell M, Atwill ER, Carychao D, Nguyen K, Quiñones B, Patel R, Walker S, Swimley M, Pierre-Jerome E. 2013. Development of a robust method for isolation of Shiga toxin-positive Escherichia coli (STEC) from fecal, plant, soil and water samples from a leafy greens production region in California. PLoS One 8:e65716.
Corinaldesi C, Danovaro R, Dell'Anno A. 2005. Simultaneous recovery of extracellular and intracellular DNA suitable for molecular studies from marine sediments. Applied and Environmental Microbiology 71:46–50.
Cortes MC, Cauchemez S, Lefrancq N, Luby SP, Jahangir Hossain M, Sazzad HMS, Rahman M, Daszak P, Salje H, Gurley ES. 2018. Characterization of the spatial and temporal distribution of Nipah virus spillover events in Bangladesh, 2007–2013. The Journal of Infectious Diseases 217:1390–1394.
Cosgrove SE. 2006. The relationship between antimicrobial resistance and patient outcomes: mortality, length of hospital stay, and health care costs. Clinical Infectious Diseases 42:S82–S89.
Costanza R, Norton BG, Haskell BD. 1992. Ecosystem health: new goals for ecosystem management. Washington, D.C., USA: Island Press.
Cotner JB, Hall EK, Scott T, Heldal M. 2010. Freshwater bacteria are stoichiometrically flexible with a nutrient composition similar to seston. Frontiers in Microbiology 1:132.
Courcoul C, Leflaive J, Ferriol J, Boulêtreau S. 2022. The sensitivity of aquatic microbial communities to a complex agricultural contaminant depends on previous drought conditions. Water Research 217:118396.
Craft JA, Stanford JA, Pusch M. 2002. Microbial respiration within a floodplain aquifer of a large gravel-bed river. Freshwater Biology 47:251–261.
Crayne MP. 2020. The traumatic impact of job loss and job search in the aftermath of COVID-19. Psychological Trauma: Theory, Research, Practice, and Policy 12:S180.
Creer S, Deiner K, Frey S, Porazinska D, Taberlet P, Thomas WK, Potter C, Bik HM. 2016. The ecologist's field guide to sequence-based identification of biodiversity. Methods in Ecology and Evolution 7:1008–1018. DOI: 10.1111/2041-210X.12574.
Cristescu ME, Hebert PDN. 2018. Uses and misuses of environmental DNA in biodiversity science and conservation. Annual Review of Ecology, Evolution, and Systematics 49:209–230.
Cross PC, Edwards WH, Scurlock BM, Maichak EJ, Rogerson JD. 2007. Effects of management and climate on elk brucellosis in the Greater Yellowstone Ecosystem. Ecological Applications 17:957–964.
Crump JA, Murdoch DR, Baker MG. 2001. Emerging infectious diseases in an island ecosystem: the New Zealand perspective. Emerging Infectious Diseases 7:767.
Culhane FE, Robinson LA, Lillebø AI. 2020. Approaches for estimating the supply of ecosystem services: concepts for ecosystem-based management in coastal and marine environments. Ecosystem-based management, ecosystem services and aquatic biodiversity: Theory, tools and applications:105–126.
Curriero FC, Patz JA, Rose JB, Lele S. 2001. The association between extreme precipitation and waterborne disease outbreaks in the United States, 1948–1994. American Journal of Public Health 91:1194–1199.
Dahmen S, Métayer V, Gay E, Madec J-Y, Haenni M. 2013. Characterization of extended-spectrum beta-lactamase (ESBL)-carrying plasmids and clones of Enterobacteriaceae causing cattle mastitis in France. Veterinary Microbiology 162:793–799.
Daly AJ, Baetens JM, De Baets B. 2018. Ecological diversity: measuring the unmeasurable.

Mathematics 6:119.
Dangendorf F. 2004. Impacts of anthropogenic and environmental factors on the distribution of zoonoses. Waterborne Zoonoses: Identification, Causes, and Control. IWA Publishing, London:46–65.
Danovaro R, Pusceddu A. 2007. Biodiversity and ecosystem functioning in coastal lagoons: does microbial diversity play any role? Estuarine, Coastal and Shelf Science 75:4–12.
Danovaro, Roberto, and Antonio Pusceddu. 2007. 'Biodiversity and Ecosystem Functioning in Coastal Lagoons: Does Microbial Diversity Play Any Role?' Estuarine, Coastal and Shelf Science 75: 4–12.
Dansted P. 2021. Animal Products Notice: Specifications for Bivalve Molluscan Shellfish for Human Consumption. Wellington, New Zealand.
Daszak P, Cunningham AA, Hyatt AD. 2000. Emerging infectious diseases of wildlife-threats to biodiversity and human health. Science 287:443–449.
Daszak P, das Neves C, Amuasi J, Hayman D, Kuiken T, Roche B, Zambrana-Torrelio C, Buss P, Dundarova H, Feferholtz Y, Foldvari G, Igbinosa E, Junglen S, Liu Q, Suzan G, Uhart M, Wannous C, Woolaston K, Mosig Reidl P, O'Brien K, Pascual U, Stoett P, Li H, Ngo HT. 2020. Workshop Report on Biodiversity and Pandemics of the Intergovernmental Platform on Biodiversity and Ecosystem Services. Bonn, Germany. https://doi.org/10.5281/zenodo.4147317.
Davidson S, Crotta S, McCabe TM, Wack A. 2014. Pathogenic potential of interferon αβ in acute influenza infection. Nature Communications 5:1–15.
Davies CM, Long JA, Donald M, Ashbolt NJ. 1995. Survival of fecal microorganisms in marine and freshwater sediments. Applied and Environmental Microbiology 61:1888–1896.
Davies PE, Harris JH, Hillman TJ, Walker KF. 2010. The Sustainable Rivers Audit: assessing river ecosystem health in the Murray-Darling Basin, Australia. Marine and Freshwater Research 61:764–777. DOI: 10.1071/mf09043.
Davies-Colley R, Valois A, Milne J. 2018. Faecal contamination and visual clarity in New Zealand rivers: correlation of key variables affecting swimming suitability. Journal of Water and Health 16:329–339.
Davies-Colley RJ, Nagels JW, Smith RA, Young RG, Phillips CJ. 2004. Water quality impact of a dairy cow herd crossing a stream. New Zealand Journal of Marine and Freshwater Research 38:569–576. DOI: 10.1080/00288330.2004.9517262.
Davis M, Midwinter AC, Cosgrove R, Death RG. 2021. Detecting genes associated with antimicrobial resistance and pathogen virulence in three New Zealand rivers. PeerJ 9:e12440.
de Oliveira LFV, Margis R. 2015. The source of the river as a nursery for microbial diversity. PLoS One 10:e0120608.
de Vries W, Erisman JW, Spranger T, Stevens CJ, van den Berg L. 2011. Nitrogen as a threat to European terrestrial biodiversity. In: Sutton MA, Howard CM, Erisman JW, Billen G, Bleeker A, Grennfelt P, van Grinsven H, Grizzetti B eds. The European nitrogen assessment: sources, effects and policy perspectives. Cambridge, UK: Cambridge University Press Cambridge, UK, 436–494.
Death RG. 1995. Spatial patterns in benthic invertebrate communities: products of habitat stability or are they habitat specific? Freshwater Biology 33:455–467.
Deiner K, Altermatt F. 2014. Transport distance of invertebrate environmental DNA in a natural river. Plos One 9:e88786. DOI: 10.1371/journal.pone.0088786.
Deiner K, Fronhofer EA, Mächler E, Walser J-C, Altermatt F. 2016. Environmental DNA reveals that rivers are conveyer belts of biodiversity information. Nature Communications 7:1–9.

Deiner, Kristy, Emanuel A Fronhofer, Elvira Mächler, Jean-Claude Walser, and Florian Altermatt. 2016. 'Environmental DNA Reveals That Rivers Are Conveyer Belts of Biodiversity Information'. Nature Communications 7: 1–9.
del Carmen Gomez Cabrera M, Young JM, Roff G, Staples T, Ortiz JC, Pandolfi JM, Cooper A. 2019. Broadening the taxonomic scope of coral reef palaeoecological studies using ancient DNA. Molecular Ecology 28:2636–2652.
del Rio-Rodriguez RE, Inglis V, Millar SD. 1997. Survival of Escherichia coli in the intestine of fish. Aquaculture Research 28:257–264.
Delannoy S, Beutin L, Fach P. 2013. Towards a molecular definition of enterohemorrhagic Escherichia coli (EHEC): detection of genes located on O island 57 as markers to distinguish EHEC from closely related enteropathogenic E. coli strains. Journal of Clinical Microbiology 51:1083–1088.
DeLong EF, Preston CM, Mincer T, Rich V, Hallam SJ, Frigaard N-U, Martinez A, Sullivan MB, Edwards R, Brito BR. 2006. Community genomics among stratified microbial assemblages in the ocean's interior. Science 311:496–503.
DeLorenzo ME, Scott GI, Ross PE. 2001. Toxicity of pesticides to aquatic microorganisms: a review. Environmental Toxicology and Chemistry: An International Journal 20:84–98.
Denaro R, Aulenta F, Crisafi F, Di Pippo F, Viggi CC, Matturro B, Tomei P, Smedile F, Martinelli A, Di Lisio V. 2020. Marine hydrocarbon-degrading bacteria breakdown poly (ethylene terephthalate)(PET). Science of The Total Environment 749:141608.
Desmarais TR, Solo-Gabriele HM, Palmer CJ. 2002. Influence of soil on fecal indicator organisms in a tidally influenced subtropical environment. Applied and Environmental Microbiology 68:1165–1172.
Destoumieux-Garzón D, Mavingui P, Boetsch G, Boissier J, Darriet F, Duboz P, Fritsch C, Giraudoux P, Le Roux F, Morand S. 2018. The one health concept: 10 years old and a long road ahead. Frontiers in Veterinary Science 5:14.
Devane M, Moriarty E, Weaver L, Cookson A, Gilpin B. 2020. Fecal indicator bacteria from environmental sources; strategies for identification to improve water quality monitoring. Water Research 185:116204.
Devane ML, Weaver L, Singh SK, Gilpin BJ. 2018. Fecal source tracking methods to elucidate critical sources of pathogens and contaminant microbial transport through New Zealand agricultural watersheds–a review. Journal of Environmental Management 222:293–303.
Dhont K, Piazza J, Hodson G. 2021. The role of meat appetite in willfully disregarding factory farming as a pandemic catalyst risk. Appetite:105279.
Diao M, Balkema C, Suárez-Muñoz M, Huisman J, Muyzer G. 2023. Succession of bacteria and archaea involved in the nitrogen cycle of a seasonally stratified lake. FEMS Microbiology Letters 370:fnad013.
Díaz-Sánchez S, Sánchez S, Sánchez M, Herrera-León S, Hanning I, Vidal D. 2012. Detection and characterization of Shiga toxin-producing Escherichia coli in game meat and ready-to-eat meat products. International Journal of Food Microbiology 160:179–182.
Ding T, Suo Y, Xiang Q, Zhao X, Chen S, Ye X, Liu D. 2017. Significance of viable but nonculturable Escherichia coli: induction, detection, and control. Journal of Microbiology and Biotechnology 27:417–428.
Ding T, Suo Y, Xiang Q, Zhao X, Chen S, Ye X, Liu D. 2017. Significance of viable but nonculturable Escherichia coli: induction, detection, and control. Journal of Microbiology and Biotechnology 27:417–428.
Dodds WK, Bouska WW, Eitzmann JL, Pilger TJ, Pitts KL, Riley AJ, Schloesser JT, Thornbrugh DJ. 2009. Eutrophication of US freshwaters: analysis of potential economic

damages. Environmental Science & Technology 43:12–19. DOI: 10.1021/es801217q.
Dodds WK, Perkin JS, Gerken JE. 2013. Human impact on freshwater ecosystem services: a global perspective. Environmental Science & Technology 47:9061–9068.
Doi H, Inui R, Akamatsu Y, Kanno K, Yamanaka H, Takahara T, Minamoto T. 2017. Environmental DNA analysis for estimating the abundance and biomass of stream fish. Freshwater Biology 62:30–39.
Dong Y, Gao M, Qiu W, Song Z. 2021. Effects of microplastic on arsenic accumulation in Chlamydomonas reinhardtii in a freshwater environment. Journal of Hazardous Materials 405:124232.
Doolittle WF, Zhaxybayeva O. 2009. On the origin of prokaryotic species. Genome Research 19:744–756.
Doré WJ, Henshilwood K, Lees DN. 2000. Evaluation of F-specific RNA bacteriophage as a candidate human enteric virus indicator for bivalve molluscan shellfish. Applied and Environmental Microbiology 66:1280–1285.
Droppo IG, King K, Tirado SM, Sousa A, Wolfaardt G, Liss SN, Warren LA. 2010. Assessing riverine sediment—pathogen dynamics: implications for the management of aquatic and human health risk. In: ICCE symposium. Warsaw, Poland: International Association of Hydrological Sciences, 245–250.
Dudgeon D, Arthington AH, Gessner MO, Kawabata ZI, Knowler DJ, Leveque C, Naiman RJ, Prieur-Richard AH, Soto D, Stiassny MLJ, Sullivan CA. 2007. Freshwater biodiversity: importance, threats, status and conservation challenges. Biological Reviews 81:163–182.
Dufour AP. 1984. Health Criteria for Fresh Recreational Waters. Document US EPAJ600-1-84-004, Research Tnangle Park, NC.
Dulu TD. 2020. Health in all policies: agriculture, land use and animal health. In: Handbook of global health. Springer, 1–14.
Dutta C, Pan A. 2002. Horizontal gene transfer and bacterial diversity. Journal of Biosciences 27:27–33.
Dwivedi D, Mohanty BP, Lesikar BJ. 2016. Impact of the linked surface water-soil water-groundwater system on transport of E. coli in the subsurface. Water, Air, & Soil Pollution 227:351.
Ebert D, Bull JJ. 2003. Challenging the trade-off model for the evolution of virulence: is virulence management feasible? Trends in Microbiology 11:15–20.
Edwards DR, Coyne MS, Vendrell PF, Daniel TC, Moore Jr PA, Murdoch JF. 1997. Fecal coliform and streptococcus concentrations in runoff from grazed pastures in northwest Arkansas. JAWRA Journal of the American Water Resources Association 33:413–422.
Effendi I, Nedi S, Pakpahan R. 2017. Detergent disposal into our environmentand its impact on marine microbes. In: IOP Conference Series: Earth and Environmental Science. IOP Publishing, 12030.
Eigenbrod F, Armsworth PR, Anderson BJ, Heinemeyer A, Gillings S, Roy DB, Thomas CD, Gaston KJ. 2010. The impact of proxy-based methods on mapping the distribution of ecosystem services. Journal of Applied Ecology 47:377–385.
Ekroth AKE, Gerth M, Stevens EJ, Ford SA, King KC. 2021. Host genotype and genetic diversity shape the evolution of a novel bacterial infection. The ISME Journal 15:1–12.
El-Wakf AM, Hassan HA, El-said FG, El-Said A. 2009. Hypothyroidism in male rats of different ages exposed to nitrate polluted drinking water. Research Journal of Medical Sciences 4:160–164.
Elmonir W, Shalaan S, Tahoun A, Mahmoud SF, Remela EMA, Eissa R, El-Sharkawy H, Shukry M, Zahran RN. 2021. Prevalence, antimicrobial resistance, and genotyping of Shiga toxin-producing Escherichia coli in foods of cattle origin, diarrheic cattle, and

diarrheic humans in Egypt. Gut Pathogens 13:1–11.
Endris AA, Addissie A, Ahmed M, Abagero A, Techane B, Tadesse M. 2022. Epidemiology of Cholera Outbreak and Summary of the Preparedness and Response Activities in Addis Ababa, Ethiopia, 2016. Journal of Environmental and Public Health 2022:e4671719.
EPA US. 2015.Method 1603: Escherichia coli (E. coli) in water by membrane filtration using modified membrane-thermotolerant Escherichia coli agar (modified mTEC). Available at https://www.epa.gov/sites/production/files/2015-08/documents/method_1603_2009.pdf (accessed October 4, 2019).
Erbilgin O, Bowen BP, Kosina SM, Jenkins S, Lau RK, Northen TR. 2017. Dynamic substrate preferences predict metabolic properties of a simple microbial consortium. BMC Bioinformatics 18:1–12.
Ericksen TH, Dufour AP. 2018. Methods to identify waterborne pathogens and indicator organisms. Waterborne Diseases in the US 195.
Erickson P, Lazarus M. 2014. Impact of the Keystone XL pipeline on global oil markets and greenhouse gas emissions. Nature Climate Change 4:778–781.
Erken M, Lutz C, McDougald D. 2013. The rise of pathogens: predation as a factor driving the evolution of human pathogens in the environment. Microbial Ecology 65:860–868.
Escobar-Páramo P, Clermont O, Blanc-Potard A-B, Bui H, Le Bouguénec C, Denamur E. 2004. A specific genetic background is required for acquisition and expression of virulence factors in Escherichia coli. Molecular Biology and Evolution 21:1085–1094.
Espinosa L, Gray A, Duffy G, Fanning S, McMahon BJ. 2018. A scoping review on the prevalence of Shiga-toxigenic Escherichia coli in wild animal species. Zoonoses and Public Health 65:911–920.
Essential Freshwater Science and Technical Advisory Group. 2019. Freshwater Science and Technical Advisory Group (STAG) report to the Minister for the Environment. Wellington, New Zealand.
Estrada-Peña A, Ostfeld RS, Peterson AT, Poulin R, de la Fuente J. 2014. Effects of environmental change on zoonotic disease risk: an ecological primer. Trends in Parasitology 30:205–214.
Etten, Julia Van, and Debashish Bhattacharya. 2020. 'Horizontal Gene Transfer in Eukaryotes: Not If, but How Much?' Trends in Genetics 36: 915–25.
European Center for Disease Prevention and Control. 2018. Annual epidemiological report for 2016 Shiga-toxin/verocytotoxin-producing Escherichia coli (STEC/VTEC) infection. Available at https://www.ecdc.europa.eu/en/publications-data/shiga-toxinverocytotoxin-producing-escherichia-coli-stecvtec-infection-annual (accessed 4 October 2019).
Evans BR, Leighton FA. 2014. A history of One Health. Revue Scientifique et Technique (International Office of Epizootics) 33:413.
Ewald PW. 1983. Host-parasite relations, vectors, and the evolution of disease severity. Annual Review of Ecology and Systematics 14:465–485.
Ewers C, Antão E-M, Diehl I, Philipp H-C, Wieler LH. 2009. Intestine and environment of the chicken as reservoirs for extraintestinal pathogenic Escherichia coli strains with zoonotic potential. Applied and Environmental Microbiology 75:184–192.
Fairbrother JM, Nadeau E. 2006. Escherichia coli: on-farm contamination of animals. Rev Sci Tech 25:555–569.
Fauci AS, Lane HC, Redfield RR. 2020.Covid-19—navigating the uncharted. Available at https://www.nejm.org/doi/full/10.1056/nejme2002387 (accessed April 20, 2021).
Feichtmayer J, Deng L, Griebler C. 2017. Antagonistic microbial interactions: contributions and potential applications for controlling pathogens in the aquatic systems. Frontiers in microbiology 8:2192.

Ferri M, Ranucci E, Romagnoli P, Giaccone V. 2017. Antimicrobial resistance: a global emerging threat to public health systems. Critical Reviews in Food Science and Nutrition 57:2857–2876.
Fisher AN, Ryan MK. 2021. Gender inequalities during COVID-19. Group Processes & Intergroup Relations 24:237–245.
Fitzgerald JR, Musser JM. 2001. Evolutionary genomics of pathogenic bacteria. Trends in Microbiology 9:547–553.
Fitzpatrick FA, Scudder BC, Lenz BN, Sullivan DJ. 2001. Effects of multi-scale environmental characteristics on agricultural stream biota in eastern Wisconsin. Journal of the American Water Resources Association 37:1489–1507.
Flotemersch JE, Shattuck SM, Aho KB, Cox CE, Cairns MR. 2019. Factors influencing social demands of aquatic ecosystems. Ecology and Society 24:1.
Fluke J, González-Pinzón R, Thomson B. 2019. Riverbed sediments control the spatiotemporal variability of E. coli in a highly managed, arid river. Frontiers in Water 1:4.
Foerstner KU, Von Mering C, Hooper SD, Bork P. 2005. Environments shape the nucleotide composition of genomes. EMBO reports 6:1208–1213.
Fong IW. 2017. Emerging zoonoses. In: Emerging Infectious Diseases of the 21st Century. Cham, Switzerland: Springer, 1–33.
Fong TT, Lipp EK. 2005. Enteric viruses of humans and animals in aquatic environments: health risks, detection, and potential water quality assessment tools. Microbiology and Molecular Biology Reviews 69:357–371.
Fonseca VG. 2018. Pitfalls in relative abundance estimation using eDNA metabarcoding.
Fonseca, Vera G. 2018. Pitfalls in Relative Abundance Estimation Using EDNA Metabarcoding. Molecular Ecology Resources. DOI:10.1111/1755-0998.12902
Foote KJ, Joy MK, Death RG. 2015. New Zealand dairy farming: milking our environment for all its worth. Environmental Management 56:709–720. DOI: 10.1007/s00267-015-0517-x.
Forouzan E, Shariati P, Maleki MSM, Karkhane AA, Yakhchali B. 2018. Practical evaluation of 11 de novo assemblers in metagenome assembly. Journal of Microbiological Methods 151:99–105.
Foster G, Evans J, Knight HI, Smith AW, Gunn GJ, Allison LJ, Synge BA, Pennycott TW. 2006. Analysis of feces samples collected from a wild-bird garden feeding station in Scotland for the presence of verocytotoxin-producing Escherichia coli O157. Applied and Environmental Microbiology 72:2265–2267.
Fraiture MA, Deckers M, Papazova N, Roosens NHC. 2020. Detection strategy targeting a chloramphenicol resistance gene from genetically modified bacteria in food and feed products. Food Control 108:106873.
Franck SM, Bosworth BT, Moon HW. 1998. Multiplex PCR for enterotoxigenic, attaching and effacing, and Shiga toxin-producing Escherichia coli strains from calves. Journal of Clinical Microbiology 36:1795–1797.
Francy DS, Myers DN, Metzker KD. 1993. Escherichia coli and fecal-coliform bacteria as indicators of recreational water quality. Columbus, Ohio: U.S. Department of the Interior, U.S. Geological Survey.
Frank SA. 1996. Models of parasite virulence. The Quarterly review of biology 71:37–78.
Fremaux B, Prigent-Combaret C, Vernozy-Rozand C. 2008. Long-term survival of Shiga toxin-producing Escherichia coli in cattle effluents and environment: an updated review. Veterinary Microbiology 132:1–18.
French R, Charon J, Lay C Le, Muller C, Holmes EC. 2022. Human land use impacts viral diversity and abundance in a New Zealand river. Virus Evolution 8:veac032.

French, Rebecca, Justine Charon, Callum Le Lay, Chris Muller, and Edward C Holmes. 2022. 'Human Land Use Impacts Viral Diversity and Abundance in a New Zealand River'. Virus Evolution 8: veac032.
Frenzen PD, Drake A, Angulo FJ. 2005. Economic cost of illness due to Escherichia coli O157 infections in the United States. Journal of Food Protection 68:2623–2630.
Frick C, Vierheilig J, Linke R, Savio D, Zornig H, Antensteiner R, Baumgartner C, Bucher C, Blaschke AP, Derx J. 2018. Poikilothermic animals as a previously unrecognized source of fecal indicator bacteria in a backwater ecosystem of a large river. Applied and Environmental Microbiology 84:AEM-00715.
Frumin GT, Gildeeva IM. 2014. Eutrophication of water bodies—A global environmental problem. Russian Journal of General Chemistry 84:2483–2488.
Frutos R, Devaux CA. 2020. Mass culling of minks to protect the COVID-19 vaccines: is it rational? New Microbes and New Infections 38:100816.
Fry GLA. 2001. Multifunctional landscapes—towards transdisciplinary research. Landscape and Urban Planning 57:159–168.
Fuchsman CA, Collins RE, Rocap G, Brazelton WJ. 2017. Effect of the environment on horizontal gene transfer between bacteria and archaea. PeerJ 5:e3865.
Fuhrman JA, Campbell L. 1998. Microbial microdiversity. Nature 393:410–411.
Fuhrman JA, Cram JA, Needham DM. 2015. Marine microbial community dynamics and their ecological interpretation. Nature Reviews Microbiology 13:133–146.
Fukushima H, Hoshina K, Gomyoda M. 1999. Long-Term Survival of Shiga Toxin-ProducingEscherichia coli O26, O111, and O157 in Bovine Feces. Applied and Environmental Microbiology 65:5177–5181.
Gagliardi J v, Karns JS. 2000. Leaching of Escherichia coli O157: H7 in diverse soils under various agricultural management practices. Applied and Environmental Microbiology 66:877–883.
Galfi H, Österlund H, Marsalek J, Viklander M. 2016. Indicator bacteria and associated water quality constituents in stormwater and snowmelt from four urban catchments. Journal of Hydrology 539:125–140.
Galvani AP. 2003. Epidemiology meets evolutionary ecology. Trends in Ecology & Evolution 18:132–139.
Gannon JT, Manilal VB, Alexander M. 1991. Relationship between cell surface properties and transport of bacteria through soil. Applied and Environmental Microbiology 57:190–193.
García-Meniño I, García V, Alonso MP, Blanco JE, Blanco J, Mora A. 2021. Clones of enterotoxigenic and Shiga toxin-producing Escherichia coli implicated in swine enteric colibacillosis in Spain and rates of antibiotic resistance. Veterinary Microbiology 252:108924.
Garg S, Kim L, Whitaker M, O'Halloran A, Cummings C, Holstein R, Prill M, Chai SJ, Kirley PD, Alden NB. 2020. Hospitalization rates and characteristics of patients hospitalized with laboratory-confirmed coronavirus disease 2019—COVID-NET, 14 States, March 1–30, 2020. Morbidity and Mortality Weekly Report 69:458.
Garlapati D, Charankumar B, Ramu K, Madeswaran P, Murthy MVR. 2019. A review on the applications and recent advances in environmental DNA (eDNA) metagenomics. Reviews in Environmental Science and Bio/Technology 18:389–411.
Garzio-Hadzick A, Shelton DR, Hill RL, Pachepsky YA, Guber AK, Rowland R. 2010. Survival of manure-borne E. coli in streambed sediment: effects of temperature and sediment properties. Water Research 44:2753–2762.
Gelvin SB. 2009. Agrobacterium in the genomics age. Plant Physiology 150:1665–1676.
Geng M, Zhang W, Hu T, Wang R, Cheng X, Wang J. 2022. Eutrophication causes microbial

community homogenization via modulating generalist species. Water Research 210:118003.
Genin S, Denny TP. 2012. Pathogenomics of the Ralstonia solanacearum species complex. Annual Review of Phytopathology 50:67–89.
Geoghegan JL, Holmes EC. 2018. The phylogenomics of evolving virus virulence. Nature Reviews Genetics 19:756–769.
Geoghegan JL, Ren X, Storey M, Hadfield J, Jelley L, Jefferies S, Sherwood J, Paine S, Huang S, Douglas J. 2020. Genomic epidemiology reveals transmission patterns and dynamics of SARS-CoV-2 in Aotearoa New Zealand. Nature Communications 11:1–7.
Gerba CP, Bitton G. 1984. Microbial pollutants: their survival and transport pattern to groundwater. Groundwater Pollution Microbiology, John Wiley and Sons, New York, New York 1984. p 65-88, 3 fig, 4 tab, 123 ref.
Germer S, Neill C, Krusche A V, Elsenbeer H. 2010. Influence of land-use change on near-surface hydrological processes: undisturbed forest to pasture. Journal of Hydrology 380:473–480.
Ghaly TM, Tetu SG, Penesyan A, Qi Q, Rajabal V, Gillings MR. 2022. Discovery of integrons in Archaea: Platforms for cross-domain gene transfer. Science Advances 8:eabq6376.
Gibbons DW, Sandbrook C, Sutherland WJ, Akter R, Bradbury R, Broad S, Clements A, Crick HQP, Elliott J, Gyeltshen N. 2021. The relative importance of COVID-19 pandemic impacts on biodiversity conservation globally. Conservation Biology:1–34.
Gibbs DS, Anderson GL, Beuchat LR, Carta LK, Williams PL. 2005. Potential role of Diploscapter sp. strain LKC25, a bacterivorous nematode from soil, as a vector of food-borne pathogenic bacteria to preharvest fruits and vegetables. Applied and Environmental Microbiology 71:2433–2437.
Gill EE, Brinkman FSL. 2011. The proportional lack of archaeal pathogens: Do viruses/phages hold the key? Bioessays 33:248–254.
Gill SR, Pop M, DeBoy RT, Eckburg PB, Turnbaugh PJ, Samuel BS, Gordon JI, Relman DA, Fraser-Liggett CM, Nelson KE. 2006. Metagenomic analysis of the human distal gut microbiome. Science 312:1355–1359.
Giller PS, Hillebrand H, Berninger UG, Gessner MO, Hawkins S, Inchausti P, Inglis C, Leslie H, Malmqvist B, Monaghan MT, Morin PJ, O'Mullan G. 2004. Biodiversity effects on ecosystem functioning: emerging issues and their experimental test in aquatic environments. Oikos 104:423–436.
Gilpin B, Walker T, Paine S, Sherwood J, Mackereth G, Wood T, Hambling T, Hewison C, Brounts A, Wilson M, Scholes P, Robson B, Lin S, Cornelius A, Rivas L, Hayman D, French N, Zhang J, Wilkinson D, Midwinter A, Biggs P, Jagroop A, Eyre R, Baker M, Jones N. 2020. A large scale waterborne Campylobacteriosis outbreak, Havelock North, New Zealand. Journal of Infection 81:390–395.
Gilpin BJ, Walker T, Paine S, Sherwood J, Mackereth G, Wood T, Hambling T, Hewison C, Brounts A, Wilson M. 2020. A large scale waterborne Campylobacteriosis outbreak, Havelock North, New Zealand. Journal of Infection 81:390–395.
Gleick PH. 1996. Water resources. In: Schneider SH ed. Encyclopedia of Climate and Weather. New York, USA: Oxford University Press US, 817–823.
Gluckman P. 2017. New Zealand's fresh waters : Values, state, trends and human impacts. Wellington, New Zealand.
Glünder G. 2002. Influence of diet on the occurrence of some bacteria in the intestinal flora of wild and pet birds. DTW. Deutsche Tierarztliche Wochenschrift 109:266–270.
Goławska O, Zając M, Maluta A, Pristas P, Hamarová Ľ, Wasyl D. 2019. Complex bacterial flora of imported pet tortoises deceased during quarantine: Another zoonotic threat?

Comparative Immunology, Microbiology and Infectious Diseases 65:154–159.
Gonggrijp MA, Santman-Berends I, Heuvelink AE, Buter GJ, Van Schaik G, Hage JJ, Lam T. 2016. Prevalence and risk factors for extended-spectrum β-lactamase-and AmpC-producing Escherichia coli in dairy farms. Journal of Dairy Science 99:9001–9013.
Gonzales Tovar J, Sarmiento Barletti JP, Larson AM, Barnes G, Tucker CM. 2021. Can multistakeholder forums empower indigenous and local communities and promote forest conservation? A comparative analysis of territorial planning in two Brazilian states with contrasting contexts. Conservation Science and Practice 3:e326.
Gopee N v, Adesiyun AA, Caesar K. 2000. A longitudinal study of Escherichia coli strains isolated from captive mammals, birds, and reptiles in Trinidad. Journal of Zoo and Wildlife Medicine 31:353–360.
Gophna U, Altman-Price N. 2022. Horizontal gene transfer in archaea—from mechanisms to genome evolution. Annual Review of Microbiology 76:481–502.
Gordon DM, Cowling A. 2003. The distribution and genetic structure of Escherichia coli in Australian vertebrates: host and geographic effects. Microbiology 149:3575–3586.
Gortázar C, Ferroglio E, Höfle U, Frölich K, Vicente J. 2007. Diseases shared between wildlife and livestock: a European perspective. European Journal of Wildlife Research 53:241.
Graczyk TK, Evans BM, Shiff CJ, Karreman HJ, Patz JA. 2000. Environmental and geographical factors contributing to watershed contamination with Cryptosporidium parvum oocysts. Environmental Research 82:263–271.
Gravesen ML. 2020. Blaming the others: ethnic identity and claim-making. In: The contested lands of Laikipia. Leiden, The Netherlands: Brill, 80–122.
Gregor J, Maršálek B. 2004. Freshwater phytoplankton quantification by chlorophyll a: a comparative study of in vitro, in vivo and in situ methods. Water Research 38:517–522.
Groisman EA, Ochman H. 1997. How Salmonella became a pathogen. Trends in Microbiology 5:343–349.
Groner E, Novoplansky A. 2003. Reconsidering diversity-productivity relationships: Directness of productivity estimates matters. Ecology Letters 6:695–699. DOI: 10.1046/j.1461-0248.2003.00488.x.
Gronewold AD, Wolpert RL. 2008. Modeling the relationship between most probable number (MPN) and colony-forming unit (CFU) estimates of fecal coliform concentration. Water Research 42:3327–3334.
Grossart H, Massana R, McMahon KD, Walsh DA. 2020. Linking metagenomics to aquatic microbial ecology and biogeochemical cycles. Limnology and Oceanography 65:S2–S20.
Gryseels S, De Bruyn L, Gyselings R, Calvignac-Spencer S, Leendertz FH, Leirs H. 2020. Risk of human-to-wildlife transmission of SARS-CoV-2. Mammal Review 51:272–292. DOI: 10.1111/mam.12225.
Gu W, Zhou T, Wilke CO. 2010. A universal trend of reduced mRNA stability near the translation-initiation site in prokaryotes and eukaryotes. PLoS Computational Biology 6:e1000664.
Guan, Y, Jia, J, Fan, X, Li, K, Wang, Z. 2022. Anthropogenic impacts on antibiotic resistance genes and their hosts from pristine to urban river using metagenomic and binning approaches. Aquatic Toxicology 249:106221.
Gülay A, Çekiç Y, Musovic S, Albrechtsen H-J, Smets BF. 2018. Diversity of iron oxidizers in groundwater-fed rapid sand filters: evidence of Fe (II)-dependent growth by Curvibacter and Undibacterium spp. Frontiers in Microbiology 9:2808.
Guo AC, Jewison T, Wilson M, Liu Y, Knox C, Djoumbou Y, Lo P, Mandal R, Krishnamurthy R, Wishart DS. 2012. ECMDB: the E. coli Metabolome Database.

Nucleic Acids Research 41:D625–D630.
Guo Y, Bandaru V, Jaruga P, Zhao X, Burrows CJ, Iwai S, Dizdaroglu M, Bond JP, Wallace SS. 2010. The oxidative DNA glycosylases of Mycobacterium tuberculosis exhibit different substrate preferences from their Escherichia coli counterparts. DNA Repair 9:177–190.
Guss AM, Rother M, Zhang JK, Kulkkarni G, Metcalf WW. 2008. New methods for tightly regulated gene expression and highly efficient chromosomal integration of cloned genes for Methanosarcina species. Archaea 2:193–203.
Guterres A. 2023. Progress towards the Sustainable Development Goals: towards a rescue plan for people and planet.
Haase P, Pauls SU, Schindehütte K, Sundermann A. 2010. First audit of macroinvertebrate samples from an EU Water Framework Directive monitoring program: human error greatly lowers precision of assessment results. Journal of the North American Benthological Society 29:1279–1291.
Haberecht HB, Nealon NJ, Gilliland JR, Holder A V, Runyan C, Oppel RC, Ibrahim HM, Mueller L, Schrupp F, Vilchez S. 2019. Antimicrobial-resistant Escherichia coli from environmental waters in Northern Colorado. Journal of Environmental and Public Health 18:e05967.
Haller L, Amedegnato E, Poté J, Wildi W. 2009. Influence of freshwater sediment characteristics on persistence of fecal indicator bacteria. Water, Air, and Soil Pollution 203:217–227.
Hamill KD, McBride GB. 2003. River water quality trends and increased dairying in Southland, New Zealand. New Zealand Journal of Marine and Freshwater Research 37:323–332.
Hamilton WP, Kim M, Thackston EL. 2005. Comparison of commercially available Escherichia coli enumeration tests: Implications for attaining water quality standards. Water Research 39:4869–4878.
Hammes WP, Vogel RF. 1995. The genus lactobacillus. In: Holzapfel WH ed. The genera of lactic acid bacteria. Boston, MA: Springer, 19–54. DOI: https://doi.org/10.1007/978-1-4615-5817-0_3.
Hansell DA, Carlson CA. 2002. Biogeochemistry of marine dissolved organic matter. London, UK: Academic Press.
Hansen C. 2019. How funding and financing affects productivity: Implications for three-waters reform and for local government funding and financing. Wellington, New Zealand.
Hardin G. 1968. The tragedy of the commons. Science 162:1243–1248.
Hardison RC. 2003. Comparative genomics. PLoS Biology 1:e58.
Harmsworth GR, Awatere S. 2013. Indigenous Māori knowledge and perspectives of ecosystems. Ecosystem services in New Zealand—conditions and trends. Manaaki Whenua Press, Lincoln, New Zealand:274–286.
Harris A, Dash J. 2010. The potential of the MERIS Terrestrial Chlorophyll Index for carbon flux estimation. Remote Sensing of Environment 114:1856–1862.
Harrison S, Baker MG, Benschop J, Death RG, French NP, Harmsworth G, Lake RJ, Lamont IL, Priest PC, Ussher JE. 2020. One Health Aotearoa: a transdisciplinary initiative to improve human, animal and environmental health in New Zealand. One Health Outlook 2:1–6.
Hartz A, Cuvelier M, Nowosielski K, Bonilla TD, Green M, Esiobu N, McCorquodale DS, Rogerson A. 2008. Survival potential of Escherichia coli and enterococci in subtropical beach sand: implications for water quality managers. Journal of Environmental Quality 37:898–905.

Harwood VJ, Staley C, Badgley BD, Borges K, Korajkic A. 2014. Microbial source tracking markers for detection of fecal contamination in environmental waters: relationships between pathogens and human health outcomes. FEMS Microbiology Reviews 38:1–40.
Häsler B, Gilbert W, Jones BA, Pfeiffer DU, Rushton J, Otte MJ. 2012. The economic value of One Health in relation to the mitigation of zoonotic disease risks. Heidelberg, Germany: Springer. DOI: 10.1007/978-3-642-36889-9.
Hauer C, Leitner P, Unfer G, Pulg U, Habersack H, Graf W. 2018. The role of sediment and sediment dynamics in the aquatic environment. Cham, Switzerland: Springer International Publishing.
Havelaar AH, Melse JM. 2003. Quantifying public health risk in the WHO Guidelines for drinking-water quality: A burden of disease approach. Bilthoven, Netherlands: Rijksinstituut voor Volksgezondheid en Milieu RIVM.
Haverkamp THA, Lossouarn J, Zhaxybayeva O, Lyu J, Bienvenu N, Geslin C, Nesbø CL. 2021. Newly identified proviruses in Thermotogota suggest that viruses are the vehicles on the highways of interphylum gene sharing. Environmental Microbiology 23:7105–7120.
Hay SI, Rao PC, Dolecek C, Day NPJ, Stergachis A, Lopez AD, Murray CJL. 2018. Measuring and mapping the global burden of antimicrobial resistance. BMC Medicine 16:1–3.
Haydon DT, Cleaveland S, Taylor LH, Laurenson MK. 2002. Identifying reservoirs of infection: a conceptual and practical challenge. Emerging Infectious Diseases 8:1468–1473.
Hayes NM, Vanni MJ, Horgan MJ, Renwick WH. 2014. Climate and land use interactively affect lake phytoplankton nutrient limitation status. Ecology 96:392–402. DOI: 10.1890/13-1840.1.
Head M, Brown R, Batchelor J, Newell M-L, Scott A, Atun R. 2020. The allocation of US $105 billion in global funding for infectious disease research between 2000 and 2017: a content analysis of investments from funders in the G20 countries. Preprints with the Lancet:1–26.
Heijnen, L, & Medema, G. 2006. Quantitative detection of E. coli, E. coli O157 and other shiga toxin-producing E. coli in water samples using a culture method combined with real-time PCR. Journal of Water and Health 4: 487–498.
Heiskary SA, Bouchard Jr. RW. 2015. Development of eutrophication criteria for Minnesota streams and rivers using multiple lines of evidence. Freshwater Science 34:574–592. DOI: 10.1086/680662.
Henderson H. 2008. Direct and indirect zoonotic transmission of Shiga toxin–producing Escherichia coli. Journal of the American Veterinary Medical Association 232:848–859.
Hendricks SP. 1993. Microbial ecology of the hyporheic zone: a perspective integrating hydrology and biology. Journal of the North American Benthological Society 12:70–78.Hernando-Amado S, Coque TM, Baquero F, Martínez JL. 2019. Defining and combating antibiotic resistance from One Health and Global Health perspectives. Nature Microbiology 4:1432–1442.
Hernando-Amado S, Coque TM, Baquero F, Martínez JL. 2019. Defining and combating antibiotic resistance from One Health and Global Health perspectives. Nature Microbiology 4:1432–1442.
Herrera D, Ellis A, Fisher B, Golden CD, Johnson K, Mulligan M, Pfaff A, Treuer T, Ricketts TH. 2017. Upstream watershed condition predicts rural children's health across 35 developing countries. Nature Communications 8:811. DOI: 10.1038/s41467-017-00775-2.
Higgins J, Zablocki J, Newsock A, Krolopp A, Tabas P, Salama M. 2021. Durable freshwater

protection: a framework for establishing and maintaining long-term protection for freshwater ecosystems and the values they sustain. Sustainability 13:1950.
Hilbi H, Weber SS, Ragaz C, Nyfeler Y, Urwyler S. 2007. Environmental predators as models for bacterial pathogenesis. Environmental Microbiology 9:563–575.
Hildebrand F, Meyer A, Eyre-Walker A. 2010. Evidence of selection upon genomic GC-content in bacteria. PLoS Genetics 6:e1001107.
Hilderbrand RH, Keller SR, Laperriere SM, Santoro AE, Cessna J, Trott R. 2020. Microbial communities can predict the ecological condition of headwater streams. PloS One 15:e0236932.
Hill WE, Carlisle CL. 1981. Loss of plasmids during enrichment for Escherichia coli. Applied and Environmental Microbiology 41:1046–1048.
Hillmann B, Al-Ghalith GA, Shields-Cutler RR, Zhu Q, Gohl DM, Beckman KB, Knight R, Knights D. 2018. Evaluating the information content of shallow shotgun metagenomics. MSystems 3:10-1128.
Hiruta N, Murase T, Okamura N. 2001. An outbreak of diarrhoea due to multiple antimicrobial-resistant Shiga toxin-producing Escherichia coli O26 [ratio] H11 in a nursery. Epidemiology & Infection 127:221–227.
Hjalten J, Nilsson C, Jorgensen D, Bell D. 2016. Forest-stream links, anthropogenic stressors, and climate change: implications for restoration planning. Bioscience 66:646–654. DOI: 10.1093/biosci/biw072.
Hmelo LR. 2017. Quorum sensing in marine microbial environments. Annual Review of Marine Science 9:257–281.
Hoekstra AY. 2014. Sustainable, efficient, and equitable water use: the three pillars under wise freshwater allocation. Wiley Interdisciplinary Reviews: Water 1:31–40. DOI: 10.1002/wat2.1000.
Hoiby N, Doring G, Schiotz PO. 1986. The role of immune complexes in the pathogenesis of bacterial infections. Annual Reviews in Microbiology 40:29.
Holcomb DA, Stewart JR. 2020. Microbial indicators of fecal pollution: recent progress and challenges in assessing water quality. Current Environmental Health Reports 7:311–324.
Honda R, Tachi C, Yasuda K, Hirata T, Noguchi M, Hara-Yamamura H, Yamamoto-Ikemoto R, Watanabe T. 2020. Estimated discharge of antibiotic-resistant bacteria from combined sewer overflows of urban sewage system. NPJ Clean Water 3:1–7.
Horton R, Beaglehole R, Bonita R, Raeburn J, McKee M, Wall S. 2014. From public to planetary health: a manifesto. The Lancet 383:847.
Howell JM, Coyne MS, Cornelius PL. 1996. Effect of sediment particle size and temperature on fecal bacteria mortality rates and the fecal coliform/fecal streptococci ratio. Journal of Environmental Quality 25:1216–1220.
Hrudey SE, Hrudey EJ, Pollard SJT. 2006. Risk management for assuring safe drinking water. Environment International 32:948–957.
Hu A, Hou L, Yu C-P. 2015. Biogeography of planktonic and benthic archaeal communities in a subtropical eutrophic estuary of China. Microbial Ecology 70:322–335.
Hu EZ, Lan XR, Liu ZL, Gao J, Niu DK. 2022. A positive correlation between GC content and growth temperature in prokaryotes. BMC Genomics 23:110.
Huang H, von Lampe M, van Tongeren F. 2011. Climate change and trade in agriculture. Food Policy 36:S9–S13.
Hubálek Z. 2003. Emerging human infectious diseases: anthroponoses, zoonoses, and sapronoses. Emerging Infectious Diseases 9:403.
Huber C, Finelli L, Stevens W. 2018. The economic and social burden of the 2014 Ebola outbreak in West Africa. The Journal of Infectious Diseases 218:S698–S704.
Huffman DE, Quinter-Betancourt W, Rose J. 2003. Emerging waterborne pathogens. In:

Mara D, Horan N eds. Handbook of Water and Wastewater Microbiology. London, UK: Academic Press, 193–208.
Hughes JM, Schmidt DJ, Finn DS. 2009. Genes in Streams: Using DNA to Understand the Movement of Freshwater Fauna and Their Riverine Habitat. Bioscience 59:573–583. DOI: 10.1525/bio.2009.59.7.8.
Hugoni M, Domaizon I, Taib N, Biderre-Petit C, Agogué H, Galand PE, Debroas D, Mary I. 2015. Temporal dynamics of active A rchaea in oxygen-depleted zones of two deep lakes. Environmental Microbiology Reports 7:321–329.
Huijbregts MAJ, Seppälä J. 2001. Life cycle impact assessment of pollutants causing aquatic eutrophication. The International Journal of Life Cycle Assessment 6:339–343.
Hunter C, McDonald A, Beven K. 1992. Input of fecal coliform bacteria to an upland stream channel in the Yorkshire Dales. Water Resources Research 28:1869–1876.
Hyytiäinen K, Bauer B, Bly Joyce K, Ehrnsten E, Eilola K, Gustafsson BG, Meier HEM, Norkko A, Saraiva S, Tomczak M. 2021. Provision of aquatic ecosystem services as a consequence of societal changes: The case of the Baltic Sea. Population Ecology 63:61–74.
Inoue H, Todo Y. 2020. The propagation of economic impacts through supply chains: The case of a mega-city lockdown to prevent the spread of COVID-19. PloS One 15:e0239251.
Irshad H, Cookson AL, Ross CM, Jaros P, Prattley DJ, Donnison A, McBride G, Marshall J, French NP. 2016. Diversity and relatedness of Shiga toxin-producing Escherichia coli and Campylobacter jejuni between farms in a dairy catchment. Epidemiology & Infection 144:1406–1417.
Isaac G, Finn S, Joe JR, Hoover E, Gone JP, Lefthand-Begay C, Hill S. 2018. Native American perspectives on health and traditional ecological knowledge. Environmental Health Perspectives 126:125002.
Ishii S, Sadowsky MJ. 2008. Escherichia coli in the environment: implications for water quality and human health. Microbes and Environments 23:101–108.
Ishii Y, Kimura S, Alba J, Shiroto K, Otsuka M, Hashizume N, Tamura K, Yamaguchi K. 2005. Extended-spectrum β-lactamase-producing shiga toxin gene (stx1)-positive Escherichia coli O26: H11: a new concern. Journal of Clinical Microbiology 43:1072–1075.
Jalliffier-Verne I, Heniche M, Madoux-Humery AS, Galarneau M, Servais P, Prévost M, Dorner S. 2016. Cumulative effects of fecal contamination from combined sewer overflows: Management for source water protection. Journal of Environmental Management 174:62–70.
Jamieson et al. 2003. Sources and persistence of fecal coliform bacteria in a rural watershed. Water Quality Research Journal 38:33–47.
Jamieson R, Gordon R, Joy D, Lee H. 2004. Assessing microbial pollution of rural surface waters: A review of current watershed scale modeling approaches. Agricultural Water Management 70:1–17.
Jamieson R, Gordon R, Tattrie S, Stratton G. 2003. Sources and persistence of fecal coliform bacteria in a rural watershed. Water Quality Research Journal 38:33–47.
Jaros P, Cookson AL, Campbell DM, Besser TE, Shringi S, Mackereth GF, Lim E, Lopez L, Dufour M, Marshall JC. 2013. A prospective case–control and molecular epidemiological study of human cases of Shiga toxin-producing Escherichia coli in New Zealand. BMC Infectious Diseases 13:450.
Jefferies S, French N, Gilkison C, Graham G, Hope V, Marshall J, McElnay C, McNeill A, Muellner P, Paine S. 2020. COVID-19 in New Zealand and the impact of the national response: a descriptive epidemiological study. The Lancet Public Health 5:e612–e623.

Jeng HC, England AJ, Bradford HB. 2005. Indicator organisms associated with stormwater suspended particles and estuarine sediment. Journal of Environmental Science and Health 40:779–791.
Jenkins M, Ahmed S, Barnes AN. 2021. A systematic review of waterborne and water-related disease in animal populations of Florida from 1999–2019. PloS One 16:e0255025.
Jerde CL, Mahon AR, Chadderton WL, Lodge DM. 2011. "Sight-unseen" detection of rare aquatic species using environmental DNA. Conservation Letters 4:150–157. DOI: 10.1111/j.1755-263X.2010.00158.x.
Johnson CK, Hitchens PL, Pandit PS, Rushmore J, Evans TS, Young CCW, Doyle MM. 2020. Global shifts in mammalian population trends reveal key predictors of virus spillover risk. Proceedings of the Royal Society B 287:20192736.
Johnson I, Hansen A, Bi P. 2018. The challenges of implementing an integrated One Health surveillance system in Australia. Zoonoses and Public Health 65:e229–e236.
Johnson J, Degeling C. 2020. More philosophical work needed in One Health on ethical frameworks and theory. Journal of Medical Ethics 46:705–706.
Johnson JR. 1991. Virulence factors in Escherichia coli urinary tract infection. Clinical Microbiology Reviews 4:80–128.
Johnson JS, Spakowicz DJ, Hong B-Y, Petersen LM, Demkowicz P, Chen L, Leopold SR, Hanson BM, Agresta HO, Gerstein M. 2019. Evaluation of 16S rRNA gene sequencing for species and strain-level microbiome analysis. Nature Communications 10:1–11.
Johnston CA, Bridgham SD, Schubauer-Berigan JP. 2001. Nutrient dynamics in relation to geomorphology of riverine wetlands. Soil Science Society of America Journal 65:557–577.
Jones I, Abrahams C, Brown L, Dale K, Edwards F, Jeffries M, Klaar M, Ledger M, May L, Milner A, Murphy J, Robertson A, Woodward G. 2013. The impact of extreme events on freshwater ecosystems. London: British Ecological Society.
Jones KE, Patel NG, Levy MA, Storeygard A, Balk D, Gittleman JL, Daszak P. 2008. Global trends in emerging infectious diseases. Nature 451:990.
Jones S, Greene N, Hueso A, Sharp H, Kennedy-Walker R. 2013. Learning from failure: lessons for the sanitation sector. London: UK Sanitation Community of Practice.
Joseph A, Cointe A, Mariani Kurkdjian P, Rafat C, Hertig A. 2020. Shiga toxin-associated hemolytic uremic syndrome: a narrative review. Toxins 12:2–46.
Jovel J, Patterson J, Wang W, Hotte N, O'Keefe S, Mitchel T, Perry T, Kao D, Mason AL, Madsen KL. 2016. Characterization of the gut microbiome using 16S or shotgun metagenomics. Frontiers in Microbiology 7:459.
Joy MK. 2015. A fish index of biotic integrity (IBI) for Horizons Regional Council. Palmerston North.
Joy MK, Rankin DA, Wöhler L, Boyce P, Canning A, Foote KJ, McNie PM. 2022. The grey water footprint of milk due to nitrate leaching from dairy farms in Canterbury, New Zealand. Australasian Journal of Environmental Management 29:177–199.
Joy, MK. 2015. A Fish Index of Biotic Integrity (IBI) for Horizons Regional Council. Palmerston North. https://envirolink.govt.nz/assets/Envirolink/1541-HZLC118-A-fish-Index-of-Biotic-Integrity-IBI-for-Horizons-Regional-Council.pdf.
Julian JP, de Beurs KM, Owsley B, Davies-Colley RJ, Ausseil AGE. 2017. River water quality changes in New Zealand over 26 years: response to land use intensity. Hydrology and Earth System Sciences 21:1149–1171. DOI: 10.5194/hess-21-1149-2017.
Kaden R, Spröer C, Beyer D, Krolla-Sidenstein P. 2014. Rhodoferax saidenbachensis sp. nov., a psychrotolerant, very slowly growing bacterium within the family Comamonadaceae, proposal of appropriate taxonomic position of Albidiferax

ferrireducens strain T118T in the genus Rhodoferax and emended description of. International Journal of Systematic and Evolutionary Microbiology 64:1186–1193.
Kaiser J, Egbetade AO, Sonibare AO, Meseko CA, Jayeola OA, Otesile EB. 2015. Implications of Ebola virus disease on wildlife conservation in Nigeria. Science 300:232–233.
Kajale S, Jani K, Sharma A. 2021. Contribution of archaea and bacteria in sustaining climate change by oxidizing ammonia and sulfur in an Arctic Fjord. Genomics 113:1272–1276.
Kalyuzhnaya MG, Lapidus A, Ivanova N, Copeland AC, McHardy AC, Szeto E, Salamov A, Grigoriev I V, Suciu D, Levine SR. 2008. High-resolution metagenomics targets specific functional types in complex microbial communities. Nature Biotechnology 26:1029–1034.
Karesh WB, Dobson A, Lloyd-Smith JO, Lubroth J, Dixon MA, Bennett M, Aldrich S, Harrington T, Formenty P, Loh EH. 2012. Ecology of zoonoses: natural and unnatural histories. The Lancet 380:1936–1945.
Karner MB, DeLong EF, Karl DM. 2001. Archaeal dominance in the mesopelagic zone of the Pacific Ocean. Nature 409:507–510.
Kasalický V, Jezbera J, Hahn MW, Šimek K. 2013. The diversity of the Limnohabitans genus, an important group of freshwater bacterioplankton, by characterization of 35 isolated strains. PloS One 8:e58209.
Kash JC, Taubenberger JK. 2015. The role of viral, host, and secondary bacterial factors in influenza pathogenesis. The American Journal of Pathology 185:1528–1536.
Kelly DJ, Hawes I. 2005. Effects of invasive macrophytes on littoral-zone productivity and foodweb dynamics in a New Zealand high-country lake. Journal of the North American Benthological Society 24:300–320.
Khachatryan L, de Leeuw RH, Kraakman MEM, Pappas N, Te Raa M, Mei H, de Knijff P, Laros JFJ. 2020. Taxonomic classification and abundance estimation using 16S and WGS—A comparison using controlled reference samples. Forensic Science International: Genetics 46:102257.
Khan MN, Mohammad F. 2014. Eutrophication: challenges and solutions. In: Eutrophication: Causes, consequences and control. Springer, 1–15.
King N, Lake R, Campbell D. 2011. Source attribution of nontyphoid salmonellosis in New Zealand using outbreak surveillance data. Journal of Food Protection 74:438–445.
Kinzelman J, McLellan SL, Daniels AD, Cashin S, Singh A, Gradus S, Bagley R. 2004. Non-point source pollution: determination of replication versus persistence of Escherichia coli in surface water and sediments with correlation of levels to readily measurable environmental parameters. Journal of Water and Health 2:103–114.
Kirchner M, Mafura M, Hunt T, Card R, Anjum MF. 2013. Antibiotic resistance gene profiling of faecal and oral anaerobes collected during an antibiotic challenge trial. Anaerobe 23:20–22.
Kirschner AKT, Zechmeister TC, Kavka GG, Beiwl C, Herzig A, Mach RL, Farnleitner AH. 2004. Integral strategy for evaluation of fecal indicator performance in bird-influenced saline inland waters. Applied and Environmental Microbiology 70:7396–7403.
Kjelleberg S, Steinberg P, Givskov M, Gram L, Manefield M, de Nys R. 1997. Do marine natural products interfere with prokaryotic AHL regulatory systems? Aquatic Microbial Ecology 13:85–93.
Klein DA, Casida Jr LE. 1967. Escherichia coli die-out from normal soil as related to nutrient availability and the indigenous microflora. Canadian Journal of Microbiology 13:1461–1470.
Kleynhans CJ. 1999. The development of a fish index to assess the biological integrity of South African rivers. Water SA-Pretoria- 25:265–278.

Knapp S, Schweiger O, Kraberg A, Asmus H, Asmus R, Brey T, Frickenhaus S, Gutt J, Kühn I, Liess M, Musche M, Pörtner H-O, Seppelt R, Klotz S, Krause G. 2017. Do drivers of biodiversity change differ in importance across marine and terrestrial systems — Or is it just different research communities' perspectives? Science of The Total Environment 574:191–203. DOI: http://dx.doi.org/10.1016/j.scitotenv.2016.09.002.

Kock RA, Begovoeva M, Ansumana R, Suluku R. 2019. Searching for the source of Ebola: the elusive factors driving its spillover into humans during the West African outbreak of 2013–2016. OIE Scientific and Technical Review 38:113–117.

Kok MTJ, Alkemade R, Bakkenes M, van Eerdt M, Janse J, Mandryk M, Kram T, Lazarova T, Meijer J, van Oorschot M. 2018. Pathways for agriculture and forestry to contribute to terrestrial biodiversity conservation: a global scenario-study. Biological Conservation 221:137–150.

Konakli K, Sudret B, Faber MH. 2016. Numerical investigations into the value of information in lifecycle analysis of structural systems. ASCE-ASME Journal of Risk and Uncertainty in Engineering Systems, Part A: Civil Engineering 2:B4015007.

Korajkic A, McMinn B, Herrmann MP, Sivaganesan M, Kelty CA, Clinton P, Nash MS, Shanks OC. 2020. Viral and bacterial fecal indicators in untreated wastewater across the contiguous United States exhibit geospatial trends. Applied and Environmental Microbiology 86:e02967-19.

Korajkic A, McMinn BR, Ashbolt NJ, Sivaganesan M, Harwood VJ, Shanks OC. 2019a. Extended persistence of general and cattle-associated fecal indicators in marine and freshwater environment. Science of the Total Environment 650:1292–1302.

Korajkic A, Wanjugi P, Brooks L, Cao Y, Harwood VJ. 2019. Persistence and decay of fecal microbiota in aquatic habitats. Microbiology and Molecular Biology Reviews 83:e00005-19.

Korenaga J, Planavsky NJ, Evans DAD. 2017. Global water cycle and the coevolution of the Earth's interior and surface environment. Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences 375:e20150393.

Koudelka GB, Arnold JW, Chakraborty D. 2018. Evolution of STEC virulence: Insights from the antipredator activities of Shiga toxin-producing E. coli. International Journal of Medical Microbiology 308:956–961.

Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bover-Cid S, Chemaly M, Davies R, De Cesare A, Herman L, Hilbert F. 2020. Pathogenicity assessment of Shiga toxin-producing Escherichia coli (STEC) and the public health risk posed by contamination of food with STEC. EFSA Journal 18:e05967.

Kraus JM. 2019. Contaminants in linked aquatic–terrestrial ecosystems: predicting effects of aquatic pollution on adult aquatic insects and terrestrial insectivores. Freshwater Science 38:919–927.

Kurenbach B, Hill AM, Godsoe W, van Hamelsveld S, Heinemann JA. 2018. Agrichemicals and antibiotics in combination increase antibiotic resistance evolution. PeerJ 6:e5801.

La Ragione RM, Best A, Woodward MJ, Wales AD. 2009. Escherichia coli O157: H7 colonization in small domestic ruminants. FEMS Microbiology Reviews 33:394–410.

Lainé N, Morand S. 2020. Linking humans, their animals, and the environment again: a decolonized and more-than-human approach to "One Health." Parasite 27:55.

Lake PS, Palmer MA, Biro P, Cole J, Covich AP, Dahm C, Gibert J, Goedkoop W, Martens K, Verhoeven JLB-S. 2000. Global change and the biodiversity of freshwater ecosystems: impacts on linkages between above-sediment and sediment biota. BioScience 50:1099–1107.

Lalzampuia H, Dutta TK, Warjri I, Chandra R. 2013. PCR-based detection of extended-spectrum β-lactamases (bla CTX-M-1 and bla TEM) in Escherichia coli, Salmonella

spp. and Klebsiella pneumoniae isolated from pigs in North Eastern India (Mizoram). Indian Journal of Microbiology 53:291–296.
Lambie SC, Kelly WJ, Leahy SC, Li D, Reilly K, McAllister TA, Valle ER, Attwood GT, Altermann E. 2015. The complete genome sequence of the rumen methanogen Methanosarcina barkeri CM1. Standards in Genomic Sciences 10:1–8.
Larned ST, Moores J, Gadd J, Baillie B, Schallenberg M. 2020. Evidence for the effects of land use on freshwater ecosystems in New Zealand. New Zealand Journal of Marine and Freshwater Research 54:551–591.
Lassaletta L, Billen G, Grizzetti B, Garnier J, Leach AM, Galloway JN. 2014. Food and feed trade as a driver in the global nitrogen cycle: 50-year trends. Biogeochemistry 118:225–241.
Laursen MF, Dalgaard MD, Bahl MI. 2017. Genomic GC-content affects the accuracy of 16S rRNA gene sequencing based microbial profiling due to PCR bias. Frontiers in Microbiology 8:1934–1937.
LAWA. 2021.Factsheet: faecal indicators. Available at https://www.lawa.org.nz/learn/factsheets/faecal-indicators/ (accessed August 7, 2022).
Lean IJ, Westwood CT, Playford MC. 2008. Livestock disease threats associated with intensification of pastoral dairy farming. New Zealand Veterinary Journal 56:261–269.
Lederberg J, McCray AT. 2001. Ome SweetOmics-A genealogical treasury of words. The Scientist 15:8.
Lee SS, Paspalof AM, Snow DD, Richmond EK, Rosi-Marshall EJ, Kelly JJ. 2016. Occurrence and Potential Biological Effects of Amphetamine on Stream Communities. Environmental Science & Technology 50:9727–9735. DOI: 10.1021/acs.est.6b03717.
Lei M, Li Y, Zhang W, Niu L, Wang L, Zhang H. 2020. Identifying ecological processes driving vertical and horizontal archaeal community assemblages in a contaminated urban river. Chemosphere 245:125615.
Leightner JE, Inoue T. 2007. Tackling the omitted variables problem without the strong assumptions of proxies. European Journal of Operational Research 178:819–840.
Lejzerowicz F, Esling P, Majewski W, Szczuciński W, Decelle J, Obadia C, Arbizu PM, Pawlowski J. 2013. Ancient DNA complements microfossil record in deep-sea subsurface sediments. Biology Letters 9:20130283.
Lekshmi M, Oishi Das SK, Nayak BB. 2018. Occurrence of human enterovirus in tropical fish and shellfish and their relationship with fecal indicator bacteria. Veterinary World 11:1285.
Lévesque B, Brousseau P, Bernier F, Dewailly É, Joly J. 2000. Study of the bacterial content of ring-billed gull droppings in relation to recreational water quality. Water Research 34:1089–1096.
Lévesque S, Dufresne PJ, Soualhine H, Domingo M-C, Bekal S, Lefebvre B, Tremblay C. 2015. A side by side comparison of Bruker Biotyper and VITEK MS: utility of MALDI-TOF MS technology for microorganism identification in a public health reference laboratory. PloS one 10:e0144878.
Levin SA. 1989. Challenges in the development of a theory of community and ecosystem structure and function. In: Roughgarden J, May RM, Levin SA eds. Perspectives in ecological theory. Princeton, New Jersey: Princeton University Press, 242-255.
Levin SA. 2009. The Princeton guide to ecology. Princeton, N.J., USA: Princeton University Press. DOI: 10.1515/9781400833023.
Lewis JS, Herrera M, Wickes B, Patterson JE, Jorgensen JH. 2007. First report of the emergence of CTX-M-type extended-spectrum β-lactamases (ESBLs) as the predominant ESBL isolated in a US health care system. Antimicrobial Agents and Chemotherapy 51:4015–4021.

Li Y, Fan P, Zhou S, Zhang L. 2017. Loop-mediated isothermal amplification (LAMP): a novel rapid detection platform for pathogens. Microbial Pathogenesis 107:54–61.
Li Z, Ye X, Chen P, Ji K, Zhou J, Wang F, Dong W, Huang Y, Zhang Z, Cui Z. 2017. Antifungal potential of Corallococcus sp. strain EGB against plant pathogenic fungi. Biological Control 110:10–17.
Liang K, Sakakibara Y. 2021. MetaVelvet-DL: a MetaVelvet deep learning extension for de novo metagenome assembly. BMC Bioinformatics 22:1–21.
Lindemann-Matthies P, Bose E. 2008. How many species are there? Public understanding and awareness of biodiversity in Switzerland. Human Ecology 36:731–742.
Lipp EK, Kurz R, Vincent R, Rodriguez-Palacios C, Farrah SR, Rose JB. 2001. The effects of seasonal variability and weather on microbial fecal pollution and enteric pathogens in a subtropical estuary. Estuaries 24:266-276.
Liu H, Fu Y, Jiang D, Li G, Xie J, Cheng J, Peng Y, Ghabrial SA, Yi X. 2010. Widespread horizontal gene transfer from double-stranded RNA viruses to eukaryotic nuclear genomes. Journal of Virology 84:11876–11887.
Liu J, Zhou R, Li L, Peters BM, Li B, Lin C, Chuang T-L, Chen D, Zhao X, Xiong Z. 2017. Viable but non-culturable state and toxin gene expression of enterohemorrhagic Escherichia coli O157 under cryopreservation. Research in Microbiology 168:188–193.
Liu S, Shen L, Lou L, Tian G, Zheng P, Hu B. 2013. Spatial distribution and factors shaping the niche segregation of ammonia-oxidizing microorganisms in the Qiantang River, China. Applied and Environmental Microbiology 79:4065–4071.
Liu, H, Yanping F, Daohong J, Guoqing L, Jiatao X, Jiasen C, Youliang P, Said AG, and Xianhong Y. 2010. Widespread Horizontal Gene Transfer from Double-Stranded RNA Viruses to Eukaryotic Nuclear Genomes. Journal of Virology 84: 11876–87.
Llames ME, Quiroga MV, Schiaffino MR. 2022. Research in ecosystem services provided by bacteria, archaea, and viruses from inland waters: synthesis of main topics and trends over the last ca. 40 years. Hydrobiologia:1–20.
Loeza-Quintana T, Abbott CL, Heath DD, Bernatchez L, Hanner RH. 2020. Pathway to Increase Standards and Competency of eDNA Surveys (PISCeS)—Advancing collaboration and standardization efforts in the field of eDNA. Environmental DNA 2:255–260.
Logue JB, Findlay SEG, Comte J. 2015. Microbial responses to environmental changes. Frontiers in Microbiology 6:1364.
Long X, Xue H, Wong JT-F. 2020. Descent of Bacteria and Eukarya from an archaeal root of life. Evolutionary Bioinformatics 16:1176934320908267.
Lor Y, Schreier TM, Waller DL, Merkes CM. 2020. Using environmental DNA (eDNA) to detect the endangered Spectaclecase Mussel (Margaritifera monodonta). Freshwater Science 39:837–847.
Louca S, Parfrey LW, Doebeli M. 2016. Decoupling function and taxonomy in the global ocean microbiome. Science 353:1272–1277.
Lu M, Wang X, Ye H, Wang H, Qiu S, Zhang H, Liu Y, Luo J, Feng J. 2021. Does public fear that bats spread COVID-19 jeopardize bat conservation? Biological Conservation 254:108952.
Lucy FE, Graczyk TK, Tamang L, Miraflor A, Minchin D. 2008. Biomonitoring of surface and coastal water for Cryptosporidium, Giardia, and human-virulent microsporidia using molluscan shellfish. Parasitology Research 103:1369. DOI: 10.1007/s00436-008-1143-9.
Lukjancenko O, Wassenaar TM, Ussery DW. 2010. Comparison of 61 sequenced Escherichia coli genomes. Microbial Ecology 60:708–720.
Lupi F, Basso B, Garnache C, Herriges JA, Hyndman DW, Stevenson RJ. 2020. Linking

agricultural nutrient pollution to the value of freshwater ecosystem services. Land Economics 96:493–509.
Lürling M, Mackay E, Reitzel K, Spears BM. 2016. Editorial – A critical perspective on geo-engineering for eutrophication management in lakes. Water Research 97:1–10. DOI: http://dx.doi.org/10.1016/j.watres.2016.03.035.
Lyautey E, Teissier S, Charcosset J-Y, Rols J-L, Garabétian F. 2003. Bacterial diversity of epilithic biofilm assemblages of an anthropised river section, assessed by DGGE analysis of a 16S rDNA fragment. Aquatic Microbial Ecology 33:217–224.
Ma T, Cheng C, Xing L, Sun Y, Wu G. 2023. Quorum sensing responses of r-/K-strategists Nitrospira in continuous flow and sequencing batch nitrifying biofilm reactors. Science of The Total Environment 857:159328.
Ma Y, Liu F, Kong Z, Yin J, Kou W, Wu L, Ge G. 2016. The distribution pattern of sediment archaea community of the Poyang Lake, the largest freshwater lake in China. Archaea 2016:9278929. DOI: 10.1155/2016/9278929.
MacDonald GK, Brauman KA, Sun S, Carlson KM, Cassidy ES, Gerber JS, West PC. 2015. Rethinking agricultural trade relationships in an era of globalization. BioScience 65:275–289.
Mackay IM. 2004. Real-time PCR in the microbiology laboratory. Clinical Microbiology and Infection 10:190–212.
Mackenbach JP, Stirbu I, Roskam A-JR, Schaap MM, Menvielle G, Leinsalu M, Kunst AE. 2008a. Socioeconomic inequalities in health in 22 European countries. New England Journal of Medicine 358:2468–2481.
MacLeod F, Kindler GS, Wong HL, Chen R, Burns BP. 2019. Asgard archaea: diversity, function, and evolutionary implications in a range of microbiomes. AIMS Microbiology 5:48.
Maddock I. 1999. The importance of physical habitat assessment for evaluating river health. Freshwater Biology 41:373–391.
Madigan MT, Martinko JM, Parker J. 1997. Brock biology of microorganisms. Upper Saddle River, NJ: Prentice Hall.
Madsen EL. 2011. Microorganisms and their roles in fundamental biogeochemical cycles. Current Opinion in Biotechnology 22:456–464.
Maechler E, Deiner K, Steinmann P, Altermatt F. 2014. Utility of environmental DNA for monitoring rare and indicator macroinvertebrate species. Freshwater Science 33:1174–1183. DOI: 10.1086/678128.
Malham SK, Rajko-Nenow P, Howlett E, Tuson KE, Perkins TL, Pallett DW, Wang H, Jago CF, Jones DL, McDonald JE. 2014. The interaction of human microbial pathogens, particulate material and nutrients in estuarine environments and their impacts on recreational and shellfish waters. Environmental Science: Processes & Impacts 16:2145–2155.
Mallin MA, McIver MR, Robuck AR, Dickens AK. 2015. Industrial swine and poultry production causes chronic nutrient and fecal microbial stream pollution. Water, Air, & Soil Pollution 226:1–13.
Malmivaara-Lämsä M, Hamberg L, Haapamäki E, Liski J, Kotze DJ, Lehvävirta S, Fritze H. 2008. Edge effects and trampling in boreal urban forest fragments–impacts on the soil microbial community. Soil Biology and Biochemistry 40:1612–1621.
Mandl JN, Ahmed R, Barreiro LB, Daszak P, Epstein JH, Virgin HW, Feinberg MB. 2015. Reservoir host immune responses to emerging zoonotic viruses. Cell 160:20–35.
Manefield M, Rasmussen TB, Henzter M, Andersen JB, Steinberg P, Kjelleberg S, Givskov M. 2002. Halogenated furanones inhibit quorum sensing through accelerated LuxR turnover. Microbiology 148:1119–1127.

Manges AR, Geum HM, Guo A, Edens TJ, Fibke CD, Pitout JDD. 2019. Global extraintestinal pathogenic Escherichia coli (ExPEC) lineages. Clinical Microbiology Reviews 32:e00135-18.
Mankin KR, Wang L, Hutchinson SL, Marchin GL. 2007. Escherichia coli sorption to sand and silt loam soil. Transactions of the ASABE 50:1159–1165.
Mantyka-Pringle CS, Jardine TD, Bradford L, Bharadwaj L, Kythreotis AP, Fresque-Baxter J, Kelly E, Somers G, Doig LE, Jones PD. 2017. Bridging science and traditional knowledge to assess cumulative impacts of stressors on ecosystem health. Environment International 102:125–137.
March JG. 2005. Parochialism in the evolution of a research community: The case of organization studies. Management and Organization Review 1:5–22.
Marino RP, Gannon JJ. 1991. Survival of fecal coliforms and fecal streptococci in storm drain sediment. Water Research 25:1089–1098.
Markland SM, LeStrange KJ, Sharma M, Kniel KE. 2015. Old friends in new places: exploring the role of extraintestinal E. coli in intestinal disease and foodborne illness. Zoonoses and Public Health 62:491–496.
Martin EA. 2015. Concise medical dictionary. Oxford, UK: Oxford University Press, USA.
Mauro SA, Opalko H, Lindsay K, Colon MP, Koudelka GB. 2013. The microcosm mediates the persistence of shiga toxin-producing Escherichia coli in freshwater ecosystems. Applied and Environmental Microbiology 79:4821–4828.
Mauvisseau Q, Kalogianni E, Zimmerman B, Bulling M, Brys R, Sweet M. 2020. eDNA-based monitoring: Advancement in management and conservation of critically endangered killifish species. Environmental DNA 2:601–613.
Maxted JR, Evans BF, Scarsbrook MR. 2003. Development of standard protocols for macroinvertebrate assessment of soft-bottomed streams in New Zealand. New Zealand Journal of Marine and Freshwater Research 37:793–807.
Mayer W V. 1968. Biology-Synthesizer of Science or Disintegrating Discipline? The American Biology Teacher 30:799–805.
Mazerolle MJ, Mazerolle MMJ. 2017. Package 'AICcmodavg.' R package.
McBride G, Till D, Ryan T, Ball A, Lewis G, Palmer S, Weinstein P. 2002. Freshwater Microbiology Research Programme Report: Pathogen Occurrence and Human Health Risk Assessment Analysis. Wellington, New Zealand: Ministry of Health.
McCulloch SP, Reiss MJ. 2017a. Bovine tuberculosis and badger culling in England: An animal rights-based analysis of policy options. Journal of Agricultural and Environmental Ethics 30:535–550.
McDonald A, Kay D, Jenkins A. 1982. Generation of fecal and total coliform surges by stream flow manipulation in the absence of normal hydrometeorological stimuli. Applied and Environmental Microbiology 44:292–300.
McDowell RW, Simpson ZP, Ausseil AG, Etheridge Z, Law R. 2021. The implications of lag times between nitrate leaching losses and riverine loads for water quality policy. Scientific Reports 11:16450.
McLeod M, Aislabie J, Ryburn J, McGill A, Taylor M. 2003. Microbial and chemical tracer movement through two Southland soils, New Zealand. Soil Research 41:1163–1169.
McMahon BJ, Morand S, Gray JS. 2018. Ecosystem change and zoonoses in the Anthropocene. Zoonoses and Public Health 65:755–765.
Melzer M, Petersen I. 2007. Mortality following bacteraemic infection caused by extended spectrum beta-lactamase (ESBL) producing E. coli compared to non-ESBL producing E. coli. Journal of Infection 55:254–259.
Mendoza H, Rubio A V, García-Peña GE, Suzán G, Simonetti JA. 2020. Does land-use change increase the abundance of zoonotic reservoirs? Rodents say yes. European

Journal of Wildlife Research 66:1–5.
Menon P, Billen G, Servais P. 2003. Mortality rates of autochthonous and fecal bacteria in natural aquatic ecosystems. Water Research 37:4151–4158.
Menzel P, Ng KL, Krogh A. 2016. Fast and sensitive taxonomic classification for metagenomics with Kaiju.
Menzi H, Oenema O, Burton C, Shipin O, Gerber P, Robinson T, Franceschini G. 2010. Impacts of intensive livestock production and manure management on the environment. Livestock in a Changing Landscape 1:139–163.
Merget B, Forbes KJ, Brennan F, McAteer S, Shepherd T, Strachan NJC, Holden NJ. 2019. Influence of plant species, tissue type, and temperature on the capacity of Shiga-toxigenic Escherichia coli to colonize, grow, and be internalized by plants. Applied and Environmental Microbiology 85:e00123-19.
Meter KJ Van, Basu NB, Veenstra JJ, Burras CL. 2016. The nitrogen legacy: emerging evidence of nitrogen accumulation in anthropogenic landscapes. Environmental Research Letters 11:35014.
Miller-Rushing AJ, Athearn N, Blackford T, Brigham C, Cohen L, Cole-Will R, Edgar T, Ellwood ER, Fisichelli N, Pritz CF. 2021. COVID-19 pandemic impacts on conservation research, management, and public engagement in US national parks. Biological Conservation 257:109038.
Ministry for the Environment. 2003. Microbiological Water Quality Guidelines for Marine and Freshwater Recreational Areas. Wellington, New Zealand.
Ministry for the Environment. 2020. National policy statement for freshwater management 2020. Wellington, New Zealand: Ministry for the Environment.
Ministry of Education. 2021. Education report: climate and the curriculum. Wellington, New Zealand.
Ministry of Health. 2017. Guidelines for Drinking-water Quality Management for New Zealand 2017. Wellington, New Zealand.
Ministry of Health. 2018. Drinking-water standards for New Zealand 2005 (revised 2018). Wellington, New Zealand.
Mirsal IA. 2008. Sources of soil pollution. In: Soil pollution. Berlin, Germany: Springer, 137–173.
Miseta A, Csutora P. 2000. Relationship between the occurrence of cysteine in proteins and the complexity of organisms. Molecular Biology and Evolution 17:1232–1239.
Mishra S, Mishra DR. 2012. Normalized difference chlorophyll index: A novel model for remote estimation of chlorophyll-a concentration in turbid productive waters. Remote Sensing of Environment 117:394–406.
Moghadam S V, Vadde KK, Phan DC, Jafarzadeh A, Kapoor V. 2022. Assessing the impact of flooding on bacterial community structure and occurrence of potentially pathogenic bacteria in Texas Rivers after Hurricane Harvey. Journal of Hazardous Materials Letters 3:100058.
Moi IM, Roslan NN, Leow ATC, Ali MSM, Rahman RNZRA, Rahimpour A, Sabri S. 2017. The biology and the importance of Photobacterium species. Applied Microbiology and Biotechnology 101:4371–4385.
Molina MC, Roa-Fuentes CA, Zeni JO, Casatti L. 2017. The effects of land use at different spatial scales on instream features in agricultural streams. Limnologica 65:14–21.
Monaghan R, Manderson A, Basher L, Spiekermann R, Dymond J, Smith C, Muirhead R, Burger D, McDowell R. 2021. Quantifying contaminant losses to water from pastoral landuses in New Zealand II. The effects of some farm mitigation actions over the past two decades. New Zealand Journal of Agricultural Research:1–25.
Monaghan RM, de Klein CAM, Muirhead RW. 2008. Prioritisation of farm scale remediation

efforts for reducing losses of nutrients and faecal indicator organisms to waterways: A case study of New Zealand dairy farming. Journal of Environmental Management 87:609–622. DOI: 10.1016/j.jenvman.2006.07.017.
Montenegro RA, Stephens C. 2006. Indigenous health in Latin America and the Caribbean. The Lancet 367:1859–1869.
Mooney W, Cullen A. 2019. Implementing the Aboriginal Waterways Assessment tool: collaborations to engage and empower First Nations in waterway management. Australasian Journal of Environmental Management 26:197–215.
Moorhouse TP, D'Cruze NC, Macdonald DW. 2021. Information about zoonotic disease risks reduces desire to own exotic pets among global consumers. Frontiers in Ecology and Evolution 9:37.
Mora A, López C, Dhabi G, López-Beceiro AM, Fidalgo LE, Díaz EA, Martínez-Carrasco C, Mamani R, Herrera A, Blanco JE. 2012. Seropathotypes, phylogroups, Stx subtypes, and intimin types of wildlife-carried, Shiga toxin-producing Escherichia coli strains with the same characteristics as human-pathogenic isolates. Applied and Environmental Microbiology 78:2578–2585.
Morand S. 2017. Infections and diseases in wildlife by non-native organisms. In: Vila M, Hulme P eds. Impact of Biological Invasions on Ecosystem Services. Cham, Switzerland: Springer, 177–190.
Moreira S, Brown A, Ha R, Iserhoff K, Yim M, Yang J, Liao B, Pszczolko E, Qin W, Leung KT. 2012. Persistence of Escherichia coli in freshwater periphyton: biofilm-forming capacity as a selective advantage. FEMS Microbiology Ecology 79:608–618.
Mori J, Smith R. 2019. Transmission of waterborne fish and plant pathogens in aquaponics and their control with physical disinfection and filtration: A systematized review. Aquaculture 504:380–395.
Morse JM, Niehaus L, Wolfe RR, Wilkins S. 2006. The role of the theoretical drive in maintaining validity in mixed-method research. Qualitative Research in Psychology 3:279–291.
Morse SS, Mazet JAK, Woolhouse M, Parrish CR, Carroll D, Karesh WB, Zambrana-Torrelio C, Lipkin WI, Daszak P. 2012. Prediction and prevention of the next pandemic zoonosis. The Lancet 380:1956–1965.
Moura A, Savageau MA, Alves R. 2013. Relative amino acid composition signatures of organisms and environments. PloS One 8:e77319.
Mtimet N, Wanyoike F, Rich KM, Baltenweck I. 2021. Zoonotic diseases and the COVID-19 pandemic: Economic impacts on Somaliland's livestock exports to Saudi Arabia. Global Food Security 28:100512.
Mubiru DN, Coyne MS, Grove JH. 2000. Mortality of Escherichia coli O157: H7 in two soils with different physical and chemical properties. Journal of Environmental Quality 29:1821–1825.
Muirhead RW, Davies-Colley RJ, Donnison AM, Nagels JW. 2004. Faecal bacteria yields in artificial flood events: quantifying in-stream stores. Water Research 38:1215–1224.
Muirhead RW. 2019. The effectiveness of streambank fencing to improve microbial water quality: a review. Agricultural Water Management 223:105684.
Murienne J, Cantera I, Cerdan A, Cilleros K, Decotte J-B, Dejean T, Vigouroux R, Brosse S. 2019. Aquatic eDNA for monitoring French Guiana biodiversity. Biodiversity Data Journal 7:e37518.
Murillo J, Villegas LM, Ulloa-Murillo LM, Rodríguez AR. 2020. Recent trends on omics and bioinformatics approaches to study SARS-CoV-2: A bibliometric analysis and mini-review. Computers in Biology and Medicine:104162.
Najafi A, Hasanpour M, Askary A, Aziemzadeh M, Hashemi N. 2018. Distribution of

pathogenicity island markers and virulence factors in new phylogenetic groups of uropathogenic Escherichia coli isolates. Folia Microbiologica 63:335–343.
Nakamura K, Murase K, Sato MP, Toyoda A, Itoh T, Mainil JG, Piérard D, Yoshino S, Kimata K, Isobe J. 2020. Differential dynamics and impacts of prophages and plasmids on the pangenome and virulence factor repertoires of Shiga toxin-producing Escherichia coli O145: H28. Microbial Genomics 6:e000323.
NASA. 2020.NASA science, solar system exploration: Earth. Available at https://solarsystem.nasa.gov/planets/earth/by-the-numbers/ (accessed April 20, 2021).NEMS Steering Group. 2019. National environmental monitoring standards. Part 2 of 4: sampling, measuring, processing and archiving of discrete river water quality data. New Zealand.
NEMS Steering Group. 2019. National environmental monitoring standards. Part 2 of 4: sampling, measuring, processing and archiving of discrete river water quality data. New Zealand.
New Zealand Government. 2017. Government inquiry into Havelock North drinking water. Auckland, New Zealand.
Nielsen EM, Skov MN, Madsen JJ, Lodal J, Jespersen JB, Baggesen DL. 2004. Verocytotoxin-producing Escherichia coli in wild birds and rodents in close proximity to farms. Applied and Environmental Microbiology 70:6944–6947.
Nielsen KM, Johnsen PJ, Bensasson D, Daffonchio D. 2007. Release and persistence of extracellular DNA in the environment. Environmental Biosafety Research 6:37–53.
Nikolay B, Salje H, Khan AKMD, Sazzad HMS, Satter SM, Rahman M, Doan S, Knust B, Flora MS, Luby SP. 2020. A Framework to Monitor Changes in Transmission and Epidemiology of Emerging Pathogens: Lessons From Nipah Virus. The Journal of Infectious Diseases 221:S363–S369.
Nishimura T, Murray JS, Boundy MJ, Balci M, Bowers HA, Smith KF, Harwood DT, Rhodes LL. 2021. Update of the Planktonic Diatom Genus Pseudo-nitzschia in Aotearoa New Zealand Coastal Waters: Genetic Diversity and Toxin Production. Toxins 13:637.
Niu L, Li Y, Wang P, Zhang W, Wang C, Li J, Wu H. 2018. Development of a microbial community-based index of biotic integrity (MC-IBI) for the assessment of ecological status of rivers in the Taihu Basin, China. Ecological Indicators 85:204–213.
Nugent G. 2011. Maintenance, spillover and spillback transmission of bovine tuberculosis in multi-host wildlife complexes: a New Zealand case study. Veterinary Microbiology 151:34–42.
Nuy JK, Lange A, Beermann AJ, Jensen M, Elbrecht V, Röhl O, Peršoh D, Begerow D, Leese F, Boenigk J. 2018. Responses of stream microbes to multiple anthropogenic stressors in a mesocosm study. Science of The Total Environment 633:1287–1301.
O'Brien A, Townsend K, Hale R, Sharley D, Pettigrove V, Huber C, Finelli L, Stevens W, Manenti R, Mori E, Di Canio V, Mercurio S, Picone M, Caffi M, Brambilla M, Ficetola GF, Rubolini D, Harrison S, Baker MG, Benschop J, Death RG, French NP, Harmsworth G, Lake RJ, Lamont IL, Priest PC, Ussher JE, Ceylan RF, Ozkan B, Mulazimogullari E, Peck JA, Frutos R, Devaux CA, Inoue H, Todo Y, Lu M, Wang X, Ye H, Wang H, Qiu S, Zhang H, Liu Y, Luo J, Feng J, Kaiser J, Egbetade AO, Sonibare AO, Meseko CA, Jayeola OA, Otesile EB. 2020a. The economic and social burden of the 2014 Ebola outbreak in West Africa. Biological Conservation 254:108728. DOI: http://dx.doi.org/10.1016/j.ecolind.2016.05.004.
O'Mullan GD, Dueker ME, Juhl AR. 2017. Challenges to managing microbial fecal pollution in coastal environments: extra-enteric ecology and microbial exchange among water, sediment, and air. Current Pollution Reports 3:1–16.
Oakes RS, Siegler RL, McReynolds MA, Pysher T, Pavia AT. 2006. Predictors of fatality in

postdiarrheal hemolytic uremic syndrome. Pediatrics 117:1656–1662.
Odonkor ST, Ampofo JK. 2013. Escherichia coli as an indicator of bacteriological quality of water: an overview. Microbiology Research 4:2.
Oita A, Malik A, Kanemoto K, Geschke A, Nishijima S, Lenzen M. 2016. Substantial nitrogen pollution embedded in international trade. Nature Geoscience 9:111–115.
Olds BP, Jerde CL, Renshaw MA, Li Y, Evans NT, Turner CR, Deiner K, Mahon AR, Brueseke MA, Shirey PD, Pfrender ME, Lodge DM, Lamberti GA. 2016. Estimating species richness using environmental DNA. Ecology and Evolution 6:4214-4226. DOI: 10.1002/ece3.2186.
Oliveira, Luiz Felipe Valter de, and Rogerio Margis. 2015. 'The Source of the River as a Nursery for Microbial Diversity'. PLoS One 10: e0120608.
Oliver DM, Clegg CD, Haygarth PM, Heathwaite AL. 2005. Assessing the potential for pathogen transfer from grassland soils to surface waters. Advances in Agronomy 85:125–180.
Olsen GJ, Lane DJ, Giovannoni SJ, Pace NR, Stahl DA. 1986. Microbial ecology and evolution: a ribosomal RNA approach. Annual Reviews in Microbiology 40:337–365.
Olson SH, Benedum CM, Mekaru SR, Preston ND, Mazet JAK, Joly DO, Brownstein JS. 2015. Drivers of emerging infectious disease events as a framework for digital detection. Emerging Infectious Diseases 21:1285.
One Health High-Level Expert Panel (OHHLEP). 2022. One Health: A new definition for a sustainable and healthy future. Plos Pathogens 18:e1010537.
Oniciuc EA, Likotrafiti E, Alvarez-Molina A, Prieto M, Santos JA, Alvarez-Ordóñez A. 2018. The present and future of whole genome sequencing (WGS) and whole metagenome sequencing (WMS) for surveillance of antimicrobial resistant microorganisms and antimicrobial resistance genes across the food chain. Genes 9:268.
Onoma AK. 2020. Epidemics, xenophobia and narratives of propitiousness. Medical Anthropology 39:382–397.
Oporto B, Ocejo M, Alkorta M, Marimón JM, Montes M, Hurtado A. 2019. Zoonotic approach to Shiga toxin-producing Escherichia coli: integrated analysis of virulence and antimicrobial resistance in ruminants and humans. Epidemiology & Infection 148:e31.
Oren A, Garrity GM. 2021. Valid publication of the names of forty-two phyla of prokaryotes. International Journal of Systematic and Evolutionary Microbiology 71:5056.
Orlova S, Rassõlkin A, Kallaste A, Vaimann T, Belahcen A. 2016. Lifecycle analysis of different motors from the standpoint of environmental impact. Latvian Journal of Physics and Technical Sciences 53:37–46.
Ormeño-Orrillo E, Martínez-Romero E. 2019. A genomotaxonomy view of the Bradyrhizobium genus. Frontiers in Microbiology 10:1334.
Pace NR. 1985. Analyzing natural microbial populations by rRNA sequences. ASM News 51:4–12.
Pachepsky YA, Shelton DR. 2011. Escherichia coli and fecal coliforms in freshwater and estuarine sediments. Critical Reviews in Environmental Science and Technology 41:1067–1110.
Paerl HW, Dyble J, Moisander PH, Noble RT, Piehler MF, Pinckney JL, Steppe TF, Twomey L, Valdes LM. 2003. Microbial indicators of aquatic ecosystem change: current applications to eutrophication studies. FEMS Microbiology Ecology 46:233–246.
Palmateer GA, McLean DE, Kutas WL, Meissner SM. 1993. Suspended paniculate/bacterial interaction in agricultural drains. Michigan: Lewis Publishers.
Pang H, Mokhtari A, Chen Y, Oryang D, Ingram DT, Sharma M, Millner PD, Van Doren JM. 2020. A Predictive Model for Survival of Escherichia coli O157: H7 and Generic E. coli in Soil Amended with Untreated Animal Manure. Risk Analysis 40:1367–1382.

Pansu J, Chapman MB, Hose GC, Chariton AA. 2021. Comparison of an extracellular v. total DNA extraction approach for environmental DNA-based monitoring of sediment biota. Marine and Freshwater Research 74:449-462.
Parliamentary Commissioner for the Environment. 2013. 'Water Quality in New Zealand: Land Use and Nutrient Pollution'. Wellington: Office of the Parliamentary Commissioner for the Environment.
Parsons M, Fisher K, Crease RP. 2021. Decolonising Blue Spaces in the Anthropocene: Freshwater Management in Aotearoa New Zealand. London, UK: Palgrave Macmillan.
Patt D, Gordan L, Diaz M, Okon T, Grady L, Harmison M, Markward N, Sullivan M, Peng J, Zhou A. 2020. Impact of COVID-19 on cancer care: how the pandemic is delaying cancer diagnosis and treatment for American seniors. JCO Clinical Cancer Informatics 4:1059–1071.
Paul JH. 1999. Microbial gene transfer: an ecological perspective. Journal of Molecular Microbiology and Biotechnology 1:45–50.
Pearez JL, Berrocal CI, Berrocal L. 1986. Evaluation of a commercial β-glucuronidase test for the rapid and economical identification of Escherichia coli. Journal of Applied Bacteriology 61:541–545.
Pecl GT, Araújo MB, Bell JD, Blanchard J, Bonebrake TC, Chen I-C, Clark TD, Colwell RK, Danielsen F, Evengård B, Falconi L, Ferrier S, Frusher S, Garcia RA, Griffis RB, Hobday AJ, Janion-Scheepers C, Jarzyna MA, Jennings S, Lenoir J, Linnetved HI, Martin VY, McCormack PC, McDonald J, Mitchell NJ, Mustonen T, Pandolfi JM, Pettorelli N, Popova E, Robinson SA, Scheffers BR, Shaw JD, Sorte CJB, Strugnell JM, Sunday JM, Tuanmu M-N, Vergés A, Villanueva C, Wernberg T, Wapstra E, Williams SE. 2017. Biodiversity redistribution under climate change: Impacts on ecosystems and human well-being. Science 355:1–9. DOI: 10.1126/science.aai9214.
Peel AJ, Wells K, Giles J, Boyd V, Burroughs A, Edson D, Crameri G, Baker ML, Field H, Wang L-F. 2019. Synchronous shedding of multiple bat paramyxoviruses coincides with peak periods of Hendra virus spillover. Emerging Microbes & Infections 8:1314–1323.
Pendleton JN, Gorman SP, Gilmore BF. 2013. Clinical relevance of the ESKAPE pathogens. Expert Review of Anti-infective Therapy 11:297–308.
Pereira HM, Leadley PW, Proença V, Alkemade R, Scharlemann JPW, Fernandez-Manjarrés JF, Araújo MB, Balvanera P, Biggs R, Cheung WWL. 2010. Scenarios for global biodiversity in the 21st century. Science 330:1496–1501.
Perkins TL, Clements K, Baas JH, Jago CF, Jones DL, Malham SK, McDonald JE. 2014. Sediment composition influences spatial variation in the abundance of human pathogen indicator bacteria within an estuarine environment. PloS One 9:e112951.
Perry M. 2012. How the signs and symptoms of common infections vary with age. Practice Nursing 23:176–182.
Perry RD, Fetherston JD. 1997. Yersinia pestis-etiologic agent of plague. Clinical Microbiology Reviews 10:35–66.
Petersen F, Hubbart JA. 2020. Physical factors impacting the survival and occurrence of Escherichia coli in secondary habitats. Water 12:1796.
Phiri BJ, Pita AB, Hayman DTS, Biggs PJ, Davis MT, Fayaz A, Canning AD, French NP, Death RG. 2020. Does land use affect pathogen presence in New Zealand drinking water supplies? Water Research 185:116229.
Picardeau M. 2013. Diagnosis and epidemiology of leptospirosis. Médecine et Maladies Infectieuses 43:1–9.
Pilliod DS, Laramie MB, MacCoy D, Maclean S. 2019. Integration of eDNA-based biological monitoring within the US Geological Survey's National Streamgage Network. JAWRA Journal of the American Water Resources Association 55:1505–1518.

Pimentel D, Berger B, Filiberto D, Newton M, Wolfe B, Karabinakis E, Clark S, Poon E, Abbett E, Nandagopal S. 2004. Water Resources: Agricultural and Environmental Issues. BioScience 54:909–918. DOI: 10.1641/0006-3568(2004)054[0909:wraaei]2.0.co;2.
Pimentel D, Cooperstein S, Randell H, Filiberto D, Sorrentino S, Kaye B, Nicklin C, Yagi J, Brian J, O'hern J. 2007. Ecology of increasing diseases: population growth and environmental degradation. Human Ecology 35:653–668.
Plough L V, Bunch AJ, Lee BB, Fitzgerald CL, Stence CP, Richardson B. 2021. Development and testing of an environmental DNA (eDNA) assay for endangered Atlantic sturgeon to assess its potential as a monitoring and management tool. Environmental DNA 3:800–814.
Plowright RK, Peel AJ, Streicker DG, Gilbert AT, McCallum H, Wood J, Baker ML, Restif O. 2016. Transmission or within-host dynamics driving pulses of zoonotic viruses in reservoir–host populations. PLoS Neglected Tropical Diseases 10:e0004796.
Plowright RK, Reaser JK, Locke H, Woodley SJ, Patz JA, Becker DJ, Oppler G, Hudson PJ, Tabor GM. 2021. Land use-induced spillover: a call to action to safeguard environmental, animal, and human health. The Lancet Planetary Health 5:e237–e245.
Poikane S, Kelly MG, Herrero FS, Pitt J-A, Jarvie HP, Claussen U, Leujak W, Solheim AL, Teixeira H, Phillips G. 2019. Nutrient criteria for surface waters under the European Water Framework Directive: Current state-of-the-art, challenges and future outlook. Science of the Total Environment 695:133888.
Pollans MJ. 2016. Drinking water protection and agricultural exceptionalism. Pace Law Faculty Publications 77:1197–1259.
Polvi LE, Lind L, Persson H, Miranda-Melo A, Pilotto F, Su X, Nilsson C. 2020. Facets and scales in river restoration: Nestedness and interdependence of hydrological, geomorphic, ecological, and biogeochemical processes. Journal of Environmental Management 265:110288.
Ponce-Soto GY, Aguirre-von-Wobeser E, Eguiarte LE, Elser JJ, Lee ZM-P, Souza V. 2015. Enrichment experiment changes microbial interactions in an ultra-oligotrophic environment. Frontiers in Microbiology 6:246.
Pont D, Hugueny B, Rogers C. 2007. Development of a fish-based index for the assessment of river health in Europe: the European Fish Index. Fisheries Management and Ecology 14:427–439.
Popoff MY, Le Minor LE. 2015. Salmonella. In: Whitman WB ed. Bergey's manual of systematics of archaea and bacteria. Hoboken, New Jersey, USA: Wiley.
Poretsky R, Rodriguez-R LM, Luo C, Tsementzi D, Konstantinidis KT. 2014. Strengths and limitations of 16S rRNA gene amplicon sequencing in revealing temporal microbial community dynamics. PloS One 9:e93827.
Porta M. 2014. A dictionary of epidemiology. Oxford, UK: Oxford university press. DOI: 10.1093/acref/9780199976720.001.0001.
Porter KDH, Reaney SM, Quilliam RS, Burgess C, Oliver DM. 2017. Predicting diffuse microbial pollution risk across catchments: The performance of SCIMAP and recommendations for future development. Science of The Total Environment 609:456–465. DOI: https://doi.org/10.1016/j.scitotenv.2017.07.186.
Postel S, Carpenter S. 1997. Freshwater ecosystem services. Washington, D.C., USA: Island Press.
Potter P, Ramankutty N, Bennett EM, Donner SD. 2010. Characterizing the spatial patterns of global fertilizer application and manure production. Earth Interactions 14:1–22.
Prescott SL. 2017. History of medicine: Origin of the term microbiome and why it matters. Human Microbiome Journal 4:24–25.

Preziusoa S, Pinhob MD, Attilia AR, Melo-Cristinob J, Ackec E, Midwinterc AC, Cuteria V, Ramirezb M. 2014. PCR based differentiation between Streptococcus dysgalactiae subsp. equisimilis strains isolated from humans and horses. Comparative Immunology, Microbiology and Infectious Diseases 37:169–172.
Probert WS, Miller GM, Ledin KE. 2017. Contaminated stream water as source for Escherichia coli O157 illness in children. Emerging Infectious Diseases 23:1216.
Procópio RE de L, Silva IR da, Martins MK, Azevedo JL de, Araújo JM de. 2012. Antibiotics produced by Streptomyces. Brazilian Journal of Infectious Diseases 16:466–471.
Prudêncio M, Costa JC. 2020. Research funding after COVID-19. Nature Microbiology 5:986.
Prüss A. 1998. Review of epidemiological studies on health effects from exposure to recreational water. International Journal of Epidemiology 27:1–9.
Public Health Surveillance: Environment Group ESR. 2019.Surveillance report: Notifiable diseases in New Zealand 2017. Available at https://surv.esr.cri.nz/surveillance/annual_surveillance.php?we_objectID=4930 (accessed 4 October 2019).
Pule M, Yahya A, Chuma J. 2017. Wireless sensor networks: A survey on monitoring water quality. Journal of Applied Research and Technology 15:562–570.
Purwanto A, Asbari M, Fahlevi M, Mufid A, Agistiawati E, Cahyono Y, Suryani P. 2020. Impact of work from home (WFH) on Indonesian teachers performance during the Covid-19 pandemic: An exploratory study. International Journal of Advanced Science and Technology 29:6235–6244.
Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, Nielsen T, Pons N, Levenez F, Yamada T. 2010. A human gut microbial gene catalogue established by metagenomic sequencing. Nature 464:59–65.
Rabinowitz P, Conti L. 2013. Links among human health, animal health, and ecosystem health. Annual Review of Public Health 34:189–204.
Rabinowitz PM, Kock R, Kachani M, Kunkel R, Thomas J, Gilbert J, Wallace R, Blackmore C, Wong D, Karesh W. 2013. Toward proof of concept of a one health approach to disease prediction and control. Emerging Infectious Diseases 19.
Raes J, Letunic I, Yamada T, Jensen LJ, Bork P. 2011. Toward molecular trait-based ecology through integration of biogeochemical, geographical and metagenomic data. Molecular Systems Biology 7:473.
Ragupathi NKD, Sethuvel DPM, Inbanathan FY, Veeraraghavan B. 2018. Accurate differentiation of Escherichia coli and Shigella serogroups: challenges and strategies. New Microbes and New Infections 21:58–62.
Rahman MM, Vu X-B. 2020. The nexus between renewable energy, economic growth, trade, urbanisation and environmental quality: a comparative study for Australia and Canada. Renewable Energy 155:617–627.
Rahme LG, Stevens EJ, Wolfort SF, Shao J, Tompkins RG, Ausubel FM. 1995. Common virulence factors for bacterial pathogenicity in plants and animals. Science 268:1899–1902.
Rajput A, Kumar M. 2017. Computational exploration of putative LuxR Solos in archaea and their functional implications in quorum sensing. Frontiers in Microbiology 8:798.
Ram S, Vajpayee P, Singh RL, Shanker R. 2009. Surface water of a perennial river exhibits multi-antimicrobial resistant shiga toxin and enterotoxin-producing Escherichia coli. Ecotoxicology and Environmental Safety 72:490–495.
Rangel JM, Sparling PH, Crowe C, Griffin PM, Swerdlow DL. 2005. Epidemiology of Escherichia coli O157: H7 outbreaks, United States, 1982–2002. Emerging Infectious Diseases 11:603.

Rapport D, Costanza R, Epstein PR, Gaudet C, Levins R. 1998. Ecosystem health. Malden, MA: Blackwell Science.
Ravva S V, Sarreal CZ, Mandrell RE. 2014. Strain differences in fitness of Escherichia coli O157: H7 to resist protozoan predation and survival in soil. PLoS One 9:e102412.
Read AF. 1994. The evolution of virulence. Trends in Microbiology 2:73–76.
Reaser JK, Witt A, Tabor GM, Hudson PJ, Plowright RK. 2021. Ecological countermeasures for preventing zoonotic disease outbreaks: when ecological restoration is a human health imperative. Restoration Ecology:e13357.
Reddy B, Dubey SK. 2021. Exploring the allochthonous pollution influence on bacterial community and co-occurrence dynamics of River Ganga water through 16S rRNA-tagged amplicon metagenome. Environmental Science and Pollution Research 28:26990–27005.
Reddy KR, Khaleel R, Overcash MR. 1981. Behavior and transport of microbial pathogens and indicator organisms in soils treated with organic wastes. Journal of Environmental Quality 10:255–266.
Reddy P. 2020. Resource management (National Environmental Standards for Freshwater) regulations 2020. Wellington, New Zealand.
Reed KD, Meece JK, Henkel JS, Shukla SK. 2003. Birds, migration and emerging zoonoses: West Nile virus, Lyme disease, influenza A and enteropathogens. Clinical Medicine & Research 1:5–12.
Reeve MA, Partridge M. 2017. The use of social media to combat research-isolation. Annals of the Entomological Society of America 110:449–456.
Reid AJ, Carlson AK, Creed IF, Eliason EJ, Gell PA, Johnson PTJ, Kidd KA, MacCormack TJ, Olden JD, Ormerod SJ. 2019. Emerging threats and persistent conservation challenges for freshwater biodiversity. Biological Reviews 94:849–873.
Reisner A, Krogfelt KA, Klein BM, Zechner EL, Molin S. 2006. In vitro biofilm formation of commensal and pathogenic Escherichia coli strains: impact of environmental and genetic factors. Journal of Bacteriology 188:3572–3581.
Richards J, Chambers T, Hales S, Joy M, Radu T, Woodward A, Humphrey A, Randal E, Baker MG. 2021. Nitrate contamination in drinking water and colorectal cancer: exposure assessment and estimated health burden in New Zealand. Environmental Research 204:112322.
Rodriguez DA, Tomasella J, Linhares C. 2010. Is the forest conversion to pasture affecting the hydrological response of Amazonian catchments? Signals in the Ji-Paraná Basin. Hydrological Processes: An International Journal 24:1254–1269.
Rodríguez RA, Gundy PM, Rijal GK, Gerba CP. 2012. The impact of combined sewage overflows on the viral contamination of receiving waters. Food and Environmental Virology 4:34–40.
Rodriguez-R LM, Tsementzi D, Luo C, Konstantinidis KT. 2020. Iterative subtractive binning of freshwater chronoseries metagenomes identifies over 400 novel species and their ecologic preferences. Environmental Microbiology 22:3394–3412.
Romanelli A, Soto DX, Matiatos I, Martínez DE, Esquius S. 2020. A biological and nitrate isotopic assessment framework to understand eutrophication in aquatic ecosystems. Science of the Total Environment 715:136909.
Roosendaal B, Gaastra W, de Graaf FK. 1984. The nucleotide sequence of the gene encoding the K99 subunit of enterotoxigenic Escherichia coli. FEMS Microbiology Letters 22:253–258.
Rose G. 2001. Sick individuals and sick populations. International Journal of Epidemiology 30:427–432.
Rose JB, Epstein PR, Lipp EK, Sherman BH, Bernard SM, Patz JA. 2001. Climate variability

and change in the United States: potential impacts on water-and foodborne diseases caused by microbiologic agents. Environmental Health Perspectives 109:211–221.
Rosselló-Mora R, Amann R. 2001. The species concept for prokaryotes. FEMS Microbiology Reviews 25:39–67.
Rousk J, Bengtson P. 2014. Microbial regulation of global biogeochemical cycles. Frontiers in Microbiology 5:103.
Rousk, Johannes, and Per Bengtson. 2014. 'Microbial Regulation of Global Biogeochemical Cycles'. Frontiers in Microbiology 5: 103.
Rousset F, Cabezas-Caballero J, Piastra-Facon F, Fernández-Rodríguez J, Clermont O, Denamur E, Rocha EPC, Bikard D. 2021. The impact of genetic diversity on gene essentiality within the Escherichia coli species. Nature Microbiology 6:301–312.
Rubin C, Myers T, Stokes W, Dunham B, Harris S, Lautner B, Annelli J. 2013. Review of institute of medicine and national research council recommendations for one health initiative. Emerging Infectious Diseases 19:1913.
Rubino F, Cohen R V, Mingrone G, le Roux CW, Mechanick JI, Arterburn DE, Vidal J, Alberti G, Amiel SA, Batterham RL. 2020a. Bariatric and metabolic surgery during and after the COVID-19 pandemic: DSS recommendations for management of surgical candidates and postoperative patients and prioritisation of access to surgery. The Lancet Diabetes & Endocrinology 8:640–648.
Rulli MC, Santini M, Hayman DTS, D'Odorico P. 2017. The nexus between forest fragmentation in Africa and Ebola virus disease outbreaks. Scientific Reports 7:41613.
Rupprecht C, Kuzmin I, Meslin F. 2017. Lyssaviruses and rabies: current conundrums, concerns, contradictions and controversies. F1000 Research 6:184.
Russell RC. 1998. Vectors vs. humans in Australia--who is on top down under? An update on vector-borne disease and research on vectors in Australia. Journal of Vector Ecology: Journal of the Society for Vector Ecology 23:1–46.
Sajjad W, Ali B, Bahadur A, Ghimire PS, Kang S. 2021. Bacterial diversity and communities structural dynamics in soil and meltwater runoff at the frontier of Baishui Glacier No. 1, China. Microbial Ecology 81:370–384.
Sakadevan K, Nguyen M-L. 2017. Livestock production and its impact on nutrient pollution and greenhouse gas emissions. Advances in Agronomy 141:147–184.
Salman MD, Steneroden K. 2015. Important public health zoonoses through cattle. In: Zoonoses-infections affecting humans and animals. Dordrecht, The Netherlands: Springer, 3–22.
Saunders DL, Meeuwig JJ, Vincent ACJ. 2002. Freshwater protected areas: strategies for conservation. Conservation Biology 16:30–41.
Sayers P, Galloway G, Penning-Rowsell E, Yuanyuan L, Fuxin S, Yiwei C, Kang W, Le Quesne T, Wang L, Guan Y. 2015. Strategic flood management: ten 'golden rules' to guide a sound approach. International Journal of River Basin Management 13:137–151. DOI: 10.1080/15715124.2014.902378.
Sayre IM. 1988. International standards for drinking water. Journal-American Water Works Association 80:53–60.
Scarsbrook MR, Boothroyd IKG, Quinn JM. 2000. New Zealand's National River Water Quality Network: long-term trends in macroinvertebrate communities. New Zealand Journal of Marine and Freshwater Research 34:289–302.
Schillinger JE, Gannon JJ. 1985. Bacterial adsorption to suspended particles in urban stormwater. Journal of the Water Pollution Control Federation 57:384–389.
Schlesinger WH. 2009. On the fate of anthropogenic nitrogen. Proceedings of the National Academy of Sciences 106:203–208.
Schmidt CE, Shringi S, Besser TE. 2016. Protozoan predation of Escherichia coli O157: H7

is unaffected by the carriage of Shiga toxin-encoding bacteriophages. PloS One 11:e0147270.
Schmidt ML, Biddanda BA, Weinke AD, Chiang E, Januska F, Props R, Denef VJ. 2020. Microhabitats are associated with diversity–productivity relationships in freshwater bacterial communities. FEMS Microbiology Ecology 96:fiaa029.
Schochetman G, Ou C-Y, Jones WK. 1988. Polymerase chain reaction. The Journal of Infectious Diseases 158:1154–1157.
Scholtz MM, Du Toit J, Neser FWC. 2014. Antagonism in the carbon footprint between beef and dairy production systems. South African Journal of Animal Science 44:17–20.
Scholz RW, Steiner G. 2015. The real type and ideal type of transdisciplinary processes: part II—what constraints and obstacles do we meet in practice? Sustainability Science 10:653–671.
Schrag SJ, Wiener P. 1995. Emerging infectious disease: what are the relative roles of ecology and evolution? Trends in Ecology & Evolution 10:319–324.
Schwarzenbach RP, Egli T, Hofstetter TB, Gunten U von, Wehrli B. 2010. Global Water Pollution and Human Health. Annual Review of Environment and Resources 35:109–136. DOI: doi:10.1146/annurev-environ-100809-125342.
Schwoerbel J. 2011. Methods of hydrobiology:(freshwater biology). Oxford, UK: Pergamon Press.
Scott D, Poynter M. 1991. Upper temperature limits for trout in New Zealand and climate change. Hydrobiologia 222:147–151. DOI: 10.1007/bf00006102.
Scrimgeour GJ, Wicklum D. 1996. Aquatic ecosystem health and integrity: problems and potential solutions. Journal of the North American Benthological Society 15:254–261.
Seet Q, Zhang L. 2011. Anti-activator QslA defines the quorum sensing threshold and response in Pseudomonas aeruginosa. Molecular Microbiology 80:951–965.
Selman M, Greenhalgh S. 2010. Eutrophication: sources and drivers of nutrient pollution. Renewable Resources Journal 26:19–26.
Semenov A v, Franz E, van Overbeek L, Termorshuizen AJ, van Bruggen AHC. 2008. Estimating the stability of Escherichia coli O157: H7 survival in manure-amended soils with different management histories. Environmental Microbiology 10:1450–1459.
Semenza JC, Hoser C, Herbst S, Rechenburg A, Suk JE, Frechen T, Kistemann T. 2012. Knowledge mapping for climate change and food- and waterborne diseases. Critical Reviews in Environmental Science and Technology 42:378–411. DOI: 10.1080/10643389.2010.518520.
Serlachius A, Badawy SM, Thabrew H. 2020. Psychosocial challenges and opportunities for youth with chronic health conditions during the COVID-19 pandemic. JMIR Pediatrics and Parenting 3:e23057.
Shade A, Handelsman J. 2012. Beyond the Venn diagram: the hunt for a core microbiome. Environmental Microbiology 14:4–12.
Shah HA, Sheraz M, Khan AU, Khan FA, Shah LA, Khan J, Khan A, Khan Z. 2020. Surface and Groundwater Pollution: The Invisible, Creeping Threat to Human Health. Civil and Environmental Engineering 16:157–169.
Shah N, Tang H, Doak TG, Ye Y. 2011. Comparing bacterial communities inferred from 16S rRNA gene sequencing and shotgun metagenomics. In: Biocomputing 2011. Bloomington, IN: World Scientific, 165–176.
Sharp JH, Clements K, Diggens M, McDonald JE, Malham SK, Jones DL. 2021. E. coli is a poor end-product criterion for assessing the general microbial risk posed from consuming norovirus contaminated shellfish. Frontiers in Microbiology 12:150.
Shaw JLA, Weyrich L, Cooper A. 2016. Using environmental (e)DNA sequencing for aquatic biodiversity surveys: a beginner's guide. Marine and Freshwater Research. DOI:

http://dx.doi.org/10.1071/MF15361.
Shehata TE, Marr AG. 1971. Effect of nutrient concentration on the growth of Escherichia coli. Journal of Bacteriology 107:210–216.
Sherris AR, Baiocchi M, Fendorf S, Luby SP, Yang W, Shaw GM. 2021. Nitrate in drinking water during pregnancy and spontaneous preterm birth: a retrospective within-mother analysis in california. Environmental Health Perspectives 129:57001.
Shin DH, Lee HJ, Hong JH, Woo EJ, Shin E, Kim Y-S, Ki HC, Lee E. 2018. A historical approach to syphilis infection in Korea. Acta Medico-Historica Adriatica: AMHA 16:185–202.
Shrader-Frechette KS. 1994. Ecosystem health: a new paradigm for ecological assessment? Trends in Ecology and Evolution 12:456–457.
Sigman DM, Casciotti KL, Andreani M, Barford C, Galanter M, Böhlke JK. 2001. A bacterial method for the nitrogen isotopic analysis of nitrate in seawater and freshwater. Analytical Chemistry 73:4145–4153.
Silk MJ, Drewe JA, Delahay RJ, Weber N, Steward LC, Wilson-Aggarwal J, Boots M, Hodgson DJ, Croft DP, McDonald RA. 2018. Quantifying direct and indirect contacts for the potential transmission of infection between species using a multilayer contact network. Behaviour 155:731–757.
Silva RDO, Barioni LG, Moran D. 2021. Fire, deforestation, and livestock: when the smoke clears. Land Use Policy 100:104949.
Simmons G, Greening G, Gao W, Campbell D. 2001. Raw oyster consumption and outbreaks of viral gastroenteritis in New Zealand: evidence for risk to the public's health. Australian and New Zealand Journal of Public Health 25:234–240.
Simon HY, Siddle KJ, Park DJ, Sabeti PC. 2019. Benchmarking metagenomics tools for taxonomic classification. Cell 178:779–794.
Singh BR. 2018. ESKAPE pathogens in animals and their antimicrobial drug resistance pattern. Journal of Dairy, Veterinary & Animal Research 7:1–10.
Sirés I, Brillas E. 2012. Remediation of water pollution caused by pharmaceutical residues based on electrochemical separation and degradation technologies: a review. Environment International 40:212–229.
Skippington E, Ragan MA. 2011. Lateral genetic transfer and the construction of genetic exchange communities. FEMS Microbiology Reviews 35:707–735.
Smith FB. 1995. The Russian Influenza in the United Kingdom, 1889–1894. Social History of Medicine 8:55–73.
Snelder TH, McDowell RW, Fraser CE. 2017. Estimation of catchment nutrient loads in New Zealand using monthly water quality monitoring data. JAWRA Journal of the American Water Resources Association 53:158–178. DOI: 10.1111/1752-1688.12492.
Sodhi MS, Tang CS. 2021. Supply chain management for extreme conditions: Research opportunities. Journal of Supply Chain Management 57:7–16.
Sogin ML, Morrison HG, Huber JA, Welch DM, Huse SM, Neal PR, Arrieta JM, Herndl GJ. 2006. Microbial diversity in the deep sea and the underexplored "rare biosphere." Proceedings of the National Academy of Sciences 103:12115–12120.
Sojka RE, Entry JA. 2000. Influence of polyacrylamide application to soil on movement of microorganisms in runoff water. Environmental Pollution 108:405–412.
Soler N, Forterre P. 2020. Vesiduction: the fourth way of HGT. Environmental Microbiology 22:2457–2460.
Soller JA, Bartrand T, Ashbolt NJ, Ravenscroft J, Wade TJ. 2010. Estimating the primary etiologic agents in recreational freshwaters impacted by human sources of faecal contamination. Water Research 44:4736–4747.
Soller JA, Schoen ME, Bartrand T, Ravenscroft JE, Ashbolt NJ. 2010. Estimated human

health risks from exposure to recreational waters impacted by human and non-human sources of faecal contamination. Water Research 44:4674–4691.
Song Y, Deng SP, Acosta-Martínez V, Katsalirou E. 2008. Characterization of redox-related soil microbial communities along a river floodplain continuum by fatty acid methyl ester (FAME) and 16S rRNA genes. Applied Soil Ecology 40:499–509.
Sowers EG, Wells JG, Strockbine NA. 1996. Evaluation of commercial latex reagents for identification of O157 and H7 antigens of Escherichia coli. Journal of Clinical Microbiology 34:1286–1289.
Spiers AJ, Buckling A, Rainey PB. 2000. The causes of Pseudomonas diversity. Microbiology 146:2345–2350.
Sprong RC, van den Brand AD, van der Aa N, van de Ven BM, Bulder AS. 2020. Combined exposure to nitrate and nitrite via food and drinking water in The Netherlands. Bilthoven, Netherlands: Rijksinstituut voor Volksgezondheid en Milieu (RIVM). DOI: 10.21945/RIVM-2020-0003.
Srivastav AL. 2020. Chemical fertilizers and pesticides: role in groundwater contamination. Oxford, UK: Butterworth-Heinemannr.
Stackebrandt E. 2006. Defining taxonomic ranks. In: Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F eds. The prokaryotes: symbiotic associations, biotechnology, applied microbiology. Berlin, Germany: Springer Science & Business Media, 229–254.
Stainton M, Capel MJ, Armstrong FA. 1977. The chemical analysis of fresh water.
Stark JD, Maxted JR. 2007. A user guide for the Macroinvertebrate Community Index. Nelson: Prepared for the Ministry for the Environment. Cawthron.
Stark JD. 1985. A macroinvertebrate community index of water quality for stony streams. Wellington LB - biomonitoring: Ministry of Works and Development.
Stark JD. 1998. SQMCI: a biotic index for freshwater macroinvertebrate coded-abundance data. New Zealand Journal of Marine and Freshwater Research 32:55–66.
Stats NZ. 2017.Agricultural production statistics: June 2017. Available at https://www.stats.govt.nz/information-releases/agricultural-production-statistics-june-2017-final (accessed 20 January 2001).
Stats NZ. 2020.Groundwater quality. Available at https://www.stats.govt.nz/indicators/groundwater-quality (accessed 22 February 2021).
Stayner LT, Almberg K, Jones R, Graber J, Pedersen M, Turyk M. 2017. Atrazine and nitrate in drinking water and the risk of preterm delivery and low birth weight in four Midwestern states. Environmental Research 152:294–303.
Stenseth N, Smith JM. 1984. Coevolution in ecosystems: Red Queen evolution or stasis? Evolution:870–880.
Stewart KA. 2019. Understanding the effects of biotic and abiotic factors on sources of aquatic environmental DNA. Biodiversity and Conservation 28:983–1001.
Stewart MK, Aitchison-Earl PL. 2020. Irrigation return flow causing a nitrate hotspot and denitrification imprints in groundwater at Tinwald, New Zealand. Hydrology and Earth System Sciences 24:3583–3601.
Stewart-Harawira MW. 2020. Troubled waters: Maori values and ethics for freshwater management and New Zealand's fresh water crisis. Wiley Interdisciplinary Reviews: Water:1–18.
Stoeckl N, Jackson S, Pantus F, Finn M, Kennard MJ, Pusey BJ. 2013. An integrated assessment of financial, hydrological, ecological and social impacts of "development" on Indigenous and non-Indigenous people in northern Australia. Biological Conservation 159:214–221. DOI: 10.1016/j.biocon.2012.12.007.
Strawn LK, Fortes ED, Bihn EA, Nightingale KK, Gröhn YT, Worobo RW, Wiedmann M,

Bergholz PW. 2013. Landscape and meteorological factors affecting prevalence of three food-borne pathogens in fruit and vegetable farms. Applied and Environmental Microbiology 79:588–600.
Strickler KM, Fremier AK, Goldberg CS. 2015. Quantifying effects of UV-B, temperature, and pH on eDNA degradation in aquatic microcosms. Biological Conservation 183:85–92.
Sugden SA. 2020. Coyotes in cities and sponges in streams: Microbiomes in the face of environmental change. University of Alberta.
Suhadolnik MLS, Salgado APC, Scholte LLS, Bleicher L, Costa PS, Reis MP, Dias MF, Ávila MP, Barbosa FAR, Chartone-Souza E. 2017. Novel arsenic-transforming bacteria and the diversity of their arsenic-related genes and enzymes arising from arsenic-polluted freshwater sediment. Scientific Reports 7:1–17.
Sun W, Xia C, Xu M, Guo J, Sun G, Wang A. 2014. Community structure and distribution of planktonic ammonia-oxidizing archaea and bacteria in the Dongjiang River, China. Research in Microbiology 165:657–670.
Sungthong R, Nakaew N. 2015. The genus Nonomuraea: a review of a rare actinomycete taxon for novel metabolites. Journal of Basic Microbiology 55:554–565.
Swaggerty CL, Corcionivoschi N, Ricke SC, Callaway TR. 2018. The first 30 years of Shiga toxin–producing Escherichia coli in cattle production: incidence, preharvest ecology, and management. In: Ricke SC, Atungulu GG, Rainwater CE, Park SH eds. Food and reed safety systems and analysis. London, UK: Elsevier, 117–131. DOI: 10.1016/B978-0-12-811835-1.00007-5.
Swei A, Couper LI, Coffey LL, Kapan D, Bennett S. 2020. Patterns, drivers, and challenges of vector-borne disease emergence. Vector-Borne and Zoonotic Diseases 20:159–170.
Taabodi M, Hashem FM, Oscar TP, Parveen S, May EB. 2019. The possible roles of Escherichia coli in the nitrogen cycle. International Journal of Environmental Research 13:597–602.
Tahamtan A, Samadizadeh S, Rastegar M, Nakstad B, Salimi V. 2020. Respiratory syncytial virus infection: why does disease severity vary among individuals? Expert Review of Respiratory Medicine 14:415–423.
Talukdar PK, Rahman M, Rahman M, Nabi A, Islam Z, Hoque MM, Endtz HP, Islam MA. 2013. Antimicrobial resistance, virulence factors and genetic diversity of Escherichia coli isolates from household water supply in Dhaka, Bangladesh. Plos One 8:e61090.
Tang X, Li J, Sun D, Fang L, Hou L, Liu M, Han P. 2023. Ammonia-oxidizing archaea and comammox Nitrospira clade B as freeze–thaw resistant nitrifiers in wetland soils. International Biodeterioration & Biodegradation 178:105570.
Tang X, Li J, Sun D, Fang L, Hou L, Liu M, Han P. 2023. Ammonia-oxidizing archaea and comammox Nitrospira clade B as freeze–thaw resistant nitrifiers in wetland soils. International Biodeterioration & Biodegradation 178:105570.
Tarr GAM, Lin CY, Lorenzetti D, Chui L, Tarr PI, Hartling L, Vandermeer B, Freedman SB. 2019. Performance of commercial tests for molecular detection of Shiga toxin-producing Escherichia coli (STEC): a systematic review and meta-analysis protocol. BMJ Open 9:e025950.
Tarr PI. 2009. Shiga toxin-associated hemolytic uremic syndrome and thrombotic thrombocytopenic purpura: distinct mechanisms of pathogenesis. Kidney International 75:S29–S32.
Tatem AJ, Rogers DJ, Hay SI. 2006. Global transport networks and infectious disease spread. Advances in Parasitology 62:293–343.
Taylor LH, Latham SM, Mark EJ. 2001. Risk factors for human disease emergence. Philosophical Transactions of the Royal Society B: Biological Sciences 356:983–989.

Tedersoo L, Albertsen M, Anslan S, Callahan B. 2021. Perspectives and benefits of high-throughput long-read sequencing in microbial ecology. Applied and Environmental Microbiology 87:e00626-21.
Tessler M, Neumann JS, Afshinnekoo E, Pineda M, Hersch R, Velho LFM, Segovia BT, Lansac-Toha FA, Lemke M, DeSalle R. 2017. Large-scale differences in microbial biodiversity discovery between 16S amplicon and shotgun sequencing. Scientific Reports 7:1–14.
Teunis PFM, Medema GJ, Kruidenier L, Havelaar AH. 1997. Assessment of the risk of infection by Cryptosporidium or Giardia in drinking water from a surface water source. Water Research 31:1333–1346.
Thaler DS. 2021. Is global microbial biodiversity increasing, decreasing, or staying the same? Frontiers in Ecology and Evolution 9:565649.
Thalinger B, Wolf E, Traugott M, Wanzenböck J. 2019. Monitoring spawning migrations of potamodromous fish species via eDNA. Scientific Reports 9:1–11.
Thawai C, He Y-W, Tadtong S. 2018. Jishengella zingiberis sp. nov., isolated from root tissue of Zingiber montanum. International Journal of Systematic and Evolutionary Microbiology 68:3345–3350.
Thomson JR, Bond NR, Cunningham SC, Metzeling L, Reich P, Thompson RM, Mac Nally R. 2012. The influences of climatic variation and vegetation on stream biota: lessons from the Big Dry in southeastern Australia. Global Change Biology 18:1582–1596. DOI: 10.1111/j.1365-2486.2011.02609.x.
Thornton PK, Gerber PJ. 2010. Climate change and the growth of the livestock sector in developing countries. Mitigation and Adaptation Strategies for Global Change 15:169–184.
Thorp JH, Thoms MC, Delong MD. 2006. The riverine ecosystem synthesis: Biocomplexity in river networks across space and time. River Research and Applications 22:123–147.
Tickner D, Opperman JJ, Abell R, Acreman M, Arthington AH, Bunn SE, Cooke SJ, Dalton J, Darwall W, Edwards G. 2020. Bending the curve of global freshwater biodiversity loss: an emergency recovery plan. BioScience 70:330–342.
Tillotson MD, Kelly RP, Duda JJ, Hoy M, Kralj J, Quinn TP. 2018. Concentrations of environmental DNA (eDNA) reflect spawning salmon abundance at fine spatial and temporal scales. Biological Conservation 220:1–11.
Tilman D, Cassman KG, Matson PA, Naylor R, Polasky S. 2002. Agricultural sustainability and intensive production practices. Nature 418:671–677.
Tortajada C, Biswas AK. 2018. Achieving universal access to clean water and sanitation in an era of water scarcity: strengthening contributions from academia. Current Opinion in Environmental Sustainability 34:21–25.
Towner KJ. 2009. Acinetobacter: an old friend, but a new enemy. Journal of Hospital Infection 73:355–363.
Tregurtha J. 2020. Annual groundwater quality survey. Kaikora, New Zealand.
Tringe SG, Rubin EM. 2005. Metagenomics: DNA sequencing of environmental samples. Nature Reviews Genetics 6:805–814.
Trøjelsgaard K, Olesen JM. 2016. Ecological networks in motion: micro- and macroscopic variability across scales. Functional Ecology 30:1926–1935a. DOI: 10.1111/1365-2435.12710.
Troth CR, Sweet MJ, Nightingale J, Burian A. 2021. Seasonality, DNA degradation and spatial heterogeneity as drivers of eDNA detection dynamics. Science of the Total Environment 768:144466.
Trujillo-González A, Villacorta-Rath C, White NE, Furlan EM, Sykes M, Grossel G, Divi UK, Gleeson D. 2021. Considerations for future environmental DNA accreditation and

proficiency testing schemes. Environmental DNA 00:1–10.
Turak E, Harrison I, Dudgeon D, Abell R, Bush A, Darwall W, Finlayson CM, Ferrier S, Freyhof J, Hermoso V, Juffe-Bignoli D, Linke S, Nel J, Patricio HC, Pittock J, Raghavan R, Revenga C, Simaika JP, De Wever A. 2017. Essential biodiversity variables for measuring change in global freshwater biodiversity. Biological Conservation 213:272–279. DOI: http://dx.doi.org/10.1016/j.biocon.2016.09.005.
Turner CR, Uy KL, Everhart RC. 2015. Fish environmental DNA is more concentrated in aquatic sediments than surface water. Biological Conservation 183:93–102.
Udmale P, Pal I, Szabo S, Pramanik M, Large A. 2020. Global food security in the context of COVID-19: A scenario-based exploratory analysis. Progress in Disaster Science 7:100120.
UNDP, IFRC. 2017. A socio-economic impact assessment of the Zika virus in Latin America and the Caribbean: with a focus on Brazil, Colombia and Suriname. New York, New York, USA.
United Nations General Assembly. 2015. Transforming our world: the 2030 Agenda for Sustainable Development. Division for Sustainable Development Goals: New York, NY, USA.
United Nations. 2015a.United Nations: sustainable development goals. Goal 6: ensure access to water and sanitation for all. Available at https://sdgs.un.org/goals/goal6 (accessed May 10, 2021).
United Nations. 2015b. The millennium development goals report 2015. New York, NY: United Nations.
United Nations. 2016. The sustainable development goals report 2016. New York, NY: United Nations. DOI: 10.29171/azu_acku_pamphlet_k3240_s878_2016.
United Nations. 2020. The sustainable development goals report. New York, NY: United Nations.
United States Environmental Protection Agency. 1985. Test methods for Escherichia coli and enterococci in water by the membrane-filter procedure. Publication EPA-600/4-85-076.
Uno H. 2013. Stream thermal heterogeneity prolongs aquatic-terrestrial subsidy and enhances riparian spider growth. Ecology 97:39–42. DOI: 10.1002/ecy.1552.
Urban L, Holzer A, Baronas JJ, Hall MB, Braeuninger-Weimer P, Scherm MJ, Kunz DJ, Perera SN, Martin-Herranz DE, Tipper ET. 2021. Freshwater monitoring by nanopore sequencing. Elife 10:e61504.
Urbanczyk H, Ast JC, Dunlap P V. 2011. Phylogeny, genomics, and symbiosis of Photobacterium. FEMS Microbiology Reviews 35:324–342.
USEPA. 2016. Drinking water contaminant candidate list (CCL) and regulatory determination 4. Washington D.C., USA. DOI: 81 FR 8109.
Ushio M, Fukuda H, Inoue T, Makoto K, Kishida O, Sato K, Murata K, Nikaido M, Sado T, Sato Y. 2017. Environmental DNA enables detection of terrestrial mammals from forest pond water. Molecular Ecology Resources 17:e63–e75.
Valat C, Haenni M, Saras E, Auvray F, Forest K, Oswald E, Madec J-Y. 2012. CTX-M-15 extended-spectrum β-lactamase in a Shiga toxin-producing Escherichia coli isolate of serotype O111: H8. Applied Environmental Microbiology 78:1308–1309.
van Elsas JD, Semenov A v, Costa R, Trevors JT. 2011. Survival of Escherichia coli in the environment: fundamental and public health aspects. The ISME Journal 5:173–183.
van Etten J, Bhattacharya D. 2020. Horizontal gene transfer in eukaryotes: not if, but how much? Trends in Genetics 36:915–925.
van Kerkhove MD, Mumford E, Mounts AW, Bresee J, Ly S, Bridges CB, Otte J. 2011. Highly pathogenic avian influenza (H5N1): pathways of exposure at the animal-human interface, a systematic review. PloS One 6:e14582.

van Valen L. 1977. The red queen. The American Naturalist 111:809–810.
Vandergeten E, Azadi H, Teklemariam D, Nyssen J, Witlox F, Vanhaute E. 2016. Agricultural outsourcing or land grabbing: a meta-analysis. Landscape Ecology 31:1395–1417.
Vasudevan S, Oturan MA. 2014. Electrochemistry: as cause and cure in water pollution—an overview. Environmental Chemistry Letters 12:97–108.
Vigneron A, Cruaud P, Lovejoy C, Vincent WF. 2022. Genomic evidence of functional diversity in DPANN archaea, from oxic species to anoxic vampiristic consortia. ISME Communications 2:4.
Vilmin L, Mogollón JM, Beusen AHW, Bouwman AF. 2018. Forms and subannual variability of nitrogen and phosphorus loading to global river networks over the 20th century. Global and Planetary Change 163:67–85.
Viñas V, Malm A, Pettersson TJR. 2019. Overview of microbial risks in water distribution networks and their health consequences: quantification, modelling, trends, and future implications. Canadian Journal of Civil Engineering 46:149–159.
Vlassov V V, Laktionov PP, Rykova EY. 2007. Extracellular nucleic acids. Bioessays 29:654–667.
Vogeleer P, Tremblay YDN, Mafu AA, Jacques M, Harel J. 2014. Life on the outside: role of biofilms in environmental persistence of Shiga-toxin-producing Escherichia coli. Frontiers in Microbiology 5:1–12.
Vollmer D, Regan HM, Andelman SJ. 2016. Assessing the sustainability of freshwater systems: A critical review of composite indicators. Ambio 45:765–780. DOI: 10.1007/s13280-016-0792-7.
von Braun J, Meinzen-Dick R. 2009. "Land Grabbing" by foreign investors in developing countries: risks and opportunities (accessed December 8, 2021).
Vorosmarty CJ, McIntyre PB, Gessner MO, Dudgeon D, Prusevich A, Green P, Glidden S, Bunn SE, Sullivan CA, Liermann CR, Davies PM. 2010. Global threats to human water security and river biodiversity. Nature 467:555–561. DOI: 10.1038/nature09440.
Vos P, Garrity G, Jones D, Krieg NR, Ludwig W, Rainey FA, Schleifer K-H, Whitman WB. 2011. Bergey's manual of systematic bacteriology: Volume 3: the Firmicutes. Baltimore, MD: Springer Science & Business Media.
Wacker S, Fossøy F, Larsen BM, Brandsegg H, Sivertsgård R, Karlsson S. 2019. Downstream transport and seasonal variation in freshwater pearl mussel (Margaritifera margaritifera) eDNA concentration. Environmental DNA 1:64–73.
Wakelin D. 1996. Immunology and genetics of zoonotic infections involving parasites. Comparative Immunology, Microbiology and Infectious Diseases 19:255–265.
Walker DI, Younger A, Stockley L, Baker-Austin C. 2018. Escherichia coli testing and enumeration in live bivalve shellfish–present methods and future directions. Food Microbiology 73:29–38.
Wall R, Dymond N, Bell A, Thornley C, Buik H, Cumming D, Petersen N. 2011. Two New Zealand outbreaks of norovirus gastroenteritis linked to commercially farmed oysters. NZ Medical Journal 124:63–71.
Wallis GP, Trewick SA. 2009. New Zealand phylogeography: evolution on a small continent. Molecular Ecology 18:3548–3580.
Wang N, Gao J, Liu Y, Wang Q, Zhuang X, Zhuang G. 2021. Realizing the role of N-acyl-homoserine lactone-mediated quorum sensing in nitrification and denitrification: A review. Chemosphere 274:129970.
Wang YJ, Deering AJ, Kim HJ. 2020. The occurrence of shiga toxin-producing E. coli in aquaponic and hydroponic systems. Horticulturae 6:1–36.
Wang YJ, J Deering A, Kim HJ. 2021. Effects of Plant Age and Root Damage on

Internalization of Shiga Toxin-Producing Escherichia coli in Leafy Vegetables and Herbs. Horticulturae 7:68.
Wang Y, Liu Q, Liu Q, Gao Q, Lu H, Meng H, Xie Y, Huang Q, Ma X, Wang H. 2018. Phylogenetic analysis and virulence determinant of the host-adapted Staphylococcus aureus lineage ST188 in China. Emerging Microbes & Infections 7:1–11.
Wanjugi P, Harwood VJ. 2013. The influence of predation and competition on the survival of commensal and pathogenic fecal bacteria in aquatic habitats. Environmental Microbiology 15:517–526.
Warburton DW, Austin JW, Harrison BH, Sanders G. 1998. Survival and recovery of Escherichia coli O157: H7 in inoculated bottled water. Journal of Food Protection 61:948–952.
Ward J V, Tockner KLB-S. 2001. Biodiversity: towards a unifying theme in river ecology. Freshwater Biology 46:807–819.
Ward MH, DeKok TM, Levallois P, Brender J, Gulis G, Nolan BT, VanDerslice J. 2005. Workgroup report: drinking-water nitrate and health—recent findings and research needs. Environmental Health Perspectives 113:1607–1614.
Ward MH, Jones RR, Brender JD, De Kok TM, Weyer PJ, Nolan BT, Villanueva CM, Van Breda SG. 2018. Drinking water nitrate and human health: an updated review. International Journal of Environmental Research and Public Health 15:1–31.
Wardeh M, Sharkey KJ, Baylis M. 2020. Integration of shared-pathogen networks and machine learning reveals the key aspects of zoonoses and predicts mammalian reservoirs. Proceedings of the Royal Society B 287:20192882.
Wargo AR, Kurath G, Scott RJ, Kerr B. 2021. Virus shedding kinetics and unconventional virulence tradeoffs. PLoS Pathogens 17:e1009528.
Warnes MGR, Bolker B, Bonebakker L, Gentleman R, Huber W. 2015. Package 'Gplots'. Various R programming tools for plotting data:12.
Webster M. 2017. Merriam-Webster Medical Dictionary. Available from: Merriam-Webster and https://en. wikipedia. org/wiki/Iatrogenesis.
Weeks ES, Death RG, Foote K, Anderson-Lederer R, Joy MK, Boyce P. 2016. Conservation Science Statement 1. The demise of New Zealand's freshwater flora and fauna: a forgotten treasure. Pacific Conservation Biology 22:110–115. DOI: http://dx.doi.org/10.1071/PC15038.
Weigel BM, Henne LJ, Martinez-Rivera LM. 2002. Macroinvertebrate-based index of biotic integrity for protection of streams in west-central Mexico. Journal of the North American Benthological Society 21:686–700.
Wein T, Hülter NF, Mizrahi I, Dagan T. 2019. Emergence of plasmid stability under non-selective conditions maintains antibiotic resistance. Nature Communications 10:1–13.
Weiskerger CJ, Whitman RL. 2018. Monitoring E. coli in a changing beachscape. Science of The Total Environment 619:1236–1246.
Weiss RA. 2002. Virulence and pathogenesis. Trends in Microbiology 10:314–317.
Weissman JL, Fagan WF, Johnson PLF. 2019. Linking high GC content to the repair of double strand breaks in prokaryotic genomes. PLoS Genetics 15:e1008493.
Wessely J, Hülber K, Gattringer A, Kuttner M, Moser D, Rabitsch W, Schindler S, Dullinger S, Essl F. 2017. Habitat-based conservation strategies cannot compensate for climate-change-induced range loss. Nature Climate Change 7:823–827.
Whitman WB, Boone DR, Koga Y, Keswani J. 2001. Taxonomy of methanogenic Archaea. In: Boone DR, Castenholz RW eds. Bergey's manual of systematic bacteriology. New York, NY, USA: Springer Verlag, 211–294.
Wickham, H. 2016. Ggplot2: Elegant Graphics for Data Analysis. New York, USA: Springer-Verlag. https://ggplot2.tidyverse.org.

Wilcock RJ, Monaghan RM, Quinn JM, Srinivasan MS, Houlbrooke DJ, Duncan MJ, Wright-Stow AE, Scarsbrook MR. 2013. Trends in water quality of five dairy farming streams in response to adoption of best practice and benefits of long-term monitoring at the catchment scale. Marine and Freshwater Research 64:401–412. DOI: http://dx.doi.org/10.1071/MF12155.
Wilcox BA, Gubler DJ. 2005. Disease ecology and the global emergence of zoonotic pathogens. Environmental Health and Preventive Medicine 10:263–272.
Wilharm G, Skiebe E, Higgins PG, Poppel MT, Blaschke U, Leser S, Heider C, Heindorf M, Brauner P, Jäckel U. 2017. Relatedness of wildlife and livestock avian isolates of the nosocomial pathogen Acinetobacter baumannii to lineages spread in hospitals worldwide. Environmental Microbiology 19:4349–4364.
Wilkinson J, Jenkins A, Wyer M, Kay D. 1995. Modelling faecal coliform dynamics in streams and rivers. Water Research 29:847–855.
Williams AP, Avery LM, Killham K, Jones DL. 2005. Persistence of Escherichia coli O157 on farm surfaces under different environmental conditions. Journal of Applied Microbiology 98:1075–1083.
Winkworth RC, Nelson BCW, Bellgard SE, Probst CM, McLenachan PA, Lockhart PJ. 2020. A LAMP at the end of the tunnel: A rapid, field deployable assay for the kauri dieback pathogen, Phytophthora agathidicida. PloS One 15:e0224007.
Wise BR, Roane TM, Mosier AC. 2020. Community composition of nitrite reductase gene sequences in an acid mine drainage environment. Microbial Ecology 79:562–575.
Wolfe ND, Dunavan CP, Diamond J. 2007. Origins of major human infectious diseases. Nature 447:279–283.
Wong Y, Othman S, Lau Y, Radu S, Chee H. 2018. Loop-mediated isothermal amplification (LAMP): a versatile technique for detection of micro-organisms. Journal of Applied Microbiology 124:626–643.
Woods RJ, Macdonald JI, Crook DA, Schmidt DJ, Hughes JM. 2010. Contemporary and historical patterns of connectivity among populations of an inland river fish species inferred from genetics and otolith chemistry. Canadian Journal of Fisheries and Aquatic Sciences 67:1098–1115.
Woodward G, Gray C, Baird DJ. 2013. Biomonitoring for the 21st Century: new perspectives in an age of globalisation and emerging environmental threats. Limnetica 32:159–173.
Woolhouse MEJ, Gowtage-Sequeria S. 2005. Host range and emerging and reemerging pathogens. Emerging Infectious Diseases 11:1842.
World Health Organization. 1978. Ebola haemorrhagic fever in Zaire, 1976. Report of an international commission. World Health Organization 56:271–293.
World Health Organization. 2011. Technical guidance on water-related disease surveillance. Copenhagen, Denmark: WHO Regional Office for Europe.
World Health Organization. 2017. Guidelines for drinking-water quality. Geneva, Switzerland: World Health Organization.
World Health Organization. 2020. World health statistics 2020: monitoring health for the SDGs, sustainable development goals. Geneva, Switzerland.
World Health Organization. 2020a. Campylobacter. Available at https://www.who.int/news-room/fact-sheets/detail/campylobacter (accessed March 11, 2021).
World Health Organization. 2020b. E. coli. Available at https://www.who.int/news-room/fact-sheets/detail/e-coli (accessed July 7, 2020).
Wright J. 2015. Managing water quality: examining the 2014 National Policy Statement. . Wellington. Parliamentary Commissioner for the Environment.
Wu T. 2021. The socioeconomic and environmental drivers of the COVID-19 pandemic: A review. Ambio 50:822–833.

Wyres KL, Holt KE. 2018. Klebsiella pneumoniae as a key trafficker of drug resistance genes from environmental to clinically important bacteria. Current Opinion in Microbiology 45:131–139.
Xia Y, Zhang M, Tsang DCW, Geng N, Lu D, Zhu L, Igalavithana AD, Dissanayake PD, Rinklebe J, Yang X. 2020. Recent advances in control technologies for non-point source pollution with nitrogen and phosphorous from agricultural runoff: current practices and future prospects. Applied Biological Chemistry 63:1–13.
Xing J, Wang H, Brookes PC, Salles JF, Xu J. 2019. Soil pH and microbial diversity constrain the survival of E. coli in soil. Soil Biology and Biochemistry 128:139–149.
Xue X, Landis AE. 2010. Eutrophication potential of food consumption patterns. Environmental Science & Technology 44:6450–6456.
Yakirevich A, Pachepsky YA, Guber AK, Gish TJ, Shelton DR, Cho KH. 2013. Modeling transport of Escherichia coli in a creek during and after artificial high-flow events: Three-year study and analysis. Water Research 47:2676–2688.
Yamaguchi Y, Park J-H, Inouye M. 2011. Toxin-antitoxin systems in bacteria and archaea. Annual Review of Genetics 45:61–79.
Yao Y, Tian H, Shi H, Pan S, Xu R, Pan N, Canadell JG. 2020. Increased global nitrous oxide emissions from streams and rivers in the Anthropocene. Nature Climate Change 10:138–142.
Yost CK, Diarra MS, Topp E. 2011. Animals and humans as sources of fecal indicator bacteria. Washington D.C., USA: American Society of Microbiology. DOI: http://dx.doi.org/10.1128/9781555816865.
Young R, Townsend C, Matthaei C. 2004. Functional indicators of river ecosystem health – an interim guide for use in New Zealand. Nelson: Cawthron Report No 870.
Young R, Wilson BR, McLeod M, Alston C. 2005. Carbon storage in the soils and vegetation of contrasting land uses in northern New South Wales, Australia. Soil Research 43:21–31.
Young, R, C Townsend, and C Matthaei. 2004. Functional Indicators of River Ecosystem Health – an Interim Guide for Use in New Zealand. Nelson: Cawthron Report No 870.
Yue Y, Wang F, Pan J, Chen X-P, Tang Y, Yang Z, Ma J, Li M, Yang M. 2022. Spatiotemporal dynamics, community assembly and functional potential of sedimentary archaea in reservoirs: coaction of stochasticity and nutrient load. FEMS Microbiology Ecology 98:fiac109.
Zeglin LH. 2015. Stream microbial diversity in response to environmental changes: review and synthesis of existing research. Frontiers in Microbiology 6:454.
Zhang G, Bai J, Tebbe CC, Zhao Q, Jia J, Wang W, Wang X, Yu L. 2021. Salinity controls soil microbial community structure and function in coastal estuarine wetlands. Environmental Microbiology 23:1020–1037.
Zhang G, Zhang F, Ding G, Li J, Guo X, Zhu J, Zhou L, Cai S, Liu X, Luo Y. 2012. Acyl homoserine lactone-based quorum sensing in a methanogenic archaeon. The ISME Journal 6:1336–1344.
Zhang J, Yang Y, Zhao L, Li Y, Xie S, Liu Y. 2015. Distribution of sediment bacterial and archaeal communities in plateau freshwater lakes. Applied Microbiology and Biotechnology 99:3291–3302.
Zhang S, Yin Y, Jones MB, Zhang Z, Deatherage Kaiser BL, Dinsmore BA, Fitzgerald C, Fields PI, Deng X. 2015. Salmonella serotype determination utilizing high-throughput genome sequencing data. Journal of Clinical Microbiology 53:1685–1692.
Zhang T, Wang P. 2022. The response of bacterial communities to organic matter in the surface sediment of the Pearl River estuary. Plankton and Benthos Research 17:45–56.
Zhang TL, Zhao QG, Zhai YS, Chen BF, Sun B. 1995. Sustainable land use in the hilly red

soil region of South-eastern China. Pedosphere 5:1–10.
Zhang X, Zhang C, Liu Y, Zhang R, Li M. 2022. Non-negligible roles of archaea in coastal carbon biogeochemical cycling. Trends in Microbiology 31:586-600. DOI: https://doi.org/10.1016/j.tim.2022.11.008.
Zhang Y. 2014. Persisters, persistent infections and the Yin–Yang model. Emerging Microbes & Infections 3:e3.
Zhao L, Zhang H, O'Gorman EJ, Tian W, Ma A, Moore JC, Borrett SR, Woodward G. 2016. Weighting and indirect effects identify keystone species in food webs. Ecology Letters 19:1032-1040. DOI: 10.1111/ele.12638.
Zheng P, Zhang Q, Zou J, Han Q, Han J, Wang Q, Yao L, Yu G, Liang Y. 2023. A new strategy for the enrichment of ammonia-oxidizing archaea in wastewater treatment systems: The positive role of quorum-sensing signalling molecules. Science of The Total Environment 873:162385.
Zhong L, Qing J, Liu M, Cai X, Li G, Chen G, Xu X, Xue K, Wang Y. 2022. Fungi and archaea control soil N2O production potential in Chinese grasslands rather than Bacteria. Frontiers in Microbiology 13:1494.
Zilber-Rosenberg I, Rosenberg E. 2008. Role of microorganisms in the evolution of animals and plants: the hologenome theory of evolution. FEMS Microbiology Reviews 32:723–735.
Zimmer-Faust AG, Thulsiraj V, Marambio-Jones C, Cao Y, Griffith JF, Holden PA, Jay JA. 2017. Effect of freshwater sediment characteristics on the persistence of fecal indicator bacteria and genetic markers within a Southern California watershed. Water Research 119:1–11.
Zinsstag J, Schelling E, Waltner-Toews D, Tanner M. 2011. From "one medicine" to "one health" and systemic approaches to health and well-being. Preventive Veterinary Medicine 101:148–156.
Zorz JK, Sharp C, Kleiner M, Gordon PMK, Pon RT, Dong X, Strous M. 2019. A shared core microbiome in soda lakes separated by large distances. Nature Communications 10:1–10.
Zou D, Li H, Du P, Wang B, Lin H, Liu H, Chen J, Li M. 2022. Distinct features of sedimentary archaeal communities in hypoxia and non-hypoxia regions off the Changjiang river estuary. Microbiology Spectrum 10:e01947-22.
Zu EK, Godar J, Lathuillière MJ, Löfgren P, Gardner T, Vasconcelos A, Meyfroidt P. 2020. The origin, supply chain, and deforestation risk of Brazil's beef exports. Proceedings of the National Academy of Sciences 117:31770–31779.