Denitrification response to nutrient enrichment in New Zealand estuaries

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Abstract

As coastal catchment land use intensifies, estuaries receive increased nutrient and sediment loads, resulting in habitats dominated by muddy organic-rich sediments. Nutrient processing and denitrification in estuarine sediments represent important ecosystem functions regenerating nutrients for primary producers, and regulating the ability to remove excess terrestrially derived nitrogen. Denitrification therefore offers resilience to estuaries through mitigating eutrophication. Biodiversity loss and increased mud content are important indicators of estuarine ecosystem degradation, and have been associated with negative effects on soft-sediment ecosystem functioning. However, the impact of these stressors on ecosystem response to nutrient enrichment is unclear. This thesis investigates the response of denitrification to nutrient enrichment with emphasis on the impact of sedimentation stress and biodiversity loss for resilience to eutrophication.

To experimentally test soft sediment ecosystem response to enrichment, an effective in situ enrichment method was required. A review of current literature was conducted highlighting a methodological gap, and lack of consistency among published studies. I developed and tested a technique for enriching estuarine sediments using slow release fertiliser. Enrichment effects (pore water ammonium concentrations) scaled with application rate, and greater elevations were observed in deeper (5-7 cm) than surface (0-2 cm) sediments. Enrichment levels were similar to eutrophic estuaries, were maintained for at least seven weeks, and enrichment levels could be partially explained by the sedimentary environment and macrofaunal community.

To test the effect of sedimentary environment on denitrification enzyme activity (DEA) response to nutrient perturbation, an in situ enrichment experiment was conducted across an intertidal sedimentary gradient. Findings show that the level of an existing stressor (sediment mud content) can influence ecosystem function response to a second stressor (nutrient enrichment). DEA was supressed by nutrient enrichment, but the effect was greater with more mud content. This

study demonstrates that increasing sediment mud content may restrict nutrient processing, facilitating ecosystem shifts toward eutrophication.

A field experiment was conducted across a heterogeneous sandflat at selected sites with a gradient in biodiversity to test the effect of macrofaunal community composition on denitrification in response to two levels of nutrient enrichment. Nutrient enrichment caused reductions in DEA as well as functional changes in the macrofaunal community. The degree of suppression of DEA following enrichment was dependent on enrichment level, and was alleviated by a key bioturbating species (medium enrichment), or the abundance and diversity of nutrient processing species (high enrichment). This study provides a prime example of the context dependent role of biodiversity in maintaining ecosystem functioning, underlining that different elements of biodiversity can become important as stress levels increase.

To investigate the controls on denitrification at a regional scale (i.e. among estuaries), DEA data and environmental co-variables from five studies across four estuaries was combined and analysed. Mud content accounted for most of the variability in DEA, but other sedimentary and macrofaunal variables were also important. DEA increased with increasing sediment mud content up to a threshold of 30% mud, above which, DEA values were variable but no longer increased. This is significant because mud content is increasing in many estuaries globally, and shows that denitrification can reach a threshold with increasing estuary degradation.

The findings of this thesis show that management of nutrients in estuarine ecosystems requires real-world understanding of the context dependent responses of denitrification, and that biodiversity loss and increasing sedimentation may reduce ecological resilience to eutrophication.

Preface

This thesis comprises four research chapters (Chapters 2-5). Chapters 3 and 5 have been published in peer-reviewed journals, and Chapters 2 and 4 are in preparation for submission. I assumed responsibility for the field and laboratory work, data analysis and writing of this thesis. The ideas in this thesis are my own, unless otherwise referenced, and were produced under the supervision of Professor Conrad Pilditch (University of Waikato), Professor Simon Thrush (University of Auckland), Professor Louis Schipper (University of Waikato), and Dr Candida Savage (University of Otago).

Chapter 2 has been published in the journal *Marine Pollution Bulletin* Volume 111: 287-294 (2016), under the title “In situ soft sediment nutrient enrichment: A unified approach to eutrophication field experiments” by E.J. Douglas, C.A. Pilditch, L.V. Hines, C Kraan, and S.F. Thrush. DOI: 10.1016/j.marpolbul.2016.06.096

Chapter 3 is under review for the journal *Estuaries and Coasts*, under the title “Sedimentary environment influences ecosystem response to nutrient enrichment” by E.J. Douglas, C.A. Pilditch, A.M. Lohrer, C. Savage, L.A. Schipper, and S.F. Thrush.

Chapter 4 has been published in the journal *Ecosystems* (2017), under the title “Macrofaunal functional diversity provides resilience to nutrient enrichment in coastal sediments” by E.J. Douglas, C.A. Pilditch, C. Kraan, L.A. Schipper, A.M. Lohrer, and S.F. Thrush. DOI: 10.1007/s10021-017-0113-4

I also contributed to an accompanying publication related to this project but not included in this thesis published in the journal *Proceedings of the Royal Society B: Biological Sciences* Volume 284: 1852 (2017), titled "Changes in the location of biodiversity-ecosystem function hot spots across the seafloor landscape with increasing sediment nutrient loading" by S.F. Thrush, J.E. Hewitt, C. Kraan, A.M. Lohrer, C.A. Pilditch, and E.J. Douglas. DOI: 10.1098/rspb.2016.2861

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Chapter One

General Introduction

1.1 Estuary nutrient enrichment and denitrification

Estuarine soft sediment ecosystems hold intrinsic ecological, cultural and economic value, however, their health and functioning is under threat from increasing anthropogenic stressors especially nutrient enrichment and sedimentation. Nitrogen is the most abundant element on earth and is essential for all life. However, reactive forms of nitrogen are now being produced and distributed at such high rates that they outweigh rates of natural processes of removal and accumulate in aquatic ecosystems (Galloway et al. 2003). Overabundance of nutrients can increase productivity and cause changes in the composition of communities and food webs, as well as ecosystem function, and ultimately lead to eutrophication (Vitousek et al. 1997, Herbert 1999, Cloern 2001).

Denitrification is an important mechanism of removal of excess bioavailable nitrogen from aquatic ecosystems and estuaries, because it regulates the amount of nitrogen available to primary producers, and therefore can lessen ecosystem shifts towards eutrophication (Seitzinger 1988, Aelion et al. 1997, Nowicki et al. 1997, Herbert 1999, Seitzinger et al. 2006, Teixeira et al. 2010). Denitrification is the conversion of electron accepting, bioavailable nitrogen compounds (nitrate and nitrite) into gaseous forms (nitrogen gas and nitrous oxide) by heterotrophic bacteria (Knowles 1982). It occurs when oxygen is absent or in low concentrations, nitrate is available, and there is a carbon source (usually in the form of electron donating organic carbon compounds) (Seitzinger et al. 2006). Nitrification (the microbial conversion of ammonium to nitrate) in the sediments supplies nitrate for denitrification (where water column nitrate is low) in many coastal marine ecosystems (Jenkins & Kemp 1984, Henriksen & Kemp 1988). The co-occurrence of these two processes is called coupled nitrification-denitrification, and is limited by the contrasting oxygen conditions that each requires (Jenkins & Kemp 1984). Nitrification requires oxygen and occurs in the top layer of sediment where oxygen

diffuses from the water column, and denitrification occurs deeper down in anoxic sediments (Figure 1.1a). In marine sediments there is usually a gradient of oxic to anoxic sediment within the top few centimetres allowing rapid transport of nutrients; this provides the site for coupled nitrification-denitrification (Jenkins & Kemp 1984) (Figure 1.1a). The nature and extent of this zone is strongly influenced by the presence of bioturbating macrofauna which facilitate movement of oxygen, nitrates and organic matter throughout the sediments (Aller 1988, Stief 2013) (Figure 1.1b).

Estuaries can be described as filters at the land/ocean interface (Anderson et al. 2013) and the biogeochemical cycling occurring within them has the potential to considerably influence nearby coastal areas (Seitzinger et al. 2006). Soft sediments in estuaries can denitrify between 10 and 80% of received terrestrial nitrogen inputs (Seitzinger 1988, Nixon et al. 1996), these habitats are therefore vital for ecosystem resilience to nutrient enrichment. Resilience can be defined as the ability of an ecosystem to maintain its functioning while withstanding disturbance (Holling 1973). Denitrification in sediments is important for mitigating the effects of excess nitrogen in coastal ecosystems, and is a potential tool for management (Seitzinger 1988, Nowicki et al. 1997, Davidson & Seitzinger 2006), but other stressors, particularly sedimentation, may impact this ability. This thesis seeks to determine how environmental variables regulate denitrification in estuary sediments, and how it is impacted by key stressors nutrient enrichment, biodiversity loss, and sedimentation. Such studies are necessary to understand how environmental change and increasing nutrients will influence denitrification in coastal ecosystems (Cornwell et al. 1999).

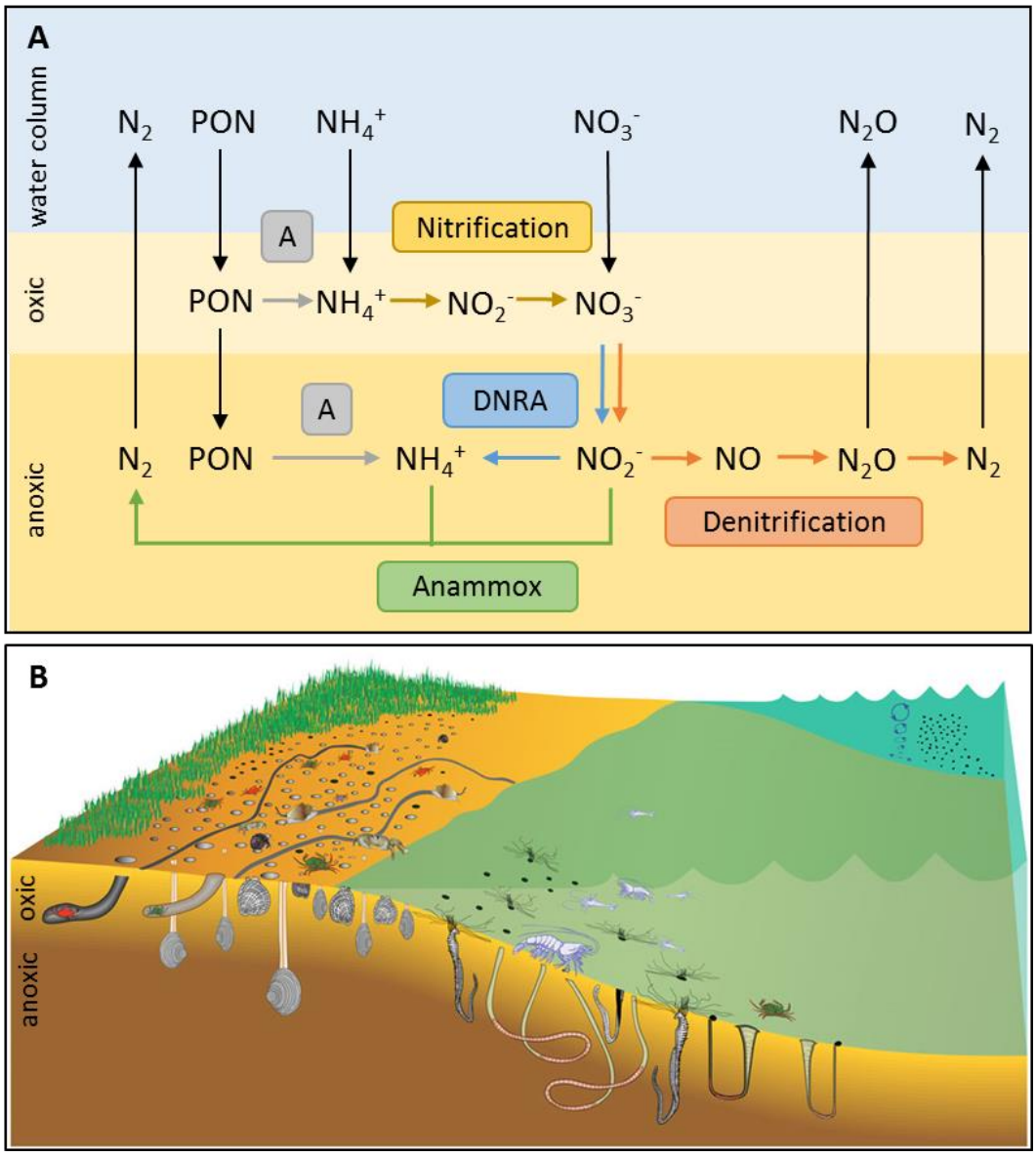

Figure 1.1 (a) Nitrogen cycling pathways in estuary sediments, showing oxic and anoxic layers. PON: particulate organic nitrogen, A: ammonification, DNRA: dissimilatory nitrate reduction to ammonium, Anammox: anaerobic ammonium oxidation. Adapted from (Stief 2013). (b) Cross section of an estuary soft sediment habitat showing oxic and anoxic sedimentary layers, depicting the complexity added by benthic macrofauna. Adapted from graphic drawn by Max Oulton, Cartographer, University of Waikato.

1.1.2 Sedimentation

Sedimentation is a major threat to estuaries in New Zealand and globally. Increasing coastal populations and intensification of land use has resulted in increases in the supply and accumulation of both nutrients and fine terrestrial sediments ('mud'; grain size <63 μm) in estuaries (Thrush et al. 2004). These inputs are likely to increase with climate change because of increased frequency and intensity of storm events (Thrush et al. 2003a, Hewitt et al. 2016). Delivery of mud to estuaries can occur through pulsed or catastrophic 'dumping' events related to major storms, or more slowly through intermittent increases in water column turbidity. This can result in sub-lethal effects to macrofauna, macrofaunal die-off, reductions in recruitment, and reductions in ecosystem function performance (Norkko et al. 2002, Cummings et al. 2003, Lohrer et al. 2004b, Billerbeck et al. 2007, Cummings et al. 2009, Pratt et al. 2014). Over longer timescales increased sediment mud content can cause degradation of estuaries through changes in or homogenisation of habitats and communities, loss of biodiversity, and reductions in or complete loss of ecosystem functions (Thrush et al. 2003b, Thrush et al. 2004, Jones et al. 2011).

An increase in the proportion of fine 'muddy' sediment causes a reduction in permeability affecting the flow of solutes and particles, and therefore has a significant influence on local biogeochemical cycling (Huettel et al. 2003, Santos et al. 2012). Muddy sediments with low permeability generally have a higher organic matter content which can increase nutrient remineralisation and microphytobenthic biomass (Lever & Valiela 2005), and influence biogeochemical processes such as denitrification (Caffrey et al. 1993). Field studies have demonstrated that ecosystem function, macrofaunal communities and behaviour vary across sedimentary gradients (Blackburn & Henriksen 1983, Edgar & Barrett 2000, Cook et al. 2004a, Cook et al. 2004b, Jones et al. 2011, Hohaia et al. 2013, Pratt et al. 2013), but less is known about the influence of sedimentation on denitrification response to nutrient enrichment (i.e. multiple stressor effects).

1.1.3 Benthic macrofauna

Loss of biodiversity is a global problem resulting in loss of ecosystem resilience to environmental change (Oliver et al. 2015). The diversity – stability hypothesis says that communities that contain more species will vary less through time in response to disturbances (Elton 1958, May 1972, Jacquet et al. 2016). Biodiversity therefore influences the ability of an ecosystem to be resilient against or to resist environmental change (Chapin et al. 2000, Naeem et al. 2012). Benthic macrofauna, particularly large species and individuals, are important for estuarine ecosystem functioning (Hewitt et al. 2006, Norkko et al. 2013). In New Zealand estuaries, two large bivalve species *Austrovenus stutchburyi* and *Macomona liliana* can make up a large proportion of the macrofaunal biomass and exert significant control on sediment biogeochemistry and ecosystem functioning (Hewitt et al. 1996, Woodin et al. 2016). The bioturbating and bioirrigating activities of benthic macrofauna control oxygen gradients as well as the movement of nitrogen solutes throughout the sediment profile (Aller 1988, Welsh 2003). Therefore, they can significantly influence the coupling of nitrification and denitrification and the degree of nitrogen removal in soft sediment ecosystems (Stief 2013). Loss of key species, such as *A. stutchburyi* and *M. liliana*, could therefore influence ecosystem response to nutrient enrichment, and if denitrification reduces, ecosystem resilience to nutrient enrichment may be lost. Changes in the composition of benthic macrofaunal communities due to increasing sediments and nutrients has been well documented (e.g. Fitch & Crowe 2012, Pratt et al. 2013), but what this means for denitrification and ecosystem resilience is unclear.

1.2 Status of denitrification research

The current literature shows that organic matter loading, water column nitrate concentration, and water column oxygenation are the primary controls on denitrification rates in aquatic ecosystems (Cornwell et al. 1999, Piña-Ochoa & Álvarez-Cobelas 2006). In most aquatic ecosystems, denitrification is coupled to nitrification in the sediments, but as nitrate and organic matter loading increase, conditions may favour direct denitrification of nitrate from the water column (Seitzinger 1988). Most of the aquatic denitrification literature comes from studies

in nutrient enriched northern hemisphere ecosystems, which have likely reached more degraded states than those in Australia and New Zealand, for which there is little data (Seitzinger 1988, Cook et al. 2004b, Gongol & Savage 2016). Comparatively, most New Zealand estuaries are pristine with low organic carbon loading (or loading has only increased in recent history), very low water column nitrate concentrations, and waters that are rarely or never low in oxygen (Thrush et al. 2006, Lohrer et al. 2010, Tay et al. 2012). The dominant (and possibly the only) pathway of denitrification is likely to be coupled to nitrification in the sediments (Cornwell et al. 1999, Gongol & Savage 2016), and the main factors influencing denitrification are expected to be different than those reported in the majority of the literature.

Denitrification is a difficult process to measure, and it is highly variable in space and time (Groffman et al. 2006). As a result, studies have been restricted to small scale field observations, and laboratory or mesocosm experiments that are difficult to extrapolate or use for modelling denitrification at ecosystem or regional scales (Boyer et al. 2006). I have found no examples of denitrification field studies that have been manipulative, and there has been little investigation of how denitrification in estuaries responds to stressors or environmental change (Fulweiler et al. 2008, Oakes et al. 2011, Bruesewitz et al. 2013).

Denitrification is influenced by numerous biotic and abiotic variables, for which individually, many of the effects are well known (see Cornwell et al. 1999). However, because of the nature of how denitrification is measured (i.e. in cores or assays in a laboratory), it is difficult to relate measurements to real world ecological variability such as benthic community composition. Studies investigating the role of the benthic macrofauna for nitrogen cycling have mostly focussed on single species and/or have been laboratory based (Kristensen et al. 1985, Kristensen et al. 1991, Pelegri et al. 1994, Gilbert et al. 1998, Webb & Eyre 2004a).

What is lacking, is the ability to predict denitrification at broad scales using estuarine characteristics and variables (i.e. sedimentary environment, macrofaunal community). Synthesis and use of denitrification measures that incorporate temporal variability and represent the status of the active denitrifier

population (e.g. denitrification enzyme activity, DEA) will add to existing literature that describes more finite controls on instantaneous denitrification rates. Measuring ecosystem functions, such as denitrification, across existing gradients can be used to predict trajectories of change and tipping points that might occur with increasing stressors and environmental degradation (e.g. increasing sediment mud content).

1.3 Approach/Measuring denitrification

There are three main methods for measuring denitrification; the isotope pairing technique, membrane inlet mass spectrometry, and denitrification enzyme activity (DEA) assays using acetylene inhibition (Seitzinger et al. 1993). The two former methods provide accurate quantification of actual denitrification rates but would be too time consuming and expensive for the field experiments and sampling programs undertaken in this PhD. The acetylene inhibition technique works through the inhibition of N_2O reduction to N_2 by acetylene (C_2H_2) making it the end product of denitrification, which is a much easier compound to measure than atmospherically abundant N_2 (Yoshinari et al. 1977, Groffman et al. 2006). This method has been widely and successfully used in denitrification studies but is not without criticism. Acetylene also inhibits the nitrification pathway which in the real world provides a source of nitrate to the denitrification process, and therefore it underestimates actual denitrification rates (Groffman et al. 2006). Denitrification in the estuaries studied in this thesis were likely to be coupled to nitrification in the sediments, and substrate amendments were necessary to detect rates. I therefore conducted DEA assays with unlimited nitrate and carbon amendments, a method sometimes referred to as denitrification potential (Smith & Tiedje 1979).

DEA assays provide a measure of the denitrification that would occur under optimal conditions (i.e. anoxic, with unlimited nitrate and carbon, and constant mixing), but without allowing for new enzyme growth. Capturing realistic and representative measures of denitrification is difficult because it is highly variable spatially and temporally, but the DEA method provides an integrative measure of the denitrification history of the sediments (that is, the duration (usually weeks to months) that the sediments have experienced conditions for denitrification). The

DEA method has been shown to be a successful measure of spatial and temporal variability in denitrification (Groffman et al. 1999), and a number of studies have successfully used it at an ecosystem scale (Livingstone et al. 2000, Wigand et al. 2004, Magalhães et al. 2005, Bruesewitz et al. 2011). DEA was used throughout this thesis as a proxy for denitrification, a tool to compare denitrification across different estuaries, and to examine the factors that govern nitrogen removal. This method was the most appropriate because it permits large sample sizes, which increased replication in experimental studies and enabled analysis of correlation with other physical and biological variables, and it also allowed comparison of denitrification across estuaries.

From a management perspective, research is required to facilitate ecosystem service mapping of denitrification, inform landscape scale nitrogen budgeting and modelling, and to initiate monitoring of denitrification in New Zealand estuaries. This calls for studies of denitrification at estuary or regional scales that can reveal patterns and generalities in where denitrification occurs, and what environmental factors controls it. Denitrification is an ecosystem service that can help to mitigate nitrogen enrichment, but response of denitrification to enrichment in estuary sediments, and what controls it, has not been demonstrated before this thesis. In order to fill these research gaps, I needed to conduct surveys of denitrification across broad environmental gradients, and field based manipulative experiments encompassing wide variability in abiotic and biotic variables.

Denitrification is highly variable in space and time, as are soft sediment ecosystems, and research that encompasses this natural heterogeneity is necessary to generate 'big-picture' conclusions that are relevant to managers (Thrush & Lohrer 2012). This thesis is unique because it presents studies testing the response of denitrification to stressors, especially nitrogen enrichment, in situ. A new technique for simulating nutrient enrichment in the field was developed, but manipulating key stressors biodiversity loss and increasing sediment mud content are more difficult in a field setting. By using natural environmental gradients as sites for enrichment experiments I could concurrently assess the influence of local biotic and abiotic variables (especially stressors) on denitrification, and on denitrification response to enrichment. In Chapter 3 I used

a gradient in sediment mud content to assess effects of sedimentation stress, and in Chapter 4 I used a gradient in macrofaunal community composition to assess effects of biodiversity loss or changing community composition. Conducting manipulative field experiments across environmental gradients encompassing real world complexity increases the generality of results (Hewitt et al. 2007, Snelgrove et al. 2014), and this approach is new to denitrification research.

1.4 Thesis overview

The main body of this thesis comprises four research chapters collectively aiming to broaden our understanding of nitrogen enrichment and denitrification in estuarine soft sediment ecosystems; a field nutrient enrichment method development (Chapter 2), two manipulative field experiments (Chapters 3 & 4), and a synthesis of ambient DEA values from five studies across four estuaries (Chapter 5) (Figure 1.2). This thesis aims to provide a greater understanding of the fate and effects of nitrogen in estuaries, and examine how denitrification in estuaries may change with changes that may occur with ecosystem stress. Specifically, increasing sediment mud content, increasing pore water nutrient concentrations, and changes in macrofaunal community.

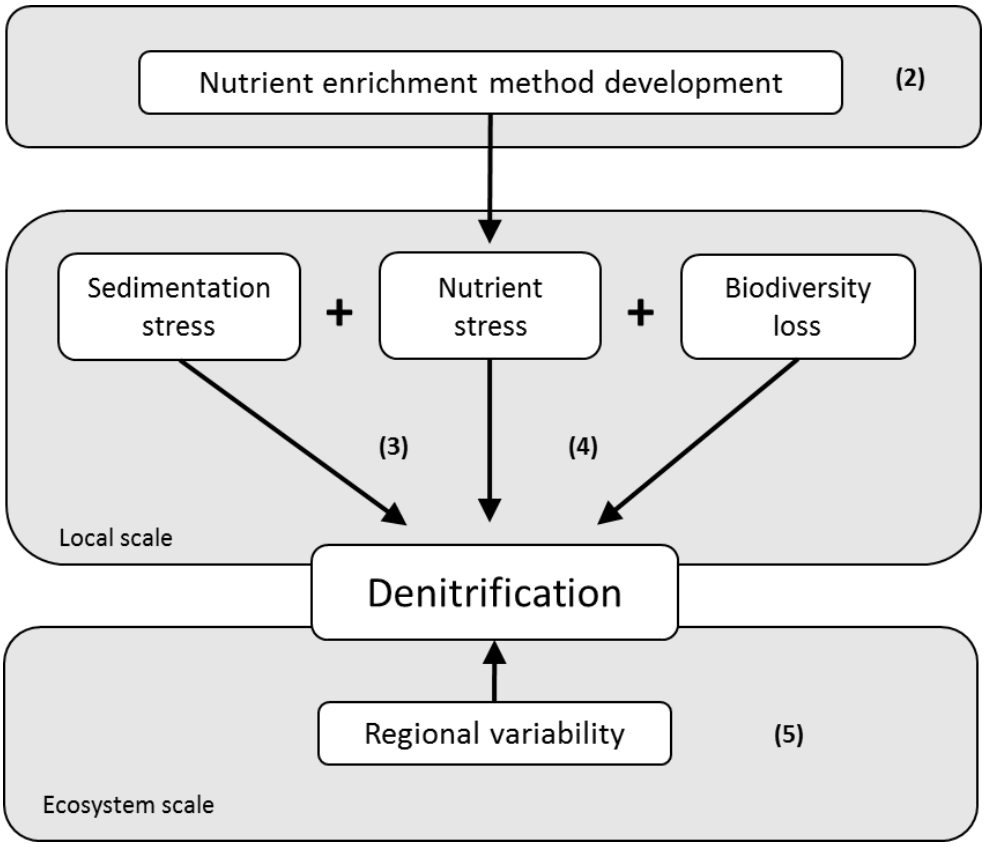

Figure 1.2 Diagram illustrating the progression of thesis chapters; (2) development of an in situ nutrient enrichment technique, manipulative studies addressing the effects of (3) nutrient stress and sedimentation stress, and (4) nutrient stress and biodiversity loss (or changing community composition) on denitrification, and (5) investigation of factors influencing variability in denitrification across estuaries. Numbers indicate the chapters where each component is addressed.

1.4.1 Chapter 2

In order to test ecosystem response to nutrient enrichment in the field, I required a simple technique that increased pore water nutrient concentration for several weeks. A methodological gap existed in the literature, therefore I developed and tested a new in situ sediment nutrient enrichment method. The method significantly increased pore water ammonium concentrations to levels representative of enriched estuaries globally, and revealed the local environmental and biological factors that control the level of enrichment.

Objectives:

1. To develop a method for simulating eutrophication in intertidal soft sediments in the field, which can be used in field experiments with low cost and time requirements.
2. To qualify the local variables that control enrichment level.

1.4.2 Chapter 3

To understand how estuary sedimentation influences ecosystem functioning, especially nutrient processing and denitrification, I conducted an enrichment experiment across an existing sedimentary gradient (0 – 24% mud) on an intertidal flat. After 6 weeks of enrichment I measured DEA, and ecosystem functions using benthic chamber incubations. I compared macrofaunal community, microphytobenthic biomass, environmental variables and ecosystem functions (community metabolism, primary productivity, nutrient processing, and DEA) in control and enriched plots. This study enabled me to demonstrate differences in responses associated with increasing sediment mud content (direct and indirect effects), and the factors that contribute to the resilience of ecosystem functions under nutrient stress.

Objectives:

1. To investigate how DEA and benthic ecosystem functioning vary across sedimentary gradients.
2. To quantify the response of DEA and benthic ecosystem functioning to sediment nutrient enrichment.

3. To investigate if sedimentary environment governs that response; ie. Is there a multiple stressor effect of sedimentation and nutrient enrichment?

1.4.3 Chapter 4

This study was part of a large in situ enrichment experiment across a sandflat with a heterogeneous landscape of macrofaunal community composition. The study was designed to investigate how abundance and diversity of macrofauna, within a functional trait group associated with nutrient processing, influence ecosystem function (especially DEA) response to nutrient enrichment. I showed that macrofauna were integral to DEA response to nutrient enrichment, and that different elements of diversity are important at different levels of stress.

Objectives:

1. To investigate the importance of benthic macrofaunal community composition for denitrification response to medium and high levels of sediment nutrient enrichment.

1.4.4 Chapter 5

This study combined data from three estuary surveys (one subtidal and two intertidal), and ambient values from the two field experiments (Chapters 3 & 4). The dataset (n = 134) represented a range of DEA values, measures of sediment properties, microphytobenthic biomass, and macrofaunal community composition. The sedimentary environment ranged from very clean coarse sands (0% mud), to cohesive, organic rich sediments with up to 52% mud content. With this dataset, I showed generality in the biotic and abiotic factors responsible for variability in DEA across multiple estuaries, and demonstrated a possible threshold in DEA with increasing sediment mud content.

Objectives:

1. To investigate the drivers of denitrification across multiple estuaries.

Chapter Two

In situ soft sediment nutrient enrichment: A unified approach to eutrophication field experiments

2.1 Introduction

Nutrient processing is deemed one of the most valuable ecosystem services globally and the majority of this occurs in coastal soft sediments (Costanza et al. 1997). This ecosystem service influences the supply and flux of nutrients within and between marine habitats and through denitrification in particular, can alleviate problems such as the loss of ecosystem functionality and biodiversity associated with excess nutrients. Indeed, excessive nutrient loading and eutrophication are stressing coastal marine environments throughout the world (Levin et al. 2015). The overabundance of nitrogen in particular (the nutrient usually limiting production (Herbert 1999, Howarth & Marino 2006) causes changes in biomass, structure, and functioning of coastal communities and food webs (Abreu et al. 2006, Howarth et al. 2011, Rabalais et al. 2014). Yet, despite being of paramount importance to global environmental wellbeing, nutrient processing in soft sediments is still poorly understood and response to perturbations are rarely tested experimentally in situ. Reliable techniques are needed to empirically test the effects of excess nutrients, and its interactions with other stressors in real world settings that embrace ecological complexity, and thereby allow broad scale inferences regarding response to change (Snelgrove et al. 2014).

Fertilisers have commonly been used to test the effects of increased nutrient loading on marine soft sediment habitats, but methodological development has been haphazard making cross-study comparisons near impossible. I extended the review of Worm et al. (2000) to include the recent literature, and found 47 enrichment studies conducted in intertidal and subtidal habitats (Appendix 1).

Approximately half of the studies tested nutrient limitation and growth in macrophytes (mainly seagrasses), and half examined nutrient enrichment effects on benthic communities and food webs. Slow release fertilisers, such as Osmocote®, were used in 33 of 47 (70%) studies, but these fertilisers varied considerably in their elemental makeup. Similarly, studies had a very wide range of application rates (between 3 and 750 g N m^{-2} (Figure 2.1); while some were based on previously published experiments or site-specific pilot studies (25 of 47), in more than 50% of studies application rates were not justified (27 of 47). Applications of fertiliser to surficial sediments were common; in 53% of studies additions were < 5 cm deep, and in many studies (36%) only the top 1 cm of sediment received fertiliser. Moreover, in only 20 of 47 studies were enrichment levels (i.e. realised treatment effect) on sediment nutrient pore water concentrations reported. Relative increases in pore water nitrogen concentrations (effect sizes) in these 20 studies ranged from 7-352 times ambient levels (Figure 2.1) but enrichment level comparisons are difficult to make because the depth of sampling (0-20 cm) was not standardised. These inconsistencies and methodological limitations indicate a need for a more informed approach to enrichment experiments that justifies fertiliser application rates, and improves understanding of the factors that may influence the resulting pore water nutrient concentrations.

Firstly, when planning manipulative field or mesocosm experiments it is useful to consider potential enrichment levels for a given application rate to avoid unrealistically high or undetectable pore water nutrient concentrations. Secondly, Worm et al. (2000) showed that enrichment level (i.e. pore water nutrient increase) could not be predicted by the initial fertiliser application rate, time since application and application depth using multiple linear regression analysis of literature studies (overall $r^2 = 0.07$, $P = 0.53$, $n = 34$). We repeated this analysis on the larger set of literature and revealed a similar result ($r^2 = 0.01$, $P = 0.92$, $n = 48$). The implication is that local environmental variables and variability in methods may strongly affect the enrichment level. I also note that previous studies have frequently overlooked co-variables or failed to assess their influence on the nutrient treatment.

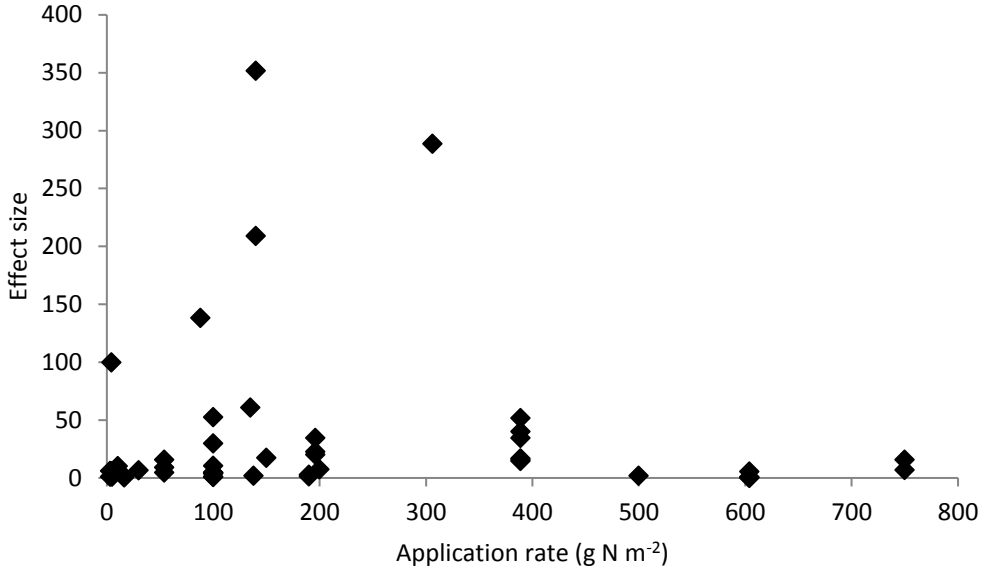

Figure 2.1 Effect size of enrichment treatment (relative to ambient pore water nitrogen concentration) as a function of fertiliser application rate in the 20 studies for which such data were reported (Appendix 1).

Marine soft sediment ecosystems vary greatly in their physical and biological makeup, and consequently their biogeochemical processes (Braeckman et al. 2014). For example, sediment properties are important to consider in studies of benthic nutrient cycling since these influence diffusion and solute transport (e.g. Blackburn & Henriksen 1983, Huettel et al. 2003, Glud 2008, Hohaia et al. 2013), as well as macrofauna behaviour and ecosystem functioning (Lohrer et al. 2004b, Woodin et al. 2012, Pratt et al. 2013). Benthic macrofauna are known to influence nitrogen cycling (Aller 1988, Kristensen et al. 1991, Laverock et al. 2011), and the presence of macrophytes and microphytobenthos are also expected to influence pore water nutrient concentrations and the level of experimental enrichment. The majority of enrichment experiments have been conducted in vegetated sediments (28 of 47) and only 10 of the 19 studies conducted in un-vegetated sediments reported significant increases in pore water concentrations (Appendix 1). My literature review shows that there is insufficient information for researchers designing enrichment experiments in un-vegetated sediments, and that there is a need to experimentally assess the role of habitat and biological processes in ameliorating pore water nutrient concentrations.

This study develops protocols that are simple and cost-effective for in situ nitrogen enrichment experiments. The method was developed based on the published literature and a recent intertidal sandflat experiment that encompassed a wide range of sediment types, macrophyte coverage, and variations in benthic macrofauna community composition (Table 2.1). The study design allowed me to document the degree to which surface and sub-surface sediment pore water nitrogen concentrations were elevated as a function of fertiliser application rate and time since application, in relation to environmental variables to serve as a guide for future studies.

Table 2.1 Sediment properties and macrofauna variables after 7 weeks of enrichment as a function of fertiliser application rate. Values are medians with minimum and maximum in parentheses (n = 28).

Variable	Control (0 g N m^{-2})	Medium (150 g N m^{-2})	High (600 g N m^{-2})
Sediment properties			
Seagrass (% cover)	16 (0-84)	20 (0-97)	21 (0-75)
OC (%)	0.9 (0.6-2.0)	0.9 (0.6-2.0)	1.0 (0.6-1.8)
Mud (% <63 µm)	1.78 (0-15)	0.62 (0-14)	0.42 (0-12)
GSM (µm)	215 (177-241)	220 (182-242)	219 (190-250)
Chl-*a* (µg g^{-1} sediment)	9.3 (3-23)	10.0 (5-32)	9.5 (5-28)
Macrofauna			
S (taxa $core^{-1}$)	26 (11-38)	23 (7-40)	26 (11-45)
N (n $core^{-1}$)	107 (19-419)	58 (8-345)	62 (22-574)
H'	2.4 (1.1-3.1)	2.4 (1.6-3.0)	2.4 (1.1-3.0)

OC = sediment organic content, Mud = sediment mud content, GSM = Grain size median, Chl-*a* = chlorophyll *a* content, S = number of species, N = number of individuals, H' = Shannon diversity

2.2 Methods

2.2.1 Experiment setup

A large scale nitrogen enrichment experiment was set up on a 300,000 m^2 area of intertidal sand flat on the Tapora Bank in the Kaipara Harbour, northern New Zealand (36° 39' S, 174° 29' E) on 20 January 2014 (Appendix 2). The study area is composed mostly of permeable sediments of varying mud (particle size < 63 µm) content (Table 2.1), and is subject to tidal flushing, wind waves, and run off from a mostly agricultural catchment. Treatment plots (1 m x 1 m) consisting of control (no addition), medium (150 g N m^{-2}) and high (600 g N m^{-2}) nitrogen enrichment were established at 28 sites (each in a 5 x 5 m area) across the study area. These application rates were based on the median and upper quartile values from the literature review (Appendix 1). We used Nutricote® N (70 d, 40-0-0 N:P:K), a controlled release coated urea fertiliser containing no phosphorus, potassium or trace elements. A nitrogen-only fertiliser was used since it is typically the limiting nutrient in these systems, and urea quickly hydrolyses to ammonium (NH_4^+) (Lomstein et al. 1989), the most common form of nitrogen in New Zealand estuaries (Tay et al. 2013).

Fertiliser was applied to each plot in a series of 20 evenly spaced 3 cm diameter 15 cm deep holes made in the sediment using a hand held corer. Each hole received an equal volume of fertiliser (which covered approximately 5-15 cm depth range within the sediment profile), and the intact sediment core plugs were replaced immediately to minimise disturbance to the sediment. For less cohesive sediments, an outer core sleeve was used to prevent holes from infilling while fertiliser was added. Control plots were similarly cored and received an equal volume (as the high treatment) of pea gravel of similar diameter to the fertiliser pellets. With this method I was able to establish 84 1 m^2 experimental plots across a 300,000 m^2 study site in one low tide (4-5 h) with a team of six people. In a preliminary study, this technique provided even elevation of pore water NH_4^+ throughout a 1 m^2 plot (1.3-2.0 fold variation in concentration between the plot centre, edge and halfway in between) when sampled four weeks after application, with enrichment effects undetectable 0.5 m beyond the plot boundary.

2.2.2 Sampling

Samples were collected four weeks (25 February 2014, pore water and sediment properties) and seven weeks (17 March 2014) after the fertiliser addition (pore water, sediment properties, macrofauna). Sampling times were chosen to allow enough time for the system to respond (based on my literature review and pilot study), and were within the 70 d release period of the fertiliser. Replicate, randomly placed sediment cores (2.6 cm dia.) from each plot were pooled and homogenised for analysis of sediment properties (n = 5, 0-2 cm depth) and pore water nutrients (n = 4, 0-2 cm and 5-7 cm depths, separated). Sediment samples were kept in the dark and transported on ice to the laboratory. At the end of the experiment, two cores (13 cm dia., 15 cm depth) were collected near the centre of each plot for analysis of the benthic macrofaunal community. Cores were sieved on a 500 µm mesh, preserved in 50% iso-propyl alcohol, and stained with Rose Bengal. All organisms were counted and identified to the lowest possible taxonomic level (usually species). The average of 2 cores was used so that there was 1 macrofaunal replicate per plot.

In the laboratory, pore water was extracted immediately by centrifuge and filtered (1.1 µm, Whatman GC glass fibre filters) prior to freezing (-20°C) (Lohrer et al., 2010). Pore water samples were later analysed for NH_4^+ using a Lachat QuickChem 8000 Series FIA+ (Zellweger Analytics Inc. Milwaukee, Wisconsin, 53218, USA) using standard operating procedures for flow injection analysis. Sediment samples were frozen at -20°C until analysis. Particle grain size was measured after removal of organic matter with 10% hydrogen peroxide, using a Malvern Mastersizer 2000 (particle size range 0.05 – 2000 µm) (Singer et al. 1988). Sediment organic matter content was determined by weight loss on ignition of dry sediments (550°C for 4 hours) according to Parker (1983). Chlorophyll *a* (Chl *a*) was extracted from freeze-dried sediment in 90% acetone, then fluorescence of samples was measured using a Turner Designs 10-AU flourometer (Arar & Collins 1997). Prior to sampling, photographs of 0.25 m^2 in the centre of each plot were taken and a random point count method used to estimate seagrass (*Zostera muelleri* Irmisch ex Asch.) coverage (%) (see Kohler & Gill 2006).

Summary statistics and univariate tests were carried out using STATISTICA version 11 (StatSoft Inc. 2012) after first identifying and removing outliers (n < 5 per treatment). When processing pore water samples, some cores were contaminated with fertiliser pellets, these were further identified as outliers in the analysis and were removed. Paired *t*-tests were used to test for differences in pore water NH_4^+ concentration between depth strata four and seven weeks after enrichment. Multivariate analyses were conducted using PRIMER 7.0 PERMANOVA+ (Clarke & Gorley 2015). A Euclidean distance matrix was generated using log (x + 1) transformed pore water concentrations from both depth strata. This matrix was then used to run a repeated measures permutational multivariate analysis of variance (PERMANOVA) to test the effects of application rate (fixed factor, 3 levels), sample time (fixed factor, 2 levels) and their interaction on multivariate pore water NH_4^+ concentration, plot was treated as a random factor (84 levels) nested within treatment. Post-hoc PERMANOVA pairwise *t*-tests were used to identify where significant treatment and time effects occurred.

To investigate whether measured environmental variables (Table 2.1) could explain variations in pore water NH_4^+ concentration, a separate Euclidean distance matrix of raw pore water concentration data (using both depth strata) from week seven was generated for each treatment. Distance-based linear models (DistLM) were run on the matrices to determine which variables were correlated with pore water NH_4^+ concentrations (e.g. as in (Pratt et al. 2015)). This multiple regression analysis uses permutation and does not assume normality, so data were left untransformed because I wanted to retain heterogeneity (and transformations did not change results). Predictor variables were however standardised (between 0 and 1) to account for differences in the magnitude and range of units. Marginal tests were used to identify individually significant correlations with pore water concentration, followed by a backwards elimination procedure, using the corrected Akaike information criterion (AICc) to select the best individual or combination of variables. AICc was the most the appropriate selection criterion since the sample size was small relative to the number of variables (Burnham & Anderson 2002).

2.3 Results

The technique successfully elevated pore water NH_4^+ concentrations for the duration of the seven week experiment, with the depth-averaged medium and high treatments respectively 1-110 and 4-580 times greater than ambient conditions (Figure 2.2). These ranges are near to (medium treatment) or greater than (high treatment) the range of values from reviewed studies using application rates between 3 and 750 g N m^{-2} (Figure 2.2). Despite high within treatment variability, there was a highly significant effect of fertiliser application rate on pore water NH_4^+ concentration (depth strata combined), and post-hoc tests revealed significant differences between all treatment levels (Table 2.2). There was also a weakly significant effect of sample date, with pore water NH_4^+ concentrations higher in week seven than week four, although plots within specific treatments did not all respond temporally in the same way (i.e. the significant plot nested in treatment effect). The lack of a significant treatment x time interaction indicates that the temporal increase in pore water NH_4^+ concentrations was a general site phenomenon, and not related solely to changes in release rate in fertilised plots. Four and seven weeks after enrichment, both fertiliser treatments showed higher pore water NH_4^+ concentrations in deeper sediments (5-7 cm) than surface sediments (0-2 cm) (paired *t*-tests $p < 0.01$). Ambient (control plot) NH_4^+ concentrations were also higher in deeper than shallower sediments although the differences were not as pronounced (paired *t*-tests $p < 0.06$; Figure 2.2).

Sediment properties and macrofaunal community characteristics varied widely across the experimental area (Table 2.1), but none of these variables were significantly correlated with pore water NH_4^+ concentration in the control and medium addition plots (Table 2.3). However, in the high addition treatment pore water NH_4^+ concentration was negatively correlated with distance from shore, organic and mud content, seagrass coverage, and benthic macrofauna diversity (Table 2.3). Sediment Chl *a* content was the only variable positively correlated with pore water NH_4^+ concentration. The most parsimonious model of pore water concentration in the high treatment included Chl *a* and number of macrofauna taxa, which collectively explained 42% of the total variation.

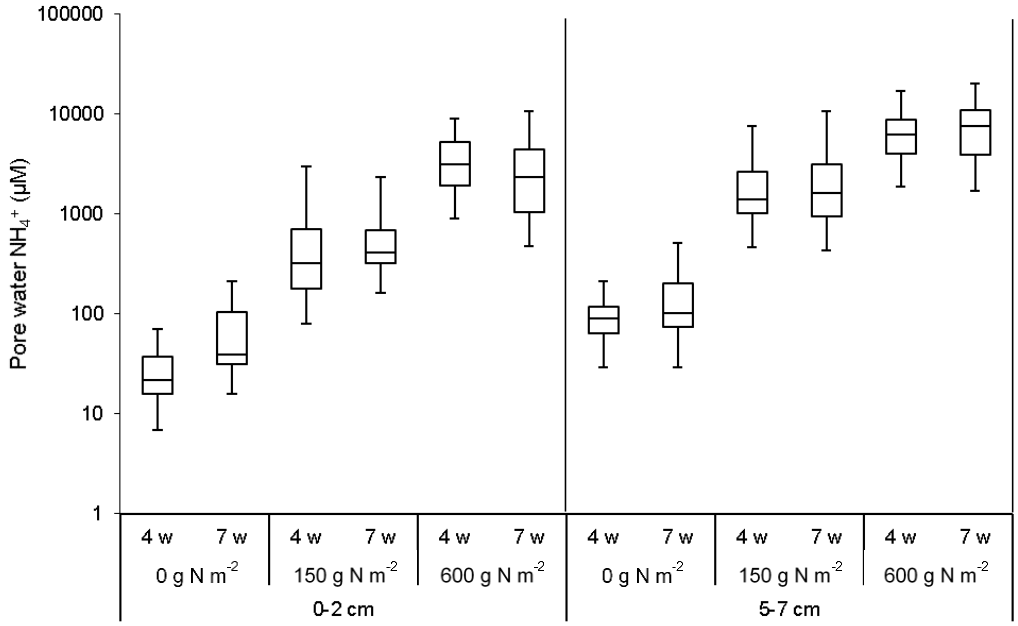

Figure 2.2 Sediment pore water NH_4^+ concentration as a function of time since fertiliser application (4 and 7 weeks), application rate (0, 150, 600 g N m^{-2}) and sample depth (0-2 and 5-7 cm). Boxes represent 25%, median and 75% distributions, with whiskers the non-outlier minimum and maximum (n=28). Note log_{10} scale of y-axis.

Table 2.2 Results of a repeated measures PERMANOVA comparing pore water NH_4^+ concentration as a function of fertiliser application rate (treatment) and sample date (time). The PERMANOVA was based on Euclidean distance of $log_{10}(x + 1)$ pore water concentrations at 0-2 and 5-7 cm depth. Post-hoc pair-wise tests are given for significant treatment effects.

Source	df	MS	Pseudo-F	Perm-*p*	Post-hoc
Treatment	2	503	162	0.001	C < M < H
Time	1	9.68	4.82	0.021	4w < 7w
Plot (Treatment)	81	3.09	1.54	0.012	
Treatment x Time	2	1.51	0.75	0.547	
Residual	81	2.01			

Treatments: C = 0, M = 150, H = 600 g N m^{-2}

Time: 4w = 4 weeks, 7w = 7 weeks

Table 2.3 Predictors of pore water NH_4^+ concentration as a function of fertiliser application rate after seven weeks.

Treatment	Variable	Pseudo-*F*	Prop.	Full model
0 g N m^{-2}	no individually significant predictors			
150 g N m^{-2}	no individually significant predictors			
600 g N m^{-2}	Distance to shore	5.42	0.20* (-)	
	OC	4.76	0.18* (-)	
	Mud	2.99	0.12 n.s. (-)	
	Chl *a*	**2.94**	**0.12 n.s. (+)**	**16 %**
	Seagrass	5.70	0.21** (-)	
	S	**7.84**	**0.26** (-)**	**30 %**
	H'	7.93	0.26** (-)	
			Total	**42 %**

Prop. is the proportion of variation explained and direction of correlation is given in parentheses. Variables in bold were those included in the best DistLM model of pore water concentration, and full model indicates the proportion of explained variance attributed to each. Variable abbreviations are given in Table 2.1. * $p \leq 0.05$, ** $p \leq 0.01$.

2.4 Discussion

In order to conduct experiments that simulate realistic eutrophic sedimentary conditions, an adequate nutrient application technique is required together with a benchmarked application rate to achieve the desired level of enrichment. Since the Worm et al. (2000) review 18 years ago there has not been sufficient improvement in methodology available in the literature to help plan enrichment experiments. I developed a technique to enrich intertidal sediments in one application, without disturbing the entire sediment profile, which can supply nutrients for at least seven weeks. This technique provides an even spread of nutrient concentrations throughout a 1 x 1 m^2 plot minimising nutrient gradients. This method is simple and cheap, can be used for both long and short-term enrichment experiments, and allows high levels of replication. Fertiliser pellets appeared intact after 7 weeks, and I expect that enrichment would have continued for at least 70 d (manufacturers estimated release period). Longer term experiments could consider using fertilisers with slower release rates to avoid repeat applications (e.g. Nutricote® N 140 d). It proved easy to use in a range of intertidal sediment types and could also be applied in other aquatic soft sediment environments, including sub-tidal and lake sediments with the use of SCUBA. Subtidal applications would be made easier with the use of fertiliser packets such as mesh bags, however biodegradable materials are recommended to avoid retrieval. The use of a dual core (i.e. an inner and outer core sleeve) may be required to prevent holes infilling and to ensure fertiliser is buried to the required depth. I recommend for all aquatic deployments workers verify that their chosen fertiliser is negatively buoyant and bury it to a depth beyond the expected mobile sediment layer.

Fertiliser type, application rate, and depth need to be carefully considered in terms of the study aims, duration, and receiving environment. I observed high variability in the enrichment level and despite measuring a large number of site specific environmental variables, much of this could not be explained. My enrichment levels tended to be higher than those measured in other studies, which could be due to shallow enrichment techniques and/or differences in pore water sampling and monitoring used in other studies (Appendix 1). Worm et al. (2000) emphasised

the importance of careful pore water sampling during experiments to be sure of a consistent and quantifiable enrichment level. A standardised sampling technique is also required since concentrations of nitrogen species typically change throughout the sediment profile (Vanderborght & Billen 1975, Zhang et al. 2013). Depending on the depth sampled, the values obtained could be very different to the desired level; in this study enrichment levels were greater in deeper than in surface sediments (Figure 2.2). Sampling the surface sediments may mean the measured enrichment is very low or undetectable, and sampling too deep may render values that are unrepresentative of the active benthos layer. Therefore, I recommend targeting a specific sediment profile area of importance to the study, and/or pooling across sediment depths which integrates the variability in enrichment level throughout the sediment profile, reduces the amount of samples to analyse, and gives more general, comparable values.

My literature review showed that many studies (53%) applied fertiliser to surface sediments (≤ 5 cm depth), mimicking eutrophication effects from the water column, but not the long term impacts of eutrophication on sediment pore water. Surface sediments are more likely to be influenced by water column hydrodynamics and pore water advection processes (reviewed by Santos et al. 2012) which may speed up nutrient release from the fertiliser. My method enriched the sediment profile at least from 0-7 cm depth, and is likely to elevate NH_4^+ availability at the sediment water interface. This zone includes the rhizoshere of seagrasses, and the layer of most macrofaunal activity in marine soft sediment habitats (Gilbert et al. 1998, Teal et al. 2008). The elevated pore water concentrations that this method delivered are equivalent to the concentrations that are measured in enriched estuaries globally (Appendix 3), simulating the long term effects of eutrophication. Unlike this method, in situ water column or surface sediment enrichment methods cannot produce this effect due to dilution and high variability in sediment-water coupling.

Many physical and biological factors influence the level of nutrient enrichment, as well as the type and severity of consequences to an ecosystem's functioning. Nutrient cycling and efflux from the sediments are influenced by the sedimentary environment (Blackburn & Henriksen 1983, Glud 2008, Santos et al. 2012), benthic

macrofauna (Bertics et al. 2010, Laverock et al. 2011), microphytobenthos (Marzocchi et al. 2018), and macrophyte communities (Kenworthy et al. 1982). These results show that primary consideration should be given to benthic macrofauna and sediment properties when estimating potential enrichment levels of experiments. In heterogeneous environments, researchers should consider the interactions and variability of site environmental and biological variables and their influence on enrichment levels. This is particularly important for studies of biological community response to enrichment. If researchers wish to achieve a specific level of enrichment, especially for studies encompassing environmental variability, a pilot study is recommended so that application rates can tailored to achieve the desired pore water concentrations and reduce variability.

In order to meaningfully progress eutrophication and nutrient cycling research, more in situ experimentation is needed. An important outcome of this work is that the same application rate can achieve very different enrichment levels even within a single habitat; I measured high variability in enrichment level across a sandflat at a scale < 1 km. This scale of variability reflects real-world complexity and should be incorporated into future experiments in order to increase generality and application of conclusions. The way to achieve this is through well replicated gradient designs that consider co-variables (Eberhardt & Thomas 1991, Thrush et al. 1997, Hewitt et al. 2007, Ellis & Schneider 2008). Many of the reviewed nutrient enrichment studies had research questions that required categorical type designs; the majority (68%) used only a single fertiliser application rate, the average number of treatment replicates was just five, and more than half the studies (57%) were conducted across spatial scales much less than 1 km (Appendix 1). Although these past studies represent an invaluable body of work, it would be complemented by experiments conducted across environmental gradients and larger spatial scales. Combining in situ assay techniques (such as sediment nutrient enrichment), with novel interaction network approaches to data analysis will provide valuable ecological tools for studies of multiple stressor effects, ecosystem resilience, and tipping points in real world settings (Thrush et al. 2014). Using previously employed methods this seems unachievable and expensive in

time and money. We have shown that such experiments can be conducted relatively easily with a simple technique that:

1. can be used for a highly replicated experiment across a large area,
2. delivers nutrient enrichment for at least seven weeks that scales with application rate,
3. requires only one initial set up,
4. has no need to build or install special diffusion devices, and is inexpensive in time and money.

Chapter Three

Sedimentary environment influences ecosystem response to nutrient enrichment

3.1 Introduction

Nutrient enrichment and sedimentation are among the primary stressors for coastal ecosystems globally (Levin et al. 2001, Hewitt et al. 2016, Sinha et al. 2017). Estuarine soft sediment ecosystems are often described as nitrogen sinks due to their high rates of nitrogen processing and ability to naturally reduce bio-available nitrogen via denitrification (Seitzinger 1988). Denitrification may play a fundamental role in ecosystem resilience to the oversupply of nitrogen but its ability to do this may be influenced by changes in the sedimentary environment. Nitrogen enrichment and sedimentation often occur in unison during periods of elevated rain runoff, and while it is clear that the 'muddying' of estuaries can negatively affect macrofaunal diversity and ecosystem functions (Pratt et al. 2013), we do not know what this means when compounded with other stressors, particularly increased nutrients. The interactive effects of these two key stressors for coastal ecosystems have rarely been investigated in a field setting (see O'Brien et al. 2009 for an exception) but such research is needed to better inform management with respect to limit setting (Chapman 2016, Hewitt et al. 2016).

Benthic macrofaunal communities and ecosystem functions can be affected by increases in mud content (Thrush et al. 2003a, Lohrer et al. 2004b, Rodil et al. 2011, Robertson et al. 2015) or nutrient enrichment in soft sediment habitats (Morris & Keough 2003b, Posey et al. 2006, Fitch & Crowe 2012). The physical and biogeochemical properties of estuary sediments change with increasing mud content (Lohrer et al. 2004b, Cummings et al. 2009) stemming from greater cohesiveness and less permeability. These sediment characteristics influence rates of pore water diffusion and solute exchange (Blackburn & Henriksen 1983, Huettel et al. 2003), ammonium (NH_4^+) adsorption (Mackin & Aller 1984), the surface area

available for microbial processes (Huettel et al. 2014), and can alter the activities and functional roles of resident macrofauna (Jones et al. 2011, Needham et al. 2011). These factors all influence processes of organic matter breakdown, community metabolism, primary production and nitrogen cycling, including denitrification (Blackburn et al. 1993, Gilbert et al. 2003, Gongol & Savage 2016).

Denitrification is carried out by heterotrophic microbes in anoxic sediments and requires both organic carbon and a source of nitrate (NO_3^-). Nitrification (microbial conversion of NH_4^+ to NO_3^-) occurs in the presence of oxygen (i.e. in the oxic sediment layer), thus the oxic-anoxic interface is an important site for coupled nitrification-denitrification. Organic matter provides an NH_4^+ source for nitrification, and its breakdown (by microbes) alters the distribution and availability of oxygen in the sediments and therefore sites for coupled nitrification-denitrification (Kemp et al. 1990, Caffrey et al. 1993). In permeable sediments, pore water advection weakens the coupling between nitrification and denitrification, and can reduce denitrification (Kessler et al. 2012). However, muddy organic rich sediments may also limit coupled nitrification-denitrification due to a reduced interface between the oxic and anoxic layers. Also, muddy sediments have a thinner oxic layer which limits nitrification, meaning NH_4^+ is more prevalent than NO_3^- in muddy sediments. Due to the lower permeability of mud, macrofaunal activities (bioturbation, bioirrigation) are important for exchange of solutes (oxygen, NH_4^+, NO_3^-) facilitating nitrogen transformation processes including nitrification and denitrification (Aller 1988). Sediments with higher mud (and organic) content are therefore expected to have differing rates of biogeochemical ecosystem functions, and differences in resilience to nutrient stress.

Field experiments across existing environmental gradients can be used to forecast ecosystem response or change under scenarios of different levels of a stressor, or stages of degradation (Pickett 1989, Thrush et al. 2003b, e.g. Pratt et al. 2013, Villnäs et al. 2013, Norkko et al. 2015). This approach has successfully been used to demonstrate effects of sedimentation on ecosystem functioning (e.g. Pratt et al. 2013), but there is an underutilised opportunity to use gradients to investigate multiple stressor effects such as mud and nutrients by manipulating one factor

across a natural gradient of the other stressor. In this study I investigated whether elevated fine sediments in an estuary sandflat (one type of stressor) would influence the ecosystem's response to increased nutrient supply (a second type of stressor). I did this using a nutrient enrichment experiment across a natural gradient of sedimentary grain size on an intertidal sandflat, measuring proxies of ecosystem function (denitrification activity, primary production and community metabolism) after a six week period of enrichment. Due to the differences in biogeochemical properties across the sedimentary gradient I expected changes in microphytobenthic biomass, macrofaunal community structure, and ecosystem function, and consequently differences in response to nutrient enrichment. Muddy sediments typically have reduced macrofaunal biodiversity and levels of ecosystem functioning (Thrush et al. 2004, Pratt et al. 2013), and I anticipated that this would mean less resilience (measured as maintenance of ecosystem function) to nutrient enrichment. In other words, I expected greater reductions in ecosystem functions in response to nutrient enrichment in areas with more mud content.

3.2 Methods

3.2.1 Experimental design/setup

An in situ sediment enrichment experiment was set up in Tuapiro estuary, Tauranga Harbour, north-eastern New Zealand (37° 29.445' S, 175° 57.007' E) in late October 2014 (austral spring). The study site encompassed a sedimentary gradient (0 - 24% mud) within a 300 x 100 m area of mid intertidal flat (Appendix 4). Differences in hydrodynamics, tidal inundation, and elevation across the study area were negligible. Duplicates of procedural control and enrichment plots (1 x 1 m) were set up in 12 locations (24 plots per treatment) to maximise the range of sediment grain size encompassed in the experimental design. Sediment enrichment was achieved using slow release (70 d) nitrogen only fertiliser (Nutricote® N 70 d, 40-0-0 N:P:K, application rate 150 g N m^{-2}) buried in the sediments in 20 evenly spaced core holes (3 cm diameter, 15 cm depth). This application rate was chosen because it was known to elevate NH_4^+ concentrations representative of eutrophic estuaries, without having adverse effects on the macrofaunal community (Douglas et al. 2016, Douglas et al. 2017, Chapters 2 & 4).

Equal volumes of fertiliser (or pea gravel for procedural disturbance controls) were placed in each hole, and the intact core plug was replaced immediately to minimise sediment profile disturbance (see Douglas et al. 2016, Chapter 2 for more detail).

Chamber incubations, followed by sediment sampling, then macrofaunal sampling were conducted after 6 weeks of enrichment, in late November 2014 (early summer). This period was based on a previous enrichment study that showed elevated pore water NH_4^+ concentrations for 7 weeks using this technique (Douglas et al. 2016, Chapter 2). I used benthic chambers to measure fluxes of solutes across the sediment-water interface and estimate community metabolism, primary productivity and nutrient regeneration rates, all commonly used proxies of ecosystem function in soft sediment habitats (Sundback et al. 2000, Rodil et al. 2011, Pratt et al. 2013, Norkko et al. 2015). Denitrification Enzyme Activity (DEA) assays (Seitzinger et al. 1993, Groffman et al. 2006, Douglas et al. 2017) were used to provide a proxy for the NO_3^- removal capacity of the resident denitrifier population and the denitrification history of sediments (i.e. conditions for denitrification in the previous weeks/months).

3.2.2 In situ chamber incubations/flux measurements

Chamber incubations were conducted over two consecutive days; on each sampling day, incubations were conducted on half the plots (one control and one enrichment plot) at each of the 12 sites across the study area. Four HOBO data loggers (5 min sampling interval) were distributed across the site to monitor light intensity and temperature during incubations. Paired light and dark chambers were used to incubate sediment (0.016 m^2) and overlying water (~0.85 L) in the centre of each plot for approximately 4 h over midday high tides. Chambers were fitted with two ports; one for water sample extraction and another inlet port to allow replacement of sampled water by ambient seawater. Sixty mL water samples were collected from each chamber (after discarding approximately 20 mL present in the sampling tube), at the beginning and end of the incubation period. Ambient seawater was incubated in three paired light and dark bottles (1.5 L vol), at the same time as the chamber incubations, in different locations across the study area to account for water column processes. Dissolved oxygen (DO) concentrations

were measured in each sample immediately after collection using an optical DO probe (PreSens Fibox PSt3). DO measurements were used to calculate gross primary productivity (GPP) and sediment oxygen consumption (SOC) (see 3.2.5 for calculations). Two replicate 15 mL water samples were then collected, after filtering through a 1.1 µm Whatman GF/C filter for nutrient analysis. Samples were frozen at -20°C until analysis.

3.2.3 Sediment Sampling

Sediment sampling for all plots was conducted after the second day of incubations were completed, as soon as possible following tidal emersion. Randomly placed cores were taken from each plot (excluding the incubated areas) for analysis of sediment pore water (5 x pooled, 0-2 cm depth, 2.6 cm dia.), sediment properties and microphytobenthic biomass (5 x pooled, 0-2 cm depth, 2.6 cm dia.), and DEA assays (5 x pooled, 0-5 cm depth, 5.3 cm dia.). Samples were stored in the dark, and transported to the laboratory on ice. Samples for sediment properties were frozen at -20°C and analysed within 6 weeks. Unfiltered seawater was collected from the site, stored on ice and then refrigerated at 4°C for DEA assays (see below).

A transparent core (5 cm dia.) was taken randomly from the centre of each plot and used to measure the depth of the colour change as a proxy for apparent Redox Potential Discontinuity (aRPD) (Danovaro 2009). Visual measurements have been shown to provide a good measure of aRPD as measured using electrodes or dissolved oxygen concentrations (Rosenberg et al. 2001, Gerwing et al. 2015). One 5 cm deep core (2.6 cm dia) hole was made in the centre of each plot, allowed to infill, and the porewater pH measured using a waterproof pHTestr® 10 (Eutech Instruments, Oakton).

Core samples for analysis of the benthic macrofaunal community were collected four days after the chamber incubations and sediment sampling. One core (13 cm dia., 15 cm depth) was taken from the position of the dark incubation chamber (this marked area was left undisturbed by the previous sediment sampling) in each plot. Immediately after collection macrofaunal core samples were sieved over a 500 µm mesh and preserved in 70% isopropyl alcohol. In the laboratory, samples

were stained (Rose Bengal), then all organisms were sorted, counted and identified to the lowest possible taxonomic level (usually species).

3.2.4 Laboratory Analyses

Within 24 h of collection, pore water was extracted from sediment (by centrifugation at 3300 rpm for 10 min), filtered (1.1 µm Whatman GF/C), then stored at -20°C until analysed. Nitrogen solute concentrations from benthic flux (NH_4^+) and pore water (NH_4^+, NO_2^-, and NO_3^-) samples were analysed using a LACHAT Quickchem 8500 series 2 Flow Injection Analyser and standard methods for seawater nutrient analysis. Benthic nitrogen flux measurements were limited to NH_4^+ because others have consistently shown that fluxes of NO_3^- and nitrite (NO_2^-) are minimal (account for less than 1% of benthic inorganic nitrogen fluxes) in northern New Zealand estuaries (Lohrer et al. 2010, Pratt et al. 2013). Sediments were analysed for organic content (%) by weight loss on ignition after drying to a constant mass at 60°C then removing the ash fraction by combusting at 550°C for 4 h. For determination of sediment grain size (% mud and grain size median (GSM)), organic matter was first removed from samples by digesting in 10% hydrogen peroxide, then measured using a Malvern Mastersizer 2000. Chlorophyll *a* (Chl *a*) and degraded (phaeophytin) biomass of microphytobenthos was measured after extraction from sediments with 90% buffered acetone, using a Turner 10-AU fluorometer, before and after acidification (Arar & Collins 1997).

DEA assays were conducted the day after sampling, using the acetylene inhibition technique (Tiedje et al. 1989, Groffman et al. 1999, Groffman et al. 2006, Douglas et al. 2017), first allowing sediment samples and water to acclimate to room temperature (20°C). Assays were composed of 60 mL homogenised sediment sample, 60 mL unfiltered site water amended with chloramphenicol (to prevent new enzyme synthesis, 0.06 g L^{-1}) and unlimited NO_3^- (10 mg L^{-1} N as KNO_3) and carbon (30 mg L^{-1} C as glucose), in 440 mL glass preserving jars with modified lids fitted with rubber septa. Jars were sealed, evacuated (by vacuum pump, 4 min), and flushed (pure N_2 for 10 min) to induce anoxia, then acetylene was added to each jar (10% of the headspace) to prevent sediment microbes from converting N_2O to N_2. Jars were kept at constant temperate (20°C), with constant mixing (25 rpm) for 2 h. Headspace gas samples were extracted from each jar 10, 30, 60 and

120 min after the addition of acetylene and analysed for N_2O concentration using Varian CP 3800 gas chromatograph equipped with a HayeSep D column and an electron capture detector. Rates of N_2O production were calculated as the increase in concentration per area of sandflat (µmol N m^{-2} h^{-1}), (calculated using the dry mass of sediment per assay jar and the sediment density).

3.2.5 Data analysis

To simultaneously account for environmental variation across the sedimentary gradient and assess the effects of nutrient addition on response variables PERMANOVAs were conducted where the treatment (nutrient enrichment) was considered a fixed factor and mud content as a continuous co-variable. This approach also enabled assessment of the interactive effects of sediment mud content and enrichment. Response variables included sediment properties, macrofaunal community characteristics and structure, proxies of ecosystem functions and denitrification activity.

Paired light and dark chamber measurements of oxygen and NH_4^+ fluxes (the difference in O_2 or NH_4^+ concentrations at the beginning and end of incubations) were used to derive the following measures of ecosystem function (Lohrer et al. 2010). Sediment oxygen consumption (SOC) which was measured as the uptake of oxygen from the water column to the sediment in dark chambers (i.e. without the effect of photosynthesis by benthic microalgae) and can be considered as a measure of community metabolism. Gross primary productivity (GPP) was measured by subtracting the flux of oxygen in the dark chamber from the flux of oxygen in the light chamber, and when normalised by the biomass of Chl *a* in the sediments provides a measure of photosynthetic efficiency ($GPP_{Chl\ a}$). The flux of NH_4^+ in dark chambers (without uptake by microalgae) can be considered as a measure of sediment nutrient regeneration. Chamber fluxes were corrected for water column processes but these made a small contribution to the total flux accounting for <5% and <1% for oxygen and NH_4^+, respectively.

In order to understand what aspects of macrofauna diversity were affected by nutrient addition, I assessed univariate measures of community characteristics (number of species (S), number of individuals (N), and numbers of adult (≥ 10 mm)

and juvenile (< 10 mm) *A. stutchburyi* and *M. liliana*) as well as a multivariate measure of macrofaunal community structure. The community structure measure was generated by combining the counts of all species into a resemblance matrix (Bray-Curtis) with treatment as a factor, after first performing a square root transformation, in order to determine effects on the macrofaunal community as a whole. *A. stutchburyi* and *M. liliana* are key bioturbating species in soft sediment ecosystems in northern New Zealand estuaries and known to be important for ecosystem functioning (Thrush et al. 2006, Sandwell et al. 2009, Pratt et al. 2013, Thrush et al. 2014, Karlson et al. 2016) so were considered separately. These were sorted into adult (≥10 mm) and juvenile (<10 mm) size classes as their activities and subsequent effects on ecosystem functions change as the grow (Hewitt et al. 1996). A Principle Coordinates Ordination (PCO) plot using a Bray-Curtis resemblance matrix of the benthic macrofaunal community was used to visualise potential differences between treatments. Vector overlays of environmental variables were used to show strength of these factors as predictors of the macrofaunal community (Pearson's correlation).

Multiple regression (using Distance based Linear Models, DistLM) was used to investigate which variables explained the observed variation in ecosystem functions with and without nutrient enrichment. DistLMs were performed on univariate Euclidean distance matrices of each ecosystem function (community metabolism (SOC), primary productivity (GPP, $GPP_{Chl\ a}$), nutrient regeneration (dark NH_4^+ flux) and DEA). I used a backwards elimination procedure with the corrected Akaike information criterion (AIC_c) and 9999 permutations to obtain the most parsimonious model. Mud was always forced to be included first in models (even if the marginal test was not significant), and where there was high collinearity among variables ($r > 0.7$), the variable explaining the least amount of variance was excluded first (Dormann et al. 2013). Predictor variables were grouped into sediment (mud), other environmental, and macrofaunal community categories. All analyses were conducted using Primer v7 with PERMANOVA+ add on (Clarke & Gorley 2015).

3.3 Results

3.3.1 Environmental variables

The study site encompassed a gradient of sediment mud content (0 – 24%, Table 3.1) which correlated with changes in other environmental variables (Appendices 5 & 6). In particular, mud content and organic content were strongly and positively correlated in both treatments ($r > 0.9$). Microphytobenthic biomass increased with increasing mud content, however the aRPD, pore water pH, and pore water nutrient concentration were similar apart from slightly higher concentrations of NO_3^- and NO_2^- in muddy sediments (Table 3.1, 3.2). Enrichment significantly increased pore water NH_4^+ concentration compared with controls (the enrichment median (532 µM) was 36 x the control median (14.6 µM); Figure 3.1), and this effect was independent of sediment mud content (Table 3.1, 3.2). Other than a small increase in pore water pH, the enrichment did not change other sediment properties, or microphytobenthic biomass. Mean light intensity was lower on sampling day 1 (8826 ± 366 Lux) than day 2 (22016 ± 1258 Lux) due to variable cloud cover but water temperatures were similar (20.4 ± 0.2 vs 19.6 ± 0.1°C). This variability did not bias flux measurements because on each sampling day, incubations were conducted in one (of two) enrichment and control plots located at each of the 12 sites.

3.3.2 Macrofaunal community

Macrofaunal community characteristics and structure changed across the sedimentary gradient but there was no effect of treatment and no significant interaction (Table 3.1, 3.2, Figure 3.2). Increasing mud content corresponded with a greater total abundance (N), fewer adult and greater numbers of juvenile *A. stutchburyi*. The total number of species did not differ across the mud gradient and neither did the abundance of adult or juvenile *M. liliana*. The main environmental variables correlated with macrofaunal community composition were sedimentary variables and microphytobenthic biomass (Figure 3.2).

Table 3.1 Sediment properties and macrofauna community variables as a function of treatment. Values are medians with minimum and maximum in parentheses (n=24 per treatment).

Variable	Control (0 g N m^{-2})	Enrichment (150 g N m^{-2})
Sediment properties		
Organic content (%)	3.2 (1.5 – 5.5)	3.3 (1.6 – 5.8)
Mud content (% < 63 µm)	3.5 (0 – 21.6)	4.0 (0 – 24.1)
Grain size median (µm)	151 (112 – 243)	151 (108 – 259)
pH	7.8 (7.6 – 8.2)	7.9 (7.6 – 8.5)
aRPD (mm)	25 (15 – 35)	20 (12 – 36)
Pore water (µM)		
NO_2^-	0.23 (0.12 – 0.49)	0.25 (0.11 – 0.94)
NO_3^-	0.96 (0.45 – 2.28)	1.03 (0.48 – 3.01)
NH_4^+	14.6 (0 – 154.2)	532 (0 – 24995)
Microphytobenthic biomass (µg g^{-1} sediment)		
Chlorophyll *a*	31.6 (15.6 – 48.7)	34.0 (14.8 – 55.0)
Phaeophytin	9.7 (4.0 – 17.2)	10.6 (2.9 – 20.6)
Macrofauna (n $core^{-1}$)		
S (taxa)	18 (14 – 23)	16 (10 – 24)
N (individuals)	124 (53 – 208)	102 (30 – 262)
A. stutchburyi (< 10 mm)	9 (1 – 42)	6 (0 – 31)
A. stutchburyi (≥ 10 mm)	6 (1 – 11)	7 (1 – 13)
M. liliana (< 10 mm)	4 (1 – 12)	3 (0 – 7)
M. liliana (≥ 10 mm)	6 (1 – 9)	5 (1 – 9)

Table 3.2 PERMANOVA test results for the effects of enrichment (treatment) and mud content (a continuous co-variable), on response variables. Significant terms are indicated in bold ($p \leq 0.05$).

	Mud		**Treatment**		**Interaction**	
	Pseudo-F	p-perm	Pseudo-F	p-perm	Pseudo-F	p-perm
Sediment properties						
pH	1.57	0.22	5.72	**0.02**	2.12	0.16
aRPD (mm)	2.36	0.13	1.84	0.19	0.30	0.59
Pore water (µM)						
NH_4^+	0.34	0.57	8.30	**0.001**	0.50	0.49
NO_2^-	6.17	**0.02**	0.96	0.36	0.07	0.79
NO_3^-	32.1	**0.0001**	0.25	0.62	0.03	0.86
Microphytobenthic biomass (µg g^{-1} sediment)						
Chlorophyll *a*	12.6	**0.001**	0.49	0.49	0.04	0.84
Phaeophytin	167	**0.0001**	0.23	0.62	0.23	0.63
Macrofauna (n $core^{-1}$)						
S	0.10	0.75	3.97	**0.05**	0.71	0.40
N	9.55	**0.004**	1.76	0.19	0.52	0.47
A. stutchburyi (< 10 mm)	11.31	**0.003**	0.88	0.37	0.64	0.43
A. stutchburyi (≥ 10 mm)	3.84	0.06	0.02	0.89	0.04	0.84
M. liliana (< 10 mm)	0.17	0.69	7.18	**0.009**	2.20	0.14
M. liliana (≥ 10 mm)	2.21	0.15	0.81	0.37	1.56	0.21
Community structure (multivariate)	11.9	**0.001**	1.72	0.13	0.41	0.84
Community metabolism						
SOC (µmol O_2 m^{-2} h^{-1})	3.53	0.06	0.51	0.48	0.28	0.61
Primary productivity						
GPP (µmol O_2 m^{-2} h^{-1})	15.3	**0.0004**	0.003	0.96	3.99	**0.05**
$GPP_{Chl\ a}$ (µmol O_2 µg Chl *a* g^{-1} dw m^{-2} h^{-1})	42.3	**0.0001**	0.27	0.61	1.81	0.19
Nutrient regeneration						
Dark NH_4^+ flux (µmol NH_4^+ m^{-2} h^{-1})	4.05	**0.05**	21.4	**0.0001**	4.8	**0.05**
DEA (µmol N m^{-2} h^{-1})	32.0	**0.0001**	4.62	**0.04**	7.42	**0.01**

Abbreviations: Macrofaunal taxonomic richness (S), macrofaunal abundance (N), sediment oxygen consumption (SOC), gross primary productivity (GPP), gross primary productivity normalised to chlorophyll *a* biomass ($GPP_{Chl\ a}$), and denitrification enzyme activity (DEA).

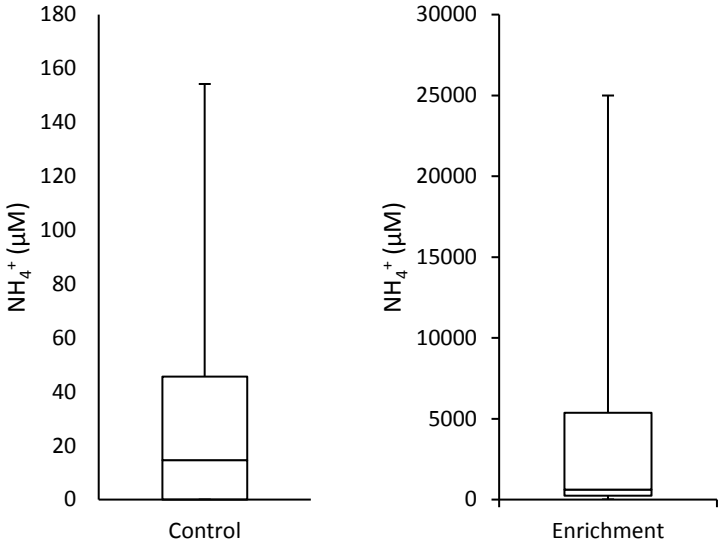

Figure 3.1 Pore water ammonium concentration in control and enrichment plots. Boxes represent 25%, median and 75% distributions, and whiskers represent minimum and maximum (n = 24). Note change of scale between plots.

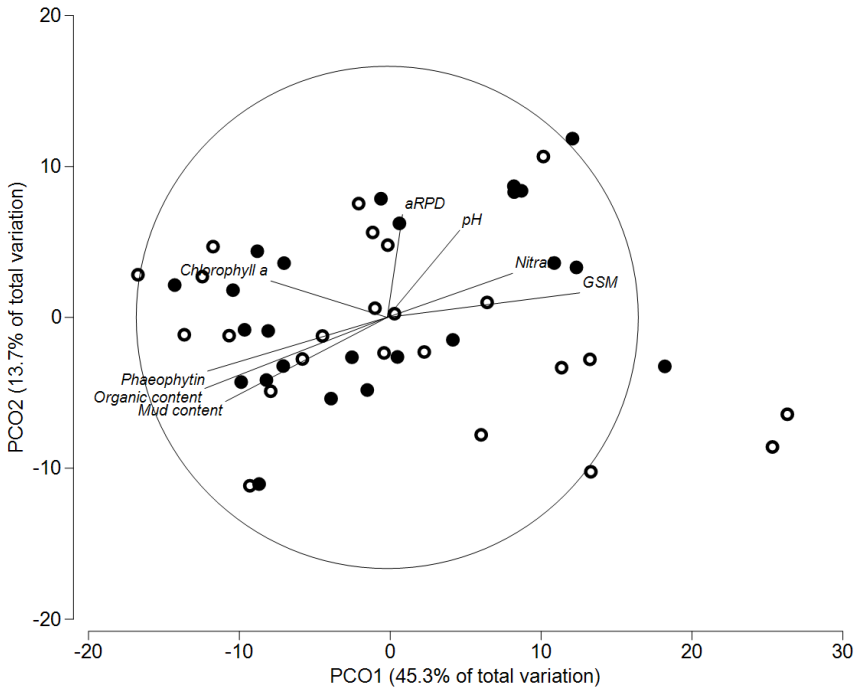

Figure 3.2 Principle coordinates ordination (Bray-Curtis similarity) showing little difference in macrofaunal community between control (black circles) and enrichment (white circles) plots. Overlaid vectors show the eight most influential environmental variables. Abbreviations: grain size median (GSM), pore water nitrate concentration (Nitrate), apparent redox potential discontinuity (aRPD).

3.3.3 Ecosystem functioning

All measures of ecosystem function varied with sediment mud content. Community metabolism (SOC) decreased with increasing mud content (by up to 49%) although this was not significant ($p = 0.06$), and there was no treatment effect (Figure 3.3, Table 3.2). The relationship between mud and nutrient regeneration (dark NH_4^+ flux) changed with nutrient enrichment (Figure 3.3, Table 3.2), as indicated by the significant interaction term. In control plots, dark NH_4^+ flux was positively related to sediment mud content but in enrichment plots this relationship was negative (Figure 3.3, Appendices 5 & 6). There was a significant interaction between mud and enrichment for gross primary productivity (GPP). In ambient sediments GPP decreased with increasing mud content but this negative relationship was counteracted in the presence of nutrients (Figure 3.3, Table 3.2, Appendices 5 & 6). When primary productivity was normalised by chlorophyll-*a* biomass ($GPP_{Chl\ a}$) (i.e. photosynthetic efficiency) there was no longer a significant interaction between mud and enrichment; in both treatments $GPP_{Chl\ a}$ decreased with increasing mud (Figure 3.3, Table 3.2). There was a significant mud x treatment interaction for DEA and on average DEA was suppressed by enrichment (Figure 3.3, Table 3.2). Although DEA was positively correlated with sediment mud content in both control and enrichment plots (Appendices 5 & 6), the response in enrichment plots was non-linear and above 10% mud content mean DEA declined by 170% compared to control plots (Figure 3.3).

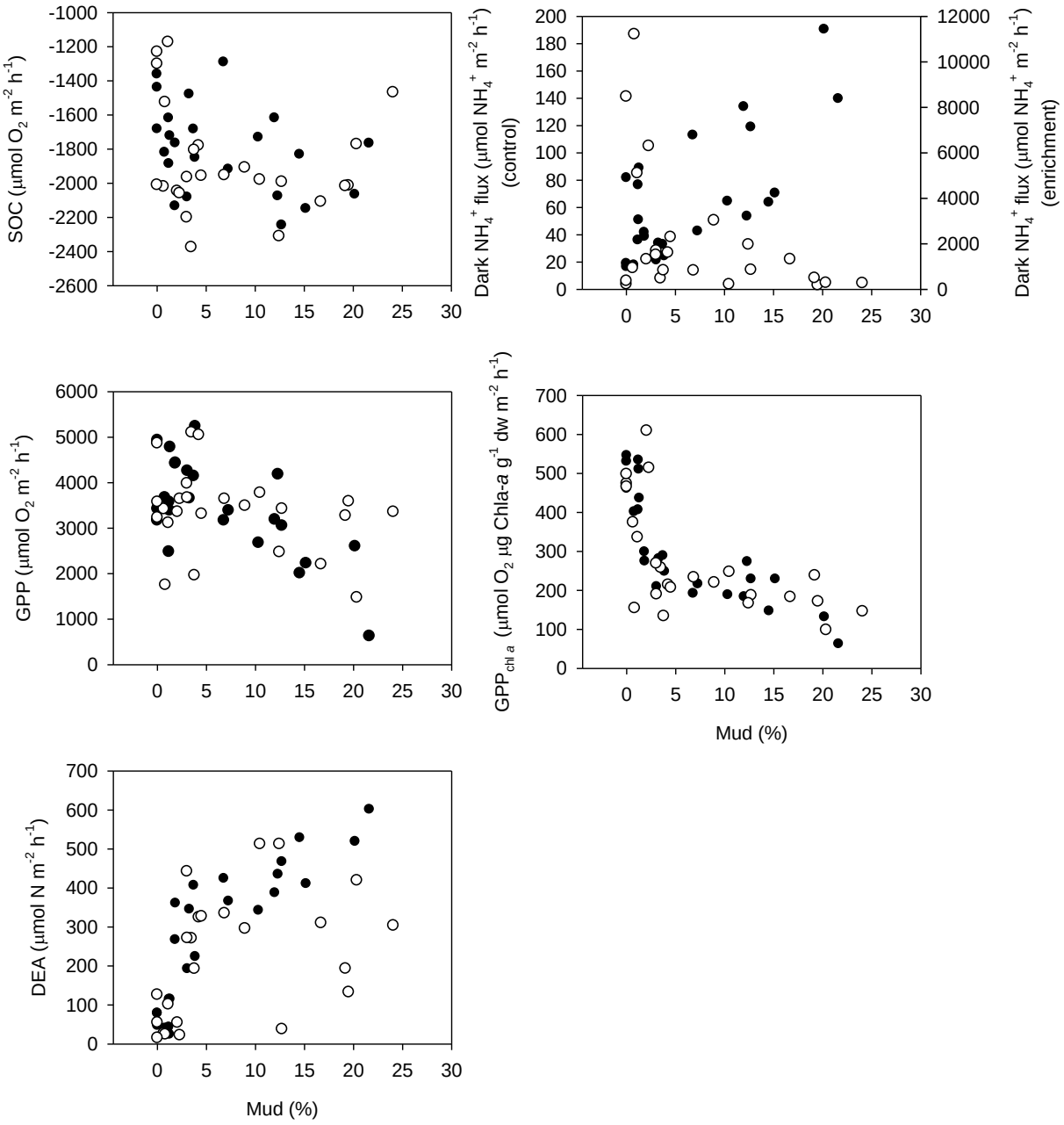

Figure 3.3 Relationships between sediment mud content and ecosystem functions for control (black circles) and enrichment (white circles) treatments. Abbreviations: SOC: sediment oxygen consumption, GPP: gross primary production, $GPP_{Chl\ a}$: gross primary production normalised by Chl *a* biomass, DEA: denitrification enzyme activity

Multiple variables were included in explanatory DistLMs of the measured ecosystem functions (Table 3.3). Community metabolism (SOC) was unaffected by nutrient enrichment and the best predictors were pore water NO_3^- and macrofaunal community characteristics (Table 3.2, 3.3). Mud was the factor explaining the largest amount of variability in $GPP_{Chl\ a}$ which was also unaffected by nutrient enrichment (Table 3.3). Other environmental variables including aRPD and pore water NO_3^- concentration accounted for a large amount of the variation in $GPP_{Chl\ a}$ (67% explained in total), followed by large bivalves (Table 3.3). For ecosystem functions that showed treatment and/or interaction effects, separate models were run for each treatment. Nutrient enrichment influenced the proportion of variability in GPP, dark NH_4^+ flux and DEA accounted for by sedimentary environment (mud), other environmental variables, and macrofaunal community (Table 3.3). Mud was the primary factor explaining variability in GPP, dark NH_4^+ flux and DEA in control plots, but with enrichment the amount of variability mud accounted for was reduced by more than half (Table 3.3). The amount of variability in these ecosystem functions accounted for by other variables, especially the macrofaunal community, became greater under enriched conditions, but the total amount of variability explained was less. Under enriched conditions, factors that positively influenced GPP and DEA were those associated with oxygenation of the sediments and pore water movement (chlorophyll *a*, aRPD, and abundance of macrofauna or large bivalves) (Table 3.3).

Table 3.3 Results of full DistLMs of ecosystem function, grouped predictor variables included in each model, and the proportion of variance each explains. Combined treatments were used for DistLMs in the absence of a significant mud x enrichment interaction or treatment effect (Table 3.2, SOC, $GPP_{Chl\ a}$). Where treatment effects occurred, DistLMs were run separately for control and enrichment plots (GPP, Dark NH_4^+ flux, DEA). Predictor variables are grouped into sediment (mud), other environmental, and macrofaunal community. Asterisks indicate significance levels of marginal tests of individual predictors included in full models *p<0.05, **p<0.01. Correlation directions are indicated in parentheses.

	Combined treatments		**Control**		**Enrichment**	
			(0 g N m^{-2})		(150 g N m^{-2})	
	Variables	Prop.	Variables	Prop.	Variables	Prop.
Community metabolism (SOC)						
Sediment	Mud (+)	0.07				
Other environmental	NO_3^- **(-)	0.21				
Macrofauna	*A. stu* (≥ 10 mm)** (+) *M. lil* (≥ 10 mm) (+)	0.26				
	Total	0.42				
Primary productivity (GPP)						
Sediment			Mud** (-)	0.47	Mud (-)	0.09
Other environmental			Chl *a* (+)	0.01		-
Macrofauna			*M. lil* (≥ 10 mm)** (+)	0.32	*M. lil* (< 10 mm)* (+) *M. lil* (≥ 10 mm)** (+)	0.34
			Total	0.71	Total	0.45
Primary productivity ($GPP_{Chl\ a}$)						
Sediment	Mud** (-)	0.48				
Other environmental	aRPD (-) NO_3^- ** (+)	0.46				
Macrofauna	*A. stu* (≥ 10 mm)** (-) *M. lil* (≥ 10 mm) (+)	0.2				
	Total	0.67				

Table 3.3 cont.

	Combined treatments		Control		Enrichment	
			(0 g N m^{-2})		(150 g N m^{-2})	
	Variables	Prop.	Variables	Prop.	Variables	Prop.
Nutrient regeneration (Dark NH_4^+ flux)						
Sediment			Mud* (+)	0.52	Mud* (-)	0.19
Other environmental			Chl *a* (+) aRPD (-) NO_3^- (-)	0.27	Chl *a* (+)	0.14
Macrofauna			*A. stu* (≥ 10 mm) (+)	0.003	S (-) N (-)	0.2
			Total	0.77	Total	0.4
DEA						
Sediment			Mud** (+)	0.73	Mud* (+)	0.17
Other environmental			NH_4^+ (+)	0.14	Chl *a*** (+) aRPD (+)	0.46
Macrofauna			*A. stu* (≥ 10 mm)* (+) *M. lil* (< 10 mm) (-)	0.33	N** (+)	0.36
			Total	0.83	Total	0.64

Abbreviations: Chlorophyll *a* (Chl *a*), apparent Redox Potential Discontinuity (aRPD), macrofaunal taxonomic richness (S), macrofaunal abundance (N), juvenile *A. stutchburyi* (*A. stu* (<10 mm)), adult *A. stutchburyi* (*A. stu* (≥10 mm)), juvenile *M. liliana* (*M. lil* (<10 mm)), adult *M. liliana* (*M. lil* (≥10 mm)), sediment oxygen consumption (SOC), gross primary productivity (GPP), gross primary productivity normalised to chlorophyll *a* biomass ($GPP_{Chl\ a}$), and denitrification enzyme activity (DEA).

3.4 Discussion

I conducted a nitrogen enrichment experiment across an existing gradient of sedimentary grain size and measured changes in the ecosystem functions of community metabolism, primary productivity, nutrient regeneration, and denitrification. This experiment has provided direct evidence that the proportion of mud in sediment can influence how estuary ecosystem functions respond to nutrient enrichment. Results indicate that high sediment mud content is detrimental to denitrification activity under nutrient enriched conditions, and furthermore, muddy sediments may restrict release of nutrients from enriched sediments making the sediments more likely to shift to a eutrophic state. The median enrichment level was 36 x ambient and independent of sediment mud content, however, nutrient effects on ecosystem functions were tightly linked to the sedimentary environment. This shows that sedimentary environment is a crucial factor affecting the response of ecosystem functions to nutrient enrichment, an important result considering these stressors often occur in unison.

I found that in control plots, higher DEA rates occurred in more muddy, organic rich sediments, and the sedimentary environment accounted for the majority of the variability in DEA. Mineralisation of organic matter may provide NH_4^+ for coupled nitrification-denitrification and an energy source (carbon) for heterotrophic denitrifiers. Organic matter is often reported as one of the direct controls of denitrification (Seitzinger et al. 2006), and other studies have similarly attributed higher denitrification rates in fine sediments to high organic carbon content (Nowicki et al. 1997, Sundback & Miles 2000). Sediment characteristics, particularly organic content have also been found to explain the majority of variability in nitrification and denitrification in lakes (Bruesewitz et al. 2012). In order for excess NH_4^+ to be transformed within the sediments it must first be nitrified, then denitrified. These processes involve different types of bacteria which require distinct physical and chemical conditions, particularly oxygen concentration (Joye & Anderson 2008), which vary substantially depending on the sedimentary environment. In cohesive sediments, organic matter mineralisation can have a negative effect on coupled nitrification-denitrification due to oxygen consumption (Eyre & Ferguson 2009). With increasing sediment mud content (and

organic content) there was increased (up to 49%) respiratory demand (community metabolism, SOC); indicative of higher microbial activity in muddy sediments from organic matter mineralisation (Kelly & Nixon 1984, Kelly et al. 1985). Rates of organic matter processing differ between muddy and sandy sediments and this has a major influence on pore water nitrogen concentrations and sediment oxygen profiles. There was no effect of enrichment on SOC, and most of the variability was explained by the abundance of large bivalves *M. liliana* and *A. stutchburyi* indicating that bioturbation stimulated microbial activity and organic matter breakdown (Sandwell et al. 2009, Woodin et al. 2016). These large species may also dominate macrofaunal biomass in the sediments thereby accounting for most of the community respiration.

The nutrient enrichment level aimed to stress the ecosystem without causing negative effects to the macrofaunal community, and pore water NH_4^+ concentrations were representative of enriched estuaries worldwide (see Douglas et al. 2016, Chapter 2). Community structure was not influenced by enrichment, but there was a small reduction in the abundance of juvenile *M. liliana*, and the number of species due to the enrichment. This may reflect species' differential sensitivity to stress, however these effects were not exacerbated by mud content (no interaction effect). Therefore, any reductions in the positive influence of macrofaunal diversity on ecosystem function and response to stress were considered consistent across the sedimentary gradient. Losses of large organisms and reductions in species diversity due to nutrient stress can lead to reduced ecosystem functioning and ecosystem resilience to stress (Chapin et al. 2000, Thrush et al. 2006, Thrush et al. 2008b, Naeem et al. 2012, Norkko et al. 2013).

The amount of variability in ecosystem functions explained by macrofaunal variables increased with nutrient enrichment (for those functions that showed a treatment effect) indicating that functional roles of some macrofauna may change under stressed conditions. Bioturbation and solute diffusion are the dominant mechanisms of pore water transport in muddy, more cohesive sediments (Huettel & Gust 1992, Joye et al. 1996, Burdige 2011), so the presence of macrofauna, especially large individuals such as bivalves will be very important in determining responses to nutrient enrichment. Macrofauna have a fundamental role in

sediment biogeochemical processes because their burrows provide sites for microbial activity, and their activity promotes sediment turnover, movement of solutes, and a spatially and temporally variable oxic-anoxic interface (Kristensen 2000, Braeckman et al. 2010). Burrow characteristics such as residence time and irrigation frequency can determine the makeup and biomass of microbial communities (Marinelli et al. 2002), and since burrow characteristics and macrofaunal behaviour vary substantially from permeable to cohesive sediments (Needham et al. 2011) this can affect biogeochemical processes and associated ecosystem functions (Yazdani Foshtomi et al. 2015). Two large bivalve species *M. liliana* and *A. stutchburyi* were important for DEA in ambient nutrient conditions, but with nutrient stress they did not contribute to explaining variability in DEA, indicating that their functional role for DEA was diminished under stress conditions. Others have also found decreasing ecosystem function performance coinciding with reduced macrofaunal density under stressed conditions making ecosystems more susceptible to stressors (Pratt et al. 2015).

Above about 10% mud content DEA was suppressed by enrichment, despite higher DEA in muddy ambient sediments (and assumed presence of larger established populations of nitrifying and denitrifying bacteria). Response of nitrifiers and denitrifiers to enrichment may be restricted in more cohesive sediments due to poor solute exchange and oxygen conditions, and this may lead to a build-up of NH_4^+ that is detrimental to nitrification (Anthonisen et al. 1976), resulting in reductions in DEA. Alternatively, this suppression of DEA at about 10% mud content may reflect a shift from denitrification to dissimilatory nitrate reduction to ammonium (DNRA). In enrichment plots, dark NH_4^+ flux increased as DEA decreased (Pearson's R = -0.34, p = 0.1), and other studies have shown that DNRA may be favoured over respiratory denitrification in NO_3^- limited coastal sediments with high organic carbon loading (see reviews by Burgin & Hamilton 2007, Giblin et al. 2013). Muddy sediments may be supporting greater populations of nitrifying and denitrifying bacteria because they have more surface area available for microbial attachment, are usually less mobile so there is less abrasion, allowing microbial biofilms to persist for longer and reach greater abundances (Belser 1979, Henriksen & Kemp 1988, Huettel et al. 2014). However, this may also limit

populations of some bacteria. In particular, nitrifying bacteria are less competitive than heterotrophic bacteria, limiting nitrification even when NH_4^+ is abundant (Henriksen & Kemp 1988), and this may have occurred in enrichment plots.

Another possible reason for differences in DEA between control and enriched plots is the response of the microbial communities to the enrichment. In agroecosystems fertilisation has been shown to change the abundance and community structure of bacterial denitrifiers (Wallenstein et al. 2006). Bacterial nitrogen turnover processes such as denitrification and NH_4^+ regeneration can be slow to recover from nutrient and contaminant stressors, and this may be due to low functional redundancy of the bacterial community (Sundback et al. 2007). Response of bacterial denitrifiers is context dependent; community composition of denitrifying bacteria can vary depending on the local environment, and different taxa may vary in their resistance to stressors (Cavigelli & Robertson 2000, Cavigelli & Robertson 2001). This may account for some of the unexplained variability in DEA across the sedimentary gradient as well as response to nutrient enrichment.

When enrichment occurs, due to lower pore water flushing and solute diffusion, muddy sediments may restrict nutrient release from the sediments which could exacerbate sediment eutrophication. Mud content was the main factor explaining variability in nutrient regeneration (dark NH_4^+ flux), but effects were dependent on treatment. Under ambient nutrient conditions dark NH_4^+ efflux was greater in muddy sediments, whereas under nutrient enriched conditions it was greater in sediments with less mud, and showed a negative relationship. In control plots, efflux rates were higher in muddy sediments presumably as a consequence of higher organic content, and higher rates of mineralisation. When NH_4^+ was in excess (i.e., in enriched plots), organic matter mineralisation was no longer the dominant source of NH_4^+, and release from the sediments was greater where there was lower sediment mud content (higher permeability). Furthermore, nitrification can be suppressed under very high NH_4^+ concentrations (Anthonisen et al. 1976), which may prevent denitrification of the excess nitrogen. This provides a clear example of an existing stressor (mud) increasing the negative impact of another stressor (nutrients).

Under nutrient enriched conditions the importance of mud for explaining variability in ecosystem functions (GPP, dark NH_4^+ flux, DEA) decreased compared with controls. Mud content can affect the oxygen profiles in the sediments and reduce rates of pore water transport. However, other factors controlling the availability and delivery of oxygen and nutrients accounted for most of the variability in enrichment plot DEA (aRPD, chlorophyll a biomass, macrofaunal abundance), enrichment plot GPP (abundance of adult and juvenile *M. liliana*), and $GPP_{Chl\ a}$ in both treatments (aRPD, pore water NO_3^-, abundance of adult *M. liliana* and *A. stutchburyi*). For DEA, this supports my assumption that coupled nitrification-denitrification is restricted by oxygen availability for nitrification of excess NH_4^+. Optimum rates of coupled nitrification-denitrification occur where there is maximum interface between oxic and anoxic sediments, and this is enhanced by the presence of macrofauna and burrow structures (Eyre & Ferguson 2009, Gilbert et al. 2016), and microphytobenthos (An & Joye 2001). Large macrofauna, especially *M. liliana*, influence solute transport through bio-irrigation (Woodin et al. 2016), and may be enhancing GPP and $GPP_{Chl\ a}$ by moving nutrient rich pore water from within the sediment to surface layers where it can be utilised by microphytobenthos. For $GPP_{Chl\ a}$ (both treatments), aRPD and pore water NO_3^- concentrations accounted for nearly as much variability as mud, indicating that the photosynthetic efficiency of the benthos is related to biogeochemical processes in the sediments.

The sedimentary environment ultimately determines the supply (through accumulation and mineralisation of organic matter) and fate of nitrogen (used by primary producers, accumulation in sediment pore water, loss through pore water flushing or diffusion to the water column, or loss through nitrification and denitrification) in soft sediment ecosystems. Context (mud) dependent responses of ecosystem functions to nutrient stress were more apparent for ecosystem functions that depended on specific biogeochemical conditions (e.g. DEA). Of all the measured ecosystem functions DEA showed the most sensitivity to both mud and nutrient stress and this may be due to the large number of indirect effects associated with the sedimentary environment which may be greater than direct effects. For example, muddy organic rich sediments provide ideal conditions for

denitrifiers (anoxia, N and C source) but these conditions can be detrimental to nitrification, on which denitrification in this system depends. Due to such sequences of indirect effects, ecosystem processes with more feedbacks may be more sensitive to stressors (i.e diversity-stability hypothesis; McCann (2000)). In particular, nitrogen cycling processes such as denitrification may be less resilient due to less functional redundancy compared with metabolism processes (Sundback et al. 2007). In this study system, muddy sediments may exacerbate eutrophication through changes in feedbacks associated with changes in macrofaunal communities and behaviour, and oxygen profiles, on which ecosystem functions linked with resilience to nutrients depend.

This is the first study, to my knowledge, that experimentally tests soft sediment ecosystem response to nutrient enrichment in different sedimentary environments, and demonstrates the combined effects of these stressors. Sediment muddiness influenced ecosystem functions and response to nutrient enrichment through direct effects on biogeochemistry associated with fine sediments, and indirect effects including differences in macrofaunal community in different sediment types. Levels of sediment mud content controlled the response of ecosystem functions to nitrogen oversupply. Although I did not measure recovery rates, recovery times after nutrient enrichment stress are likely to be longer when there is a high level of an existing stressor (e.g. mud) (Sundback et al. 2007). Isolating effects of stressors on ecosystem functioning is extremely difficult in real world settings since rarely do stressors occur independent of others or without being directly or indirectly affected by environmental factors. Anticipating how ecosystems will respond to accelerating stressors (e.g. nitrogen oversupply) under different stress regimes (e.g. sedimentation) will be critical for the preservation of healthy estuary ecosystems and the services they provide.

Chapter Four

Macrofaunal functional diversity provides resilience to nutrient enrichment in coastal sediments

4.1 Introduction

Enrichment of the ocean through anthropogenic alteration of the nitrogen cycle is leading to degradation of marine ecosystems and the services they provide (Nixon 1998). This occurs because nitrogen is essential for primary production and its oversupply in (generally) nitrogen-limited systems can cause blooms of algae, increases in organic matter, alteration of nutrient ratios, and changes to habitats, communities and food webs (Vitousek et al. 1997). Most of the terrestrial nitrogen received by the marine environment is removed through denitrification in coastal sediments (estimated up to 80%), a natural ecosystem process that removes bioavailable nitrogen (Galloway et al. 2003). Denitrification (D_N) can therefore provide resilience to eutrophication, which is recognised as a global threat to the functioning of coastal ecosystems and the goods and services they provide (Vitousek et al. 1997, Laursen et al. 2002).

Benthic macrofauna, such as bivalves and polychaetes, play a critical role in coastal marine nitrogen cycling. Particle and water transport related to feeding and movement activity (i.e. bioturbation) promotes transport of nutrients and oxygen throughout the sediment profile enhancing rates of nitrogen transformation (Kristensen et al. 1985, Kristensen et al. 1991, Pelegri et al. 1994, Gilbert et al. 1998, Webb & Eyre 2004b, Laverock et al. 2011). In sediments with an oxic layer and low water column nutrient concentrations, nitrification and D_N are often coupled (Sloth et al. 1995, Seitzinger et al. 2006). The distinct oxygen conditions these processes require (i.e., presence of oxygen for nitrification; anoxia for D_N) means that the interface between the oxic and anoxic sediments is an important site for coupled D_N. The activities of benthic macrofauna cause this interface to be

dynamic in space and time (Volkenborn et al. 2010, Volkenborn et al. 2012), enhancing coupled D_N (Stief 2013). Moreover, bioturbation can also enhance un-coupled D_N by increasing the supply of nitrate to sediments from the water column (Kristensen et al. 1991, Nogaro & Burgin 2014). However, if macrofauna are negatively affected by nutrients and/or other stressors their positive influence on D_N will be diminished.

Degradation of biodiversity through loss of species can reduce an ecosystem's ability to withstand stress or adapt to changing conditions (Villnäs et al. 2013). Species loss can be deleterious to key ecosystem processes contributing to feedback loops that invoke changes in community and overall ecosystem function, which and may lead to ecosystems reaching thresholds or tipping points resulting in shifts to alternate states (Thrush et al. 2006, Thrush et al. 2014, van Nes et al. 2016). Given the complex interaction between bioturbating macrofauna and nitrogen cycling and that species with traits relevant to nutrient processing will vary in their sensitivity to stress (i.e. response diversity) (Elmqvist et al. 2003, Hewitt et al. 2010, Mori et al. 2013, de Juan et al. 2014), non-linear responses to losses in biodiversity and ultimately resilience are likely (Naeem et al. 1994, Chapin et al. 2000). Identification of the elements of macrofaunal diversity that contribute to D_N is necessary to understand the potential ecosystem response to nutrient oversupply and to adequately conserve the necessary aspects of biodiversity. These elements include both local- (alpha), and landscape-scale (gamma) diversity that contribute to the overall heterogeneity of communities (beta diversity), which can provide a measure of ecosystem stability (Doak et al. 1998, Thrush et al. 2008a). As diversity and density of marcofauna decreases, D_N rates are also likely to decrease, which may in turn further intensify eutrophication impacts, creating a strong feedback (Loreau et al. 2001, Folke et al. 2004, Hewitt et al. 2010, Hewitt & Thrush 2010).

Nitrogen loading to coastal ecosystems is increasing globally (Galloway et al. 2008) and there is a pressing need to understand how it alters D_N and interactions with macrofaunal diversity in real world settings. While field studies have quantified D_N in a range of coastal habitats (e.g. Piehler & Smyth 2011, Eyre et al. 2013, Foster & Fulweiler 2014) they do not make linkages to macrofauna diversity or the

diversity response to nutrient stress, and the consequences for D_N are absent. Similarly, despite a considerable amount of research examining aquatic sediment nitrogen cycling (reviewed by Huettel et al. 2014), and much highlighting the importance of macrofauna (reviewed by Stief 2013), studies have so far not been able to address potential feedbacks between biodiversity and stressors. To date nutrient enrichment field experiments have tested the responses of macrofaunal communities (Morris & Keough 2003b, Posey et al. 2006, Fitch & Crowe 2012), while others have measured effects on ecosystem functions including D_N (Koop-Jakobsen & Giblin 2010, Oakes et al. 2011, Vieillard & Fulweiler 2012), but no study has combined the two and assessed the role of macrofauna in D_N response to nutrient enrichment.

I simulated eutrophication in situ using sediment nutrient enrichment in experimental plots across a sandflat with a heterogeneous landscape of macrofaunal community abundance and diversity. The study focused on two species of shellfish (*Austrovenus stutchburyi* and *Macomona liliana*) recognised as key species for nutrient processing (Thrush et al. 2006, Sandwell et al. 2009, Jones et al. 2011, Pratt et al. 2013, Thrush et al. 2014), as well as a group of 46 other species with functional traits important for nutrient processing (Greenfield et al. 2016). I used denitrification enzyme activity (DEA) assays to provide a proxy for nutrient processing and nitrogen removal; a proven method for comparisons of denitrification activity in aquatic systems that permits large sample sizes (Barnes & Owens 1998, Livingstone et al. 2000, Bernot et al. 2003, Wall et al. 2005, Teixeira et al. 2010, Bruesewitz et al. 2011, Jones et al. 2011). I expected treatments that caused substantive increases in pore water ammonium (NH_4^+) concentrations would be detrimental to the diversity of nutrient processing macrofauna (Pearson & Rosenberg 1978, Gray et al. 2002), leading to reductions in DEA. Alternatively, increased pore water NH_4^+ concentrations could enhance DEA via coupled D_N provided surface sediment remained oxygenated by macrofauna and/or in permeable sediments by advective pore water flushing due to physical processes (Huettel et al. 2014).

4.2 Methods

4.2.1 Experimental design

Twenty-eight sites across a 300, 000 m^2 intertidal sandflat in the Kaipara Harbour were selected based on a macrofauna community survey at the study site (Kraan et al. 2015) and an analysis of species functional traits that characterise life history, morphology and behaviours that influence sediment biogeochemistry and stability (Greenfield et al. 2016). From Greenfield et al. I identified a functional group of 46 species that possessed traits that influence sediment biogeochemistry (e.g. deposit feeding, free mobility, and burrow building) and therefore are important for nutrient processing. The selected sites encompassed a spectrum of abundance and species richness of this functional group as well as sediment properties (Table 4.1) to maximise the variation in nutrient processing capacity. The experiment ran for seven weeks (established 20 Jan 2014, sampled 17 March 2014) and at each site, 1 procedural control and 2 nutrient enrichment treatment plots (1 x 1 m) were established in a 5 x 5 m area by adding slow release fertiliser (or pea gravel for controls) buried in the sediments. Fertiliser (Nutricote® N (70 d, 40-0-0 N:P:K)) was applied to each plot in a series of 20 evenly spaced 3 cm diameter 15 cm deep holes made in the sediment using a hand held corer. Each hole received an equal volume of fertiliser (or pea gravel) and the intact sediment core plugs were replaced immediately to minimise disturbance to the sediment (see Douglas et al. 2016, Chapter 2 for details). I considered the control plots to be representative of ambient sediments because less than 2% of the plot area was impacted and previously, with a similar level of disturbance, no procedural effects on intertidal macrofaunal community composition, benthic respiration, nutrient fluxes and primary production were found when sampled after 4-7 d (Gladstone-Gallagher et al. 2014, Gladstone-Gallagher et al. 2016). Moreover, photographs of plots taken four and seven weeks after disturbance indicated no trace of coring, even in plots containing seagrass. Application rates (medium 150 g N m^{-2}, high 600 g N m^{-2}) were based on a literature review of previous enrichment experiments, and resulted in significantly elevated pore water NH_4^+ concentrations for at least seven weeks in surface (0-2 cm) and deeper (5-7 cm) sediments (Table 4.1) (Douglas et al. 2016, Chapter 2).

Table 4.1 Sediment properties and macrofaunal variables in control (0 g N m^{-2}), medium (150 g N m^{-2}) and high (600 g N m^{-2}) treatments.

Variable	Control (0 g N m^{-2})	Medium (150 g N m^{-2})	High (600 g N m^{-2})
Sediment properties			
Seagrass (% cover)	16 (0-84)	20 (0-97)	21 (0-75)
OC (%)	0.9 (0.6-2.0)	0.9 (0.6-2.0)	1.0 (0.6-1.8)
Mud (% < 63 μm)	1.78 (0-15)	0.62 (0-14)	0.42 (0-12)
GSM (μm)	215 (177-241)	220 (182-242)	219 (190-250)
Microphytobenthic biomass (μg g^{-1} sediment)			
Chl-*a*	9.3 (3-23)	10.0 (5-32)	9.5 (5-28)
Phaeophytin	4.4 (1.5-18)	6.4 (1.6-22)	4.0 (1.1-19)
Pore water NH_4^+ (μM)			
Surface sediments (0-2 cm)	24 (0-198)	253 (0-2210)	1849 (111-10239)
Deeper sediments (5-7 cm)	74 (15-484)	1209 (99-10275)	5846 (565-18842)
Macrofauna (n $core^{-1}$)			
S (taxa)	10 (6-16)	10 (4-15)	8 (3 -16)
N (individuals)	60 (15-376)	39 (12 -519)	32 (7-301)
A. stutchburyi (< 10 mm)	6 (0-91)	2 (0-99)	2 (0-64)
A. stutchburyi (≥ 10 mm)	1 (0-22)	1 (0-14)	1 (0-21)
M. liliana (< 10 mm)	5 (1-25)	4 (0-14)	2 (0-9)
M. liliana (≥ 10 mm)	2 (0-4)	1 (0-3)	1 (0-6)

Values are medians with minimum and maximum in parentheses (n=28).
Variables: OC = sediment organic content, Mud = sediment mud content, GSM = Grain size median, Chl-*a* = chlorophyll *a* content, S = number functional group species, N = number of functional group individuals.

4.2.3 Sample collection and analyses

All sampling was conducted on March 17th 2014, seven weeks after fertiliser enrichment. For DEA analyses, five sediment cores (0-5 cm depth, 5.3 cm dia.) were collected from each plot, pooled, transported on ice to the laboratory, kept at 4°C, and analysed within 48 h of collection (n = 1 replicate per plot). Prior to conducting assays, samples were brought to room temperature (20°C). DEA assays were used as a proxy for D_N to give a relative measure of sediment nutrient processing and nitrogen removal capacity. DEA assays were conducted using the chloramphenicol-amended acetylene inhibition technique (Tiedje et al. 1989, Groffman et al. 1999, Bruesewitz et al. 2006, Groffman et al. 2006). This method does not measure actual denitrification rates since acetylene inhibits nitrification, however it measures the activity of the resident denitrifier population under optimal conditions (total anoxia, constant mixing, unlimited nitrate and organic carbon) but without allowing new enzyme growth.

Assays were conducted in glass jars (440 mL volume) with lids fitted with a *n*-butyl rubber septa. Homogenised wet sediment samples (60 mL) were placed into jars with 54 mL unfiltered seawater from the site. Chloramphenicol was added to prevent new enzyme synthesis at a final concentration of 0.06 g L^{-1}. Assays were amended with unlimited carbon (30 mg L^{-1} C as glucose) and nitrate (10 mg L^{-1} N as KNO_3). Anaerobic conditions were obtained by sealing the jars, evacuating with a vacuum pump for 4 min, then purging with pure N_2 gas for 10 min. Pure acetylene was added as 10% of the headspace volume to prevent the conversion of N_2O to N_2. Assay jars were placed on shakers at 125 rpm and incubated at 20°C for 2 h. Headspace gas samples (6 mL) were collected at 10, 30, 60 and 120 min after the addition of acetylene. Gas samples were analysed using a Varian CP 3800 gas chromatograph equipped with a HayeSep D column and an electron capture detector.

Sediment dry mass (DM) in each assay jar was determined (after 48 h at 60 °C) and N_2O production rates (µg g DM^{-1} h^{-1}) calculated from the linear increase in concentration over time ($r^2 > 0.8$). DEA was expressed per unit area of sand flat (µmol N m^{-2} h^{-1}) by multiplying the production rate by the sediment density (g DM cm^{-3}, determined by drying a known volume of the assay sediment) and sample

depth (5 cm). My analysis had a minimum DEA detection limit of 1 µmol N m^{-2} h^{-1} and in preliminary testing replicate subsamples (n=5) from homogenized sediment had a coefficient of variation (mean/standard deviation) of 7% whereas the coefficient of variation between five replicate 1 m^2 plots in a 25 m^2 area at five sites was between 10-15%.

Environmental variables were characterised as follows. Seagrass (*Zostera muelleri* Irmisch ex. Asch.) coverage (%) was estimated using photographs (taken before sampling) of the central 0.25 m^2 of each plot and a random point count method (see Kohler & Gill 2006). Sediment cores from each plot were collected for analysis of pore water NH_4^+ (n = 4, 2.6 cm dia., 0-2 cm and 5-7 cm depths, separated and depth sections pooled), sediment organic content, mud content (% < 63 µm), grain size median, chlorophyll-*a*, phaeophytin (n = 5, 2.6 cm dia., pooled, 0-2 cm depth), and macrofauna community composition (n = 2, 13 cm dia. 0-15 cm depth). For DEA and sediment analyses there was one replicate per plot, for macrofauna there was two replicates per plot. Laboratory protocols are described in detail elsewhere (Douglas et al. 2016, Chapter 2), but briefly; pore water was extracted by centrifugation, filtered (1.1 µm Whatman GC glass fibre filter), frozen (-20°C), then analysed for NH_4^+ concentration using a Lachat QuickChem 8000 Series FIA+ (Lohrer et al. 2010), sediment grain size was analysed with a Malvern Mastersizer 2000 after removal of organic matter (Singer et al. 1988), sediment organic content was determined by loss on ignition (550°C, 4 h) (Parker 1983) and microphytobenthic biomass was determined by extraction of pigments from freeze dried sediment (90% acetone) and measuring fluorescence using a Turner Designs 10-AU flourometer (Arar & Collins 1997). Macrofaunal cores were sieved (500 µm mesh), preserved (50% iso-propyl alcohol), stained (Rose Bengal), and then all organisms were counted and identified (usually to species level).

4.2.4 Statistical analysis

A permutational multivariate analysis of variance (PERMANOVA, using a Euclidean distance matrix) was used to test for significant treatment effects on environmental variables (seagrass cover, sediment properties and microphytobenthic biomass). Due to the experimental design (i.e. the spatial scale and selection of sites to maximise macrofauna diversity) there was, as expected,

high inter-site variability in DEA, macrofauna and environmental variables (Figure 4.1, Table 4.1). To compensate for this natural heterogeneity and reveal potential treatment effects I normalised site specific treatment response parameters by the corresponding control plot values so effect size was relative to the site specific background level. Normalisation assumes control plot values are representative of a site, a justifiable assumption given the small inter-plot distances (2 m) and strong positive correlations between control and treatment plot sediment properties and primary producer biomass/coverage (Pearson's $r > 0.75$, $p < 0.001$; raw data in Appendix 7). Treatment response variables (DEA and macrofauna community measures) were also correlated (Appendix 8). Control normalised ($_{CN}$) DEA and community values were tested for differences from control values (i.e. $DEA_{CN} \neq 1$; one sample *t*-tests) and between fertilizer addition treatments (medium vs. high; two sample *t*-tests) using Statistica 11 (StatSoft Inc. 2012).

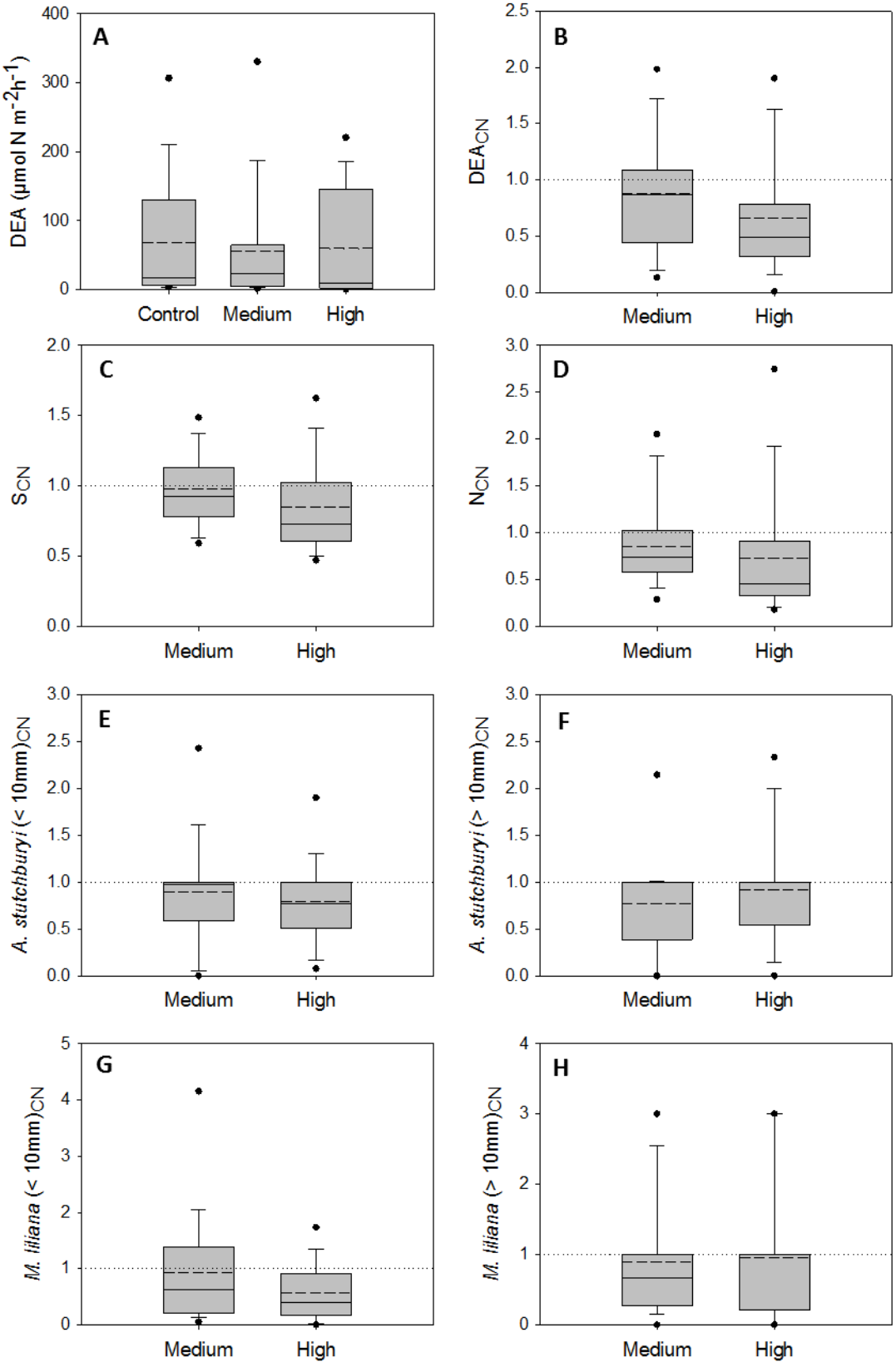

Figure 4.1 The effect of nutrient enrichment treatment on a) DEA, and control normalised b) DEA (DEA_{CN}), c) number of functional group species (S_{CN}), d) number of functional group individuals (N_{CN}), e) juvenile (< 10 mm) and f) adult (≥ 10 mm) *A. stutchbury*i_{CN} abundance, and g) juvenile (< 10 mm) and h) adult (≥ 10 mm) *M. liliana*$_{CN}$ abundance. Boxes are 25th and 75th percentiles, whiskers show 10th and 90th percentiles, black dots show 5th and 95th percentiles. Solid line is median, dashed line is mean, and in the normalised plots the dotted line is provided for reference to the control value.

Distance based Linear Models (DistLM) were used to identify significant individual predictors (marginal tests), and then the best combination of predictor variables (backwards elimination procedure) of DEA_{CN} at different levels of nutrient enrichment. Predictor variables included environmental variables and univariate measures of macrofaunal community composition. I used the corrected Akaike information criterion (AIC_c) which is the most appropriate selection criterion when the number of variables is large compared to the sample size (Burnham & Anderson 2002). Predictor variables were normalised (between -2 and 2) to enable comparison among variables with different units without altering the distribution. Where there was co-linearity among variables ($r > 0.7$), the variable explaining the lesser amount of variability was excluded from full models (Dormann et al. 2013). Variance partitioning analysis (Borcard et al. 1992, Anderson & Cribble 1998) was used to determine how much of the model variance was attributed to grouped predictor variables; sediment pore water NH_4^+ concentration (surface (0-2 cm) and deep (5-7 cm)), environmental variables (seagrass cover, sediment organic content (OC), median grain size (GSM), sediment mud content (% < 63 µm; mud), chlorophyll *a* (Chl *a*), phaeophytin, distance from shore), and macrofaunal community variables (see below). All multivariate analyses were conducted using PRIMER 7.0 with PERMANOVA+ add-on (Clarke & Gorley 2015) with untransformed data.

For measures of macrofaunal community composition I just considered the group of 46 species identified by Greenfield et al. (2016) with traits important for nutrient processing. On average this functional group comprised 52% of the taxa and 63% of the abundance, and preliminary analyses indicated that this group had greater effects on DEA than the macrofaunal community considered as a whole. I included in analyses the number of species (within group diversity, S) and individuals (within group abundance, N) belonging to this functional group, as well as the abundances of juvenile (< 10 mm) and adult (≥ 10 mm) *A. stutchburyi* and *M. liliana*. *A. stutchburyi* and *M. liliana* were included as separate predictors as both species have been shown to strongly influence ecosystem functioning (i.e. are key species) on New Zealand sandflats (Thrush et al. 2006, Sandwell et al. 2009, Jones et al. 2011, Pratt et al. 2013, Thrush et al. 2014) and I separated adults and

juveniles because impacts on ecosystem differs with size (Hewitt et al. 1997, Norkko et al. 2013).

4.3 Results

4.3.1 Nutrient enrichment effect on DEA

Nutrient treatment (150 g N m^{-2} and 600 g N m^{-2}) significantly increased pore water NH_4^+ concentrations throughout the sediment profile (Douglas et al. 2016, Chapter 2), but had no significant effects on sediment properties, seagrass cover or microphytobenthic biomass (Table 4.1; all PERMANOVA Pseudo-F = 0.77, p > 0.5, not shown). There was substantial variability in DEA values in all treatments across the study site, with control plot values ranging from 7.6 to 183.2 µmol N m^{-2} h^{-1} (Figure 4.1a). The site specific DEA response to enrichment (DEA_{CN}) ranged from 0.12 to 2.0 in medium treatment plots (i.e. 12 to 200% of control values), and 0.001 and 1.9 in high treatment plots (i.e. 0.1 and 190% of control values). In the medium treatment 18 of 28 sites, and in the high treatment 21 of 28 sites, DEA values were less than in controls (i.e. $DEA_{CN} < 1$) indicating that DEA was, on average, suppressed by enrichment (Figure 4.1b). In approximately 25% of treatment plots, enrichment enhanced DEA by > 20%. Reductions in DEA_{CN} were only significant in the high treatment, however reductions were greater in the high compared with the medium treatment (although not significant (p = 0.07); Figure 4.1b, Table 4.2).

Table 4.2 Treatment effects on control normalised ($_{CN}$) DEA and macrofaunal community measures.

	Treatment		Difference from control		Difference between treatment means	
Variable		**Mean**	***t***	***p***	***t***	***p***
DEA_{CN}	Medium	0.87	-0.13	0.20	***1.86***	0.07
	High	**0.66**	**-3.41**	**0.002**		
S_{CN}	Medium	0.98	-0.45	0.66	**2.85**	**0.008**
	High	**0.85**	**-2.50**	**0.02**		
N_{CN}	Medium	0.86	-1.60	0.12	1.44	0.16
	High	**0.73**	**-2.05**	**0.05**		
A. stutchburyi (<10 mm)$_{CN}$	Medium	0.89	-1.01	0.32	1.26	0.22
	High	**0.79**	**-2.54**	**0.02**		
A. stutchburyi (≥ 10 mm)$_{CN}$	Medium	**0.77**	**-2.13**	**0.04**	-0.92	0.37
	High	0.91	-0.77	0.45		
M. liliana (< 10 mm)$_{CN}$	Medium	0.94	-0.30	0.76	***1.94***	0.06
	High	**0.56**	**-4.54**	**0.0001**		
M. liliana (≥ 10 mm)$_{CN}$	Medium	0.89	-0.72	0.48	-0.26	0.80
	High	0.95	-0.30	0.77		

Test results for differences between treatments and controls (one sample *t*-test), and between medium and high treatment (two sample *t*-test).
Variables: control normalised DEA_{CN} = Denitrification Enzyme Activity, S_{CN} = number functional group species, N_{CN} = number of functional group individuals.
Significant differences ($p \leq 0.05$) are indicated in bold.

4.3.2 Predictors of DEA

DEA was significantly correlated with a number of environmental variables (Appendices 9-11). In general, sites with higher control plot DEA were those with more sediment OC and mud, smaller median grain size, more seagrass coverage, and more phaeophytin biomass. Control plot DEA was significantly correlated with DEA in both treatment plots (Appendix 8), i.e. sites with naturally high DEA were also high following enrichment. Normalisation of medium and high treatment DEA by control values effectively removes spatial environmental influences and consequently these variables (and control plot DEA) did not explain a substantial proportion of DEA_{CN} (Table 4.3, Appendices 10 & 11). The predictors included in the full models of DEA_{CN} differed depending on the level of enrichment (Table 4.3, Figure 4.2). In the medium treatment, surface sediment pore water NH_4^+ concentration had a positive effect on DEA_{CN}, but community variables explained more of the response. Key bioturbators showed a strong influence on medium treatment DEA_{CN}; together, juvenile and adult *M. liliana* and adult *A. stutchburyi* made up 32% of the total 54% explained variance. The effects of these two species on DEA_{CN} were different, *M. liliana* positive and *A. stutchburyi* negative (Table 4.3). Unlike in the medium treatment, pore water NH_4^+ concentration was not an important predictor of DEA_{CN} in the high treatment; only community variables were included in the full model explaining 39% of the variance, and key species did not have a significant role (Table 4.3, Figure 4.2b). Most (37%) of the explained variance was attributed to the abundance of nutrient processing species (N) which was positively correlated with DEA_{CN}. The amount of unexplained variance in DEA_{CN} increased with the level of nutrient enrichment from 46 to 61%.

Table 4.3. DistLM results for treatment plot control normalised DEA (DEA_{CN}).

Treatment	Group	Variable	Pseudo-*F*	Prop.	Full model
Medium	Pore water	NH_4^+ (0-2 cm)	7.16	0.21* (+)	19%
	Community	*M. liliana* (< 10 mm)	5.19	0.16* (+)	
		M. liliana (≥ 10 mm)	2.56	0.09 n.s. (+)	32%
		A. stutchburyi (≥ 10 mm)	3.09	0.11 n.s. (-)	
				Total	54%
High	Environment	Mud	3.68	0.12* (+)	-
		Phaeophytin	2.81	0.10 n.s. (+)	-
	Community	S	5.98	0.19** (+)	-
		N	10.98	0.30* (+)	37%
		M. liliana (≥ 10 mm)	0.50	0.02 n.s. (-)	9%
				Total	39%

Prop. is the proportion of variability in DEA_{CN} explained by each variable when considered individually. Significance levels are *$p \leq 0.05$, and **$p \leq 0.01$, and correlation directions are in parentheses.
Full model shows the variables included in the best DistLM model of DEA_{CN} and the variance attributed to each.
Variables: NH_4^+ (0-2 cm) = surface sediment pore water ammonium concentration (μM), Mud = sediment mud content (%), phaeophytin (μg g^{-1} sediment), S = number functional group species, N = number of functional group individuals.

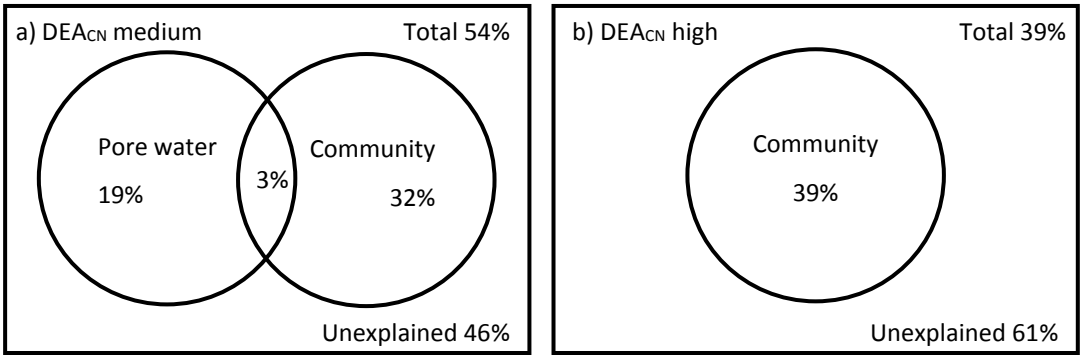

Figure 4.2 Diagrams presenting partitioning of variance in DEA_{CN} in a) medium and b) high treatment attributed to unique and shared effects of measures of community and pore water ammonium concentration (realised treatment effect). Results from variance partitioning analysis of full DistLM models as described in Table 4.3.

4.3.3 Nutrient enrichment effect on the macrofaunal community

Analysis of control normalised measures of the nutrient processing functional group composition revealed significant treatment effects (Table 4.2, Figure 4.1c-h). The number of species (S_{CN}) was lower in the high than control and medium treatments, and there were reductions in the total abundance (N_{CN}), but this was only significant in the high treatment. The abundance of key bioturbating species were also negatively impacted with nutrient enrichment. Adult and juvenile *A. stutchburyi* densities were reduced in the medium and high treatments respectively. For *M. liliana*, only juveniles (which were numerically dominant) were affected, and only in the high treatment (Table 4.2).

4.4 Discussion

I examined the role of macrofauna diversity in moderating nutrient oversupply using an indirect measure of nutrient processing capacity (DEA) across 28 sites with substantial natural variability in the community composition of nutrient processors. DEA was spatially highly variable which was expected given the heterogeneity of the sandflat and sites with naturally high DEA were also high following nutrient enrichment. By normalising treatment plot DEA by control values I revealed the response to nutrient addition and demonstrate in a real world setting that benthic macrofaunal diversity is important to the preservation of denitrification (D_N) following nutrient stress. This is significant because D_N is a process that can mitigate eutrophication, and nutrient enrichment commonly has negative effects on benthic macrofauna (Pearson & Rosenberg 1978).

Fertilizer addition on average suppressed DEA (i.e. $DEA_{CN} < 1$) especially in the high treatment, and I assume this suppression was due to inhibition of nitrification (although I did not measure this process directly). Most of the D_N in this system is likely to be coupled to nitrification because control plot DEA strongly correlates with sediment organic content (suggesting organic matter mineralisation is the primary source of N; Appendix 9) (Sloth et al. 1995, Seitzinger et al. 2006), and New Zealand estuaries typically have low pore water and water column nitrate concentrations (Lohrer et al. 2004a, Thrush et al. 2006, Lohrer et al. 2010). Nitrification inhibition would occur if the enriched sediments became periodically anoxic or the oxic layer depth decreased (preventing or reducing nitrification of NH_4^+ even when present in great quantity) (Joye & Hollibaugh 1995, Magalhães et al. 2005, Foster & Fulweiler 2014). Shifts towards anaerobic conditions may have been caused by the NH_4^+ induced reduction in the abundance of bioturbating species (Table 4.2, Figure 4.1c-h) which would reduce oxygenation of the sediments (Diaz & Rosenberg 1995, Diaz & Rosenberg 2008, Glud 2008) and further exacrbated by dead macrofauna stimulating microbial metabolsim during decay (Kelly & Nixon 1984, Blackburn et al. 1993). (But note there was no detectable enrichment of sediment organic content in treatment plots that could be related to macrofauna mortality (Table 4.1)).

Although enrichment supressed DEA_{CN} at most sites, the response represented a continuum from inhibition to enhancement. DistLM showed that 39-54% of response to enrichment could be explained, and most of it by macrofaunal diversity. It is difficult to speculate on the source(s) of the unexplained variation in DEA_{CN}, but on a dynamic intertidal sandflat spatial and temporal variations in sediment biogeochemistry caused by hydrodynamic forcing (Green & Coco 2014, Huettel et al. 2014), foraging and excretion by large predators (e.g. Thrush et al. 1994, Hines et al. 1997, Jauffrais et al. 2015), detrital inputs (e.g. Eyre & Ferguson 2002, Eyre et al. 2013) and microbial diversity (e.g. Yazdani Foshtomi et al. 2015) could all contribute, as could any initial small scale variation between plots within a site. Nevertheless the fact that a substantial proportion of the DEA response could be explained by macrofauna diversity despite the complexity of the field setting emphasises its importance in regulating the effects of enrichment.

When NH_4^+ was supplied in the medium treatment, the density of *M. liliana* was critical in mediating the response of DEA. Both the concentration of surface sediment pore water NH_4^+ and abundances of *M. liliana* were significantly positively correlated with DEA_{CN}. This agrees with my expectation that factors that promote the coupling of nitrification and D_N (i.e. bioturbation-induced increases in sediment oxygenation, solute transport, etc.) would lessen the negative effect of enrichment on DEA (i.e. DEA_{CN} declines from 1 would be less). *M. liliana* is a surface deposit feeding bivalve known to influence sedimentary oxygen and nitrogen fluxes (Thrush et al. 2006, Volkenborn et al. 2012, Pratt et al. 2015). The feeding and burrowing behaviour of this species injects pulses of oxygen rich water into sediments as well as creating hydrostatic pressure gradients in the sediment profile. This increases the oxic-anoxic interface (both spatially and temporally), accelerates solute exchange, and forces nutrient-rich anoxic water shallower in the sediment profile (and into the oxic nitrification zone) (Volkenborn et al. 2012). Others have shown that under well flushed conditions (i.e. via bioturbation and/or in permeable sediments advective pore water flushing) nitrification is positively correlated with NH_4^+ concentrations (Caffrey et al. 2003, Huettel et al. 2014), in this case bioturbation by *M. liliana* appears to be the flushing mechanism.

Adult *M. liliana* (≥ 10 mm) live deep in the anoxic zone of the sediments (about 10 cm depth) (Hewitt et al. 1997) and therefore are likely to have a strong positive influence on coupled D_N. In this study adult *M. liliana* did not show significant individual effects on DEA_{CN}, this is unsurprising given that they were in low densities, and sampling two 0.013 m^2 area cores per plot was unlikely give an accurate representation of the resident individuals. Despite this, adult *M. liliana* still featured in models explaining variance in DEA_{CN} in both treatments, suggesting an influence on the activity of the resident denitrifier population. The grouping of juvenile *M. liliana* included all those < 10 mm, encompassing young juveniles (≤ 5 mm) that occupy surface sediments (< 2 cm depth, within typical oxic zones) and larger juveniles (5 - 10 mm) that occupy sediments between 2 and 10 cm depth (Hewitt et al. 1997), below the typical oxic depth of these types of sediments. Juveniles (< 10 mm) showed a strong positive effect on medium treatment DEA_{CN} and despite being shallower dwelling than adults, their activities are likely to increase oxic zones and the transport of nutrient rich pore water (relative to un-bioturbated sediments) also facilitating coupled D_N. The negative effect of adult *A. stutchburyi* on medium treatment DEA_{CN} may also be explained by the species' biology. *A. stutchburyi* are surface dwelling filter feeders that 'bulldoze' the top layer of sediments; activity that may enhance pore water NH_4^+ efflux and bypass the nitrification process, and/or enhance efflux of nitrate making it unavailable for denitrification.

Negative ecosystem effects increased with increased nutrient enrichment (i.e. from medium to high); in particular loss of key species and decreases in DEA performance. The high nutrient treatment reduced the abundance of juvenile *M. liliana* and subsequently the positive influence on DEA_{CN} seen in the medium treatment was gone. With reduced abundance of this key species under high nutrient stress, the fundamental role in explaining DEA_{CN} (and supporting coupled D_N) was taken up by the remaining community of nutrient processing macrofauna. Both the diversity (S) and abundance (N) of the functional group were significantly positively correlated with DEA_{CN} indicating that both are important for maintaining coupled D_N (and therefore nitrogen removal) under high nutrient stress (albeit at reduced efficiency). It is possible that pore water NH_4^+ concentrations, particularly

in high treatments, reached a threshold where nitrification was either saturated or suppressed (Anthonisen et al. 1976, Henriksen & Kemp 1988). Maintenance of nutrient processing from bioturbation is important for resistance to negative feedbacks that cause nitrification inhibition. This study has shown that different elements of biodiversity, especially functional group species abundance and diversity, and key species size and abundance, are important for ecosystem functioning under increasing nutrient stress. Nutrient stress caused reduced diversity of nutrient processors which may lead to reductions in ecosystem resilience to nutrient enrichment. Such effects may be further exacerbated by multiple stressor effects associated with habitat loss, pollution and fisheries exploitation (Rothschild et al. 1994, Thrush & Dayton 2002, Lohrer et al. 2004a, Solan et al. 2004).

Land use intensification and terrestrial nutrient loading to the marine environment will continue to increase therefore maintenance of soft sediment nutrient processing will be paramount for coastal ecosystem resilience to eutrophication. This in situ study has demonstrated that under nutrient stressed conditions, key species, and then abundance and diversity of a functional group govern an essential nitrogen removal process that may ultimately mitigate shifts towards eutrophication. Furthermore, these results provide an example of how community response diversity contributes to ecosystem resilience to nutrient enrichment stress (Elmqvist et al. 2003, Mori et al. 2013). Increasing stress to soft sediment ecosystems can cause loss of bioturbators, decoupling of processes and changes in ecosystem functioning (Lohrer et al. 2011, Pratt et al. 2013). This is a concern for sediment nitrogen removal given the demonstrated dependence of soft-sediment ecosystem processes on macrobenthic communities. Although both the medium and high levels of nutrient stress led to reductions in nutrient processing, the effects were greater with the higher level of stress, due to the reduced abundance of a key species and decoupling of processes that occurred in this treatment type. This supports the notion that losses of large or functional species that play pivotal roles in ecosystem processes leads to loss of ecosystem resilience (Thrush et al. 2006, Norkko et al. 2013), with implications for future management of coastal ecosystems. If stress thresholds are crossed, causing

reductions in key nutrient processing species and functional diversity, there may be long-term effects on ecosystem resilience to eutrophication. This could contribute to tipping points and major regime shifts in coastal ecosystems (Thrush et al. 2014). This chapter highlights the importance of biodiversity and community composition for ecosystem resilience to stress.

Chapter Five

Environmental drivers of Denitrification Enzyme Activity in oligotrophic temperature estuaries

5.1 Introduction

Nitrogen pollution and the eutrophication of estuarine and coastal ecosystems are a global threat (Gruber & Galloway 2008) and recent analyses highlight that we may be near or in fact over a tipping point (Rockström et al. 2009a, 2009b). For estuaries, increasing nutrient inputs are often associated with inputs of terrestrially derived sediments, and these stressors do not always act in isolation (Galloway et al. 2008, Hewitt et al. 2016) . Collectively these stressors (along with others e.g. fishing, invasive species, habitat modifications) can result in eutrophication, biodiversity loss, decreased ecosystem functioning, reductions in ecosystem services, and may result in reduced capacity to process nitrogen (Valiela et al. 1992, Vitousek et al. 1997, Lohrer et al. 2004b). The interaction effects of nitrogen and other stressors may be greater or different than individual stressor effects, and together these stressors may increase ecosystem sensitivity to tipping points (Thrush et al. 2014, Valiela & Bartholomew 2014). Transformation of nitrogen underpins a number of important estuarine ecosystem services and estuaries are a globally important nitrogen sink significantly influencing the amount of nitrogen transported from land to sea (Seitzinger 1988). The efficiency of this sink is due in part to the denitrification that occurs in estuary soft sediment ecosystems removing excess bioavailable nitrogen. The environmental factors that control denitrification vary considerably in space and time and thus it is difficult to estimate, quantify or predict denitrification rates at estuary scales (Cornwell et al. 1999, Groffman et al. 2006). Despite the importance of denitrification to the nitrogen cycle, factors affecting variability at scales

relevant to management are poorly understood (Davidson & Seitzinger 2006, Piña-Ochoa & Álvarez-Cobelas 2006).

Denitrification is an anaerobic process carried out by a diverse array of heterotrophic microbes (i.e. denitrifiers) in terrestrial and aquatic ecosystems resulting in nitrogen loss through conversion of bioavailable nitrogen (NO_3^-) to gaseous end products (N_2O or N_2). Drivers of denitrification have been studied across and within different ecosystems, and the first order variation in denitrification rates can be attributed to carbon and nitrogen availability (reviewed by Cornwell et al. 1999). Organic matter loading has long been recognised as a primary factor controlling sediment denitrification rates in coastal ecosystems (Caffrey et al. 1993, Cornwell et al. 1999). Physical sediment characteristics such as grain size are also known to influence denitrification rates in marine systems (Kessler et al. 2012), freshwater streams (Opdyke & David 2007), wetlands (Palta et al. 2014), floodplains (Pinay et al. 2000) and forest soils (Groffman & Tiedje 1989), and in general, the importance of the sedimentary environment for biogeochemical processes is significant (Huettel et al. 2014).

Denitrification in many aquatic, and particularly estuarine, ecosystems is diffusion dominated (Seitzinger et al. 2006), so delivery of nitrate to the denitrification zone is likely to be strongly influenced by factors such as macrofaunal bioturbation and sediment porosity. When benthic macrofauna are present, bioturbation (which moves and mixes sediments and pore water), and bioirrigation activity (which brings oxygenated water (and nitrate) from the water column) mean that denitrification is no longer limited by solute diffusion. Changing sedimentary conditions, especially increases in organic matter and fine sediments, are key stressors for coastal environments worldwide, yet generally, co-variables representing sediment type and benthic macrofaunal communities are often not reported in denitrification studies (For exceptions see Wall et al. 2005, Gongol & Savage 2016, Humphries et al. 2016). Consequently, these important co-variables are absent from meta-analyses of denitrification across larger spatial scales or ecosystems (e.g. Piña-Ochoa & Álvarez-Cobelas 2006). Because benthic macrofauna are highly sensitive to environmental changes, there are implications for the resilience of denitrification when stressors occur.

Factors that control denitrification can vary considerably depending on the scale of the study, and particularly between site specific studies (Piña-Ochoa & Álvarez-Cobelas 2006). Much of the aquatic denitrification literature has focussed on anthropogenically impacted ecosystems, and these studies have shown that availability of water column nitrate is the major factor controlling denitrification in both freshwater and marine systems (Seitzinger 1988, Nielsen et al. 1995, Kana et al. 1998, Piña-Ochoa & Álvarez-Cobelas 2006, Rissanen et al. 2013). More studies addressing the spatial heterogeneity of denitrification in ecosystems are needed, as many studies may have under-sampled and failed to describe spatial variability (Piña-Ochoa & Álvarez-Cobelas 2006). Further, spatial models for mapping habitats and ecosystem services are becoming a widely used management tool (e.g. Middelburg et al. 1996, Nixon et al. 1996, Burkhard et al. 2013). However, studies modelling benthic denitrification in aquatic ecosystems largely focus on variables such as organic carbon availability, water column nitrate concentrations, and water residence times (Seitzinger & Giblin 1996, Boyer et al. 2006), rather than variables sensitive to change such as macrofaunal communities and sediment type. This has occurred because observational measurements of other variables that might control denitrification at appropriate scales are lacking (Cornwell et al. 1999, Boyer et al. 2006, Fennel et al. 2009). Small scale or laboratory studies also do not encompass a high degree of spatial variability or complexity and therefore do not have the ability to explain denitrification in terms of habitat attributes such as benthic community or sediment type (Thrush & Lohrer 2012). Studies encompassing natural environmental gradients (including variation in sediment characteristics and macrofaunal diversity) are needed to assist modellers to predict shifts in ecosystem service delivery resulting from environmental change or shifts in community composition (Snelgrove et al. 2014).

The aim of this study was to determine the drivers of denitrification across estuaries in northern New Zealand, to determine where denitrification is occurring based on abiotic and biotic variables. A specific objective was to identify possible environmental controls on a proxy for estuarine denitrification activity (i.e. denitrification enzyme activity, DEA). The DEA assay methodology uses an acetylene block technique and provides a measure of denitrification under optimal

conditions. It therefore represents the nitrogen removal capacity of the existing denitrifier community, but does not quantify actual in situ denitrification rates (Smith & Tiedje 1979, Seitzinger et al. 1993, Groffman et al. 1999, Groffman et al. 2006) which are more time consuming and expensive to obtain. My measurements span broad sedimentary gradients allowing me to elucidate how DEA may respond to changing environmental conditions. This study is unique, because unlike previous meta-analyses of denitrification (Seitzinger 1988, Cornwell et al. 1999, Piña-Ochoa & Álvarez-Cobelas 2006, Seitzinger et al. 2006), there is a consistency of methodology across the entire dataset and I have measured a large number of explanatory co-variables including macrofauna, which have normally been ignored. Accordingly, this study advances our understanding of the role of biological and physical factors in driving spatial variability in denitrification within and among estuaries.

5.2 Methods

5.2.1 Study sites and data compilation

Data were compiled from two published and three unpublished studies carried out in four bar-built estuaries with extensive intertidal areas, and catchments with variable land use (Figure 5.1, Table 5.1). In total, the dataset consisted of samples collected from 134 plots (1 m^2) during austral summer months (December-March) between 2013 and 2015 (see Appendix 12 for details). For the Mahurangi dataset, microphytobenthic biomass and macrofaunal community information was only collected in 16 of the 32 plots. For investigating DEA-sediment properties relationships the full dataset of 134 plots was used, but for analyses involving faunal and microphytobenthic variables a reduced dataset of 118 plots was used. The estuaries contained overlapping gradients in abiotic (especially sediment properties) and biotic factors (Table 5.2) allowing data to be pooled and analysed for patterns across estuaries.

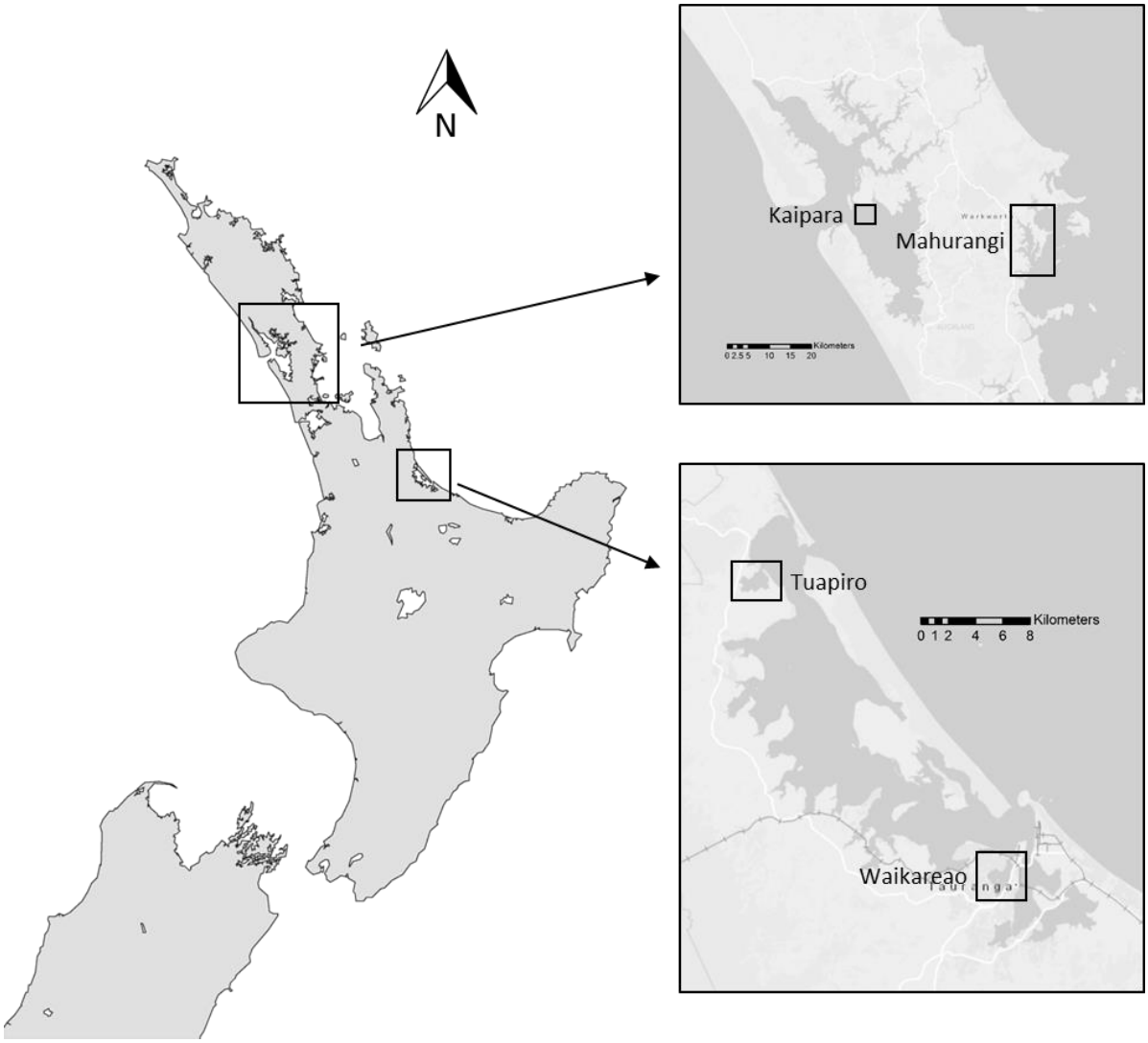

Figure 5.1 Locations of the four estuaries included in this study. See Appendices 4, 12 & 13 for details of sampling locations within estuaries.

Table 5.1 Data sources, sampling protocols and estuary characteristics. Refer to Appendix 10 for further details.

Estuary	Site location		Sample date	Plots	Sampling and data source	Water temperature	Estuary size	Catchment size	Catchment land use			
				n		°C	ha	ha	%			
									Agri	Hort	Veg	Urb
Tuapiro	37° 29’ S	175° 57' E	Jan 2014	25	5 mid-intertidal sites (5 plots in 5 x 5 m area) from head to mouth of estuary. Unpublished survey data.	22.6 ± 0.2	240[1]	7,675[1]	32[1]	15[1]	53[1]	<1[1]
			Dec 2014	24	Control plots from; 12 sites (n=2) across ~300 m of intertidal flat. Douglas et al. (in prep.) (Chapter 3 of this thesis).	20 ± 0.1						
Waikareao	37° 41’ S	176° 9’E	Dec 2013	25	5 intertidal sites (5 plots in 5 x 5 m area) from head to mouth of estuary. Unpublished survey data.	20.6 ± 0.2	200[2]	7,404[3]	41[3]	6[3]	35[3]	18[3]
Kaipara	36° 39' S	174° 29' E	Mar 2014	28	Control plots from 28 sites across 30 ha of intertidal flat. Douglas et al. (2017) (Chapter 4 of this thesis).	20.5 ± 0.04	95,000[4]	626587[5]	71[5]	<1[5]	29[5]	<1[5]
Mahurangi	36° 27' S	174° 43' E	Mar 2015	32	8 plots from 4 subtidal sites from head to mouth of estuary. Unpublished survey data.	23.1 ± 0.1	596[6]	11,500[7]	64[7]	0[7]	28[7]	5[7]

[1]BOPRC (2012b), [2]Tay et al. (2012), [3]BOPRC (2012a), [4]Kirschberg (2007), [5]Hume et al. (2007), [6]McLay (1976), [7]Boffa Miskell Limited (2008)
Land use abbreviations: Agri –agriculture, Hort – horticulture, Veg – native or exotic vegetation/forest, Urb – urban or intensive rural.

Table 5.2 Ranges (min-max) of environmental and biological variables used in analyses. See Appendix 10 for the raw data.

Estuary	Sampled plots	Mud	OC	GSM	Chl *a*	Phaeo	S	N	LB
	n	%	%	µm	µg g^{-1}	µg g^{-1}	n core $^{-1}$	n core $^{-1}$	n $core^{-1}$
Kaipara	28	0 – 14.5	0.6 – 2.0	177 – 241	3.6 – 23.2	1.5 – 17.9	9 – 29	19 – 419	1 – 41
Tuapiro	49	0 – 21.6	1.5 – 5.6	112 – 462	6.0 – 46.5	1.6 – 9.7	12 – 29	53 – 599	0 – 134
Waikareao	25	3.7 – 33.9	1.9 – 4.5	130 – 445	6.0 – 37.2	4.0 – 19.2	6 – 27	100 – 557	0 - 86
Mahurangi	16	16.7 – 52.2	3.8 – 5.7	65 – 151	2.6 – 9.9	13 – 4.7	4 – 24	50 – 263	0 – 2
All	118	0 – 52.2	0.6 – 5.7	65 – 462	2.6 – 37.2	1.3 – 19.2	4 – 29	19 – 599	0 – 134

OC: sediment organic content, Mud: sediment mud content, GSM: grain size median, Chl *a*: chlorophyll *a*, Phaeo: phaeophytin, S: number of species, N: number of individuals, LB: number of large bivalves *A. stutchburyi* and *M. liliana.*

5.2.2 Denitrification Enzyme Activity

Surveys of Tuapiro and Waikareao estuaries were conducted first and measured DEA across sedimentary gradients (mud content: 3.4 – 34%, organic content, 1.6 – 5.6%). Measurements of substrate (carbon and nitrate) limitation of denitrifying bacteria were also undertaken because most denitrification studies show nitrate and organic carbon to be the main factors controlling denitrification (e.g. Caffrey et al. 1993, Herbert 1999, Piña-Ochoa & Álvarez-Cobelas 2006). This dataset included samples from 25 plots (5 plots at each of 5 sites from head to mouth) in each estuary (see Appendix 10). Methods for DEA sample collection and assays are described in full in Douglas et al. (2017) (Chapter 4), but briefly, 5 replicate sediment cores (0-5 cm depth, 5.3 cm dia.) were taken randomly from each plot and pooled. Unfiltered seawater was collected from each site, and water and sediment were stored on ice for transport to the laboratory where they were stored at 4°C. Four assays were conducted on each sample from the Waikareao and Tuapiro surveys: control (no amendment), +C (amended with 30 mg L^{-1} C as glucose), +N (amended with 10 mg L^{-1} N as KNO_3), and +N+C (amended with 30 mg L^{-1} C as glucose and 10 mg L^{-1} N as KNO_3). The results of these initial surveys indicated that DEA in Waikareao and Tuapiro estuaries was nitrate not carbon limited, and rates without nitrate amendment (control and +C treatments) were very low (Figure 5.2, Table 5.3). Because of this only fully amended DEA assays (+N+C) were conducted in the other studies (Tuapiro experiment, Kaipara, and Mahurangi), and hereafter DEA refers to +N+C assays (unless stated).

Assays were conducted at room temperature (20°C) within 48 h of sample collection using the chloramphenicol amended acetylene inhibition technique (Tiedje et al. 1989, Groffman et al. 2006). Gas samples (6 mL) were collected from each assay over a 2 h time course (10, 30, 60, 120 min) under anoxic conditions with constant mixing (125 rpm) after the addition of acetylene (which blocks conversion of N_2O to N_2). Gas samples were analysed for N_2O concentration using a Varian CP 3800 gas chromatograph equipped with a HayeSep D column and an electron capture detector (ECD). DEA rates (i.e. N_2O production) were expressed per unit area of sand flat (μmol N m^{-2} h^{-1}; Douglas et al. (2017), Chapter 4).

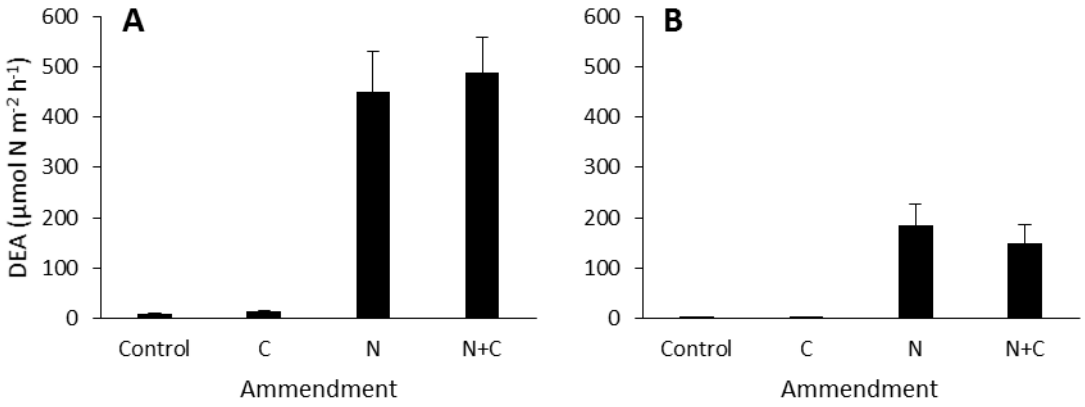

Figure 5.2 Mean DEA values in surveys of (a) Waikareao and (b) Tuapiro estuaries with different substrate amendment treatments. Error bars represent standard error (n=25).

Table 5.3 Results of PERMANOVA comparing DEA with different substrate amendments in Waikareao and Tuapiro estuaries. Post-hoc pair-wise tests are given to show differences among treatments.

Source	df	MS	Pseudo-F	Perm-p	Post-hoc
Waikareao					
Treatment	3	1750000	24.34	0.0001	Control = +C < +N = +N+C
Residual	96	71816			
Total	99				
Tuapiro					
Treatment	3	231000	12.31	0.0001	Control = +C < +N = +N+C
Residual	96	18753			
Total	99				

5.2.3 Ecosystem properties

Sampling of each plot was performed as described in Douglas et al. (2016) (Chapter 2). Briefly, sediment properties and microphytobenthic biomass were characterised from 5 pooled surface sediment samples (2.6 cm dia., 0-2 cm depth), and macrofauna from one sediment core (or 2 for the Kaipara study, averaged) (13 cm dia., 15 cm depth) retained on a 500 µm mesh. For measurements of sediment grain size (grain size median (GSM) and mud content (mud)), organic matter was first removed from samples using 10% hydrogen peroxide, then samples were analysed using a Malvern Mastersizer 2000 (Singer et al. 1988) to derive median grain size and proportion of mud (particle sizes <63 µm). Sediment samples were dried to a constant mass (60°C, 48 h), then organic content (OC) was determined by weight loss on ignition (550°C for 4 h) (Parker 1983). Microphytobenthic biomass (Chlorophyll *a* (Chl *a*) and phaeophytin (phaeo)) was determined after extraction of pigments from freeze-dried sediments using 90% acetone, then a Turner Designs 10-AU flourometer was used to measure fluorescence (Arar & Collins 1997). Preserved (50 % isopropyl alcohol) macrofauna were stained with Rose Bengal and in the laboratory, all organisms were counted and identified to the lowest possible taxonomic level (usually species).

5.2.4 Data Analysis

To test for significant differences in DEA with different substrate amendment treatments (control, +C, +N, +N+C) from Waikareao and Tuapiro survey samples, PERMANOVAs were performed using PERMANOVA+ add-on for PRIMER v7 (Clarke & Gorley 2015). Post-hoc pair-wise t-tests were used to identify which treatments were significantly different from each other. Bivariate scatter plots and a matrix of Pearson's correlation coefficients were used to explore the spread of DEA data across the ranges of predictor variables, and to ascertain patterns and relationships among predictors, and between predictors and DEA. Macrofaunal variables included number of species (S), total abundance (N), and number of large bivalves (LB, *Austrovenus stutchburyi* and *Macomona liliana*). Large species such as *A. stutchburyi* and *M. liliana* are known to strongly influence soft sediment ecosystem functions, and S and N are commonly used indexes of macrofaunal

community composition (Thrush et al. 2006, Sandwell et al. 2009, Jones et al. 2011, Norkko et al. 2013, Pratt et al. 2013).

Relationships between DEA and sedimentary variables, mud content and OC, were investigated using bivariate regression models. To identify environmental and macrofaunal community variables explaining differences in DEA across the estuaries, a multiple regression analysis (using a distance based linear model (DistLM)) was performed (PERMANOVA+ add-on for PRIMER v7). The model used a Euclidean distance matrix of DEA values with a backwards selection procedure and the corrected Akaike information criterion (AICc) to first identify significant individual predictors (marginal tests), and then the best combination of predictors of DEA (full model). I considered the effects of multicollinearity on the models, however no variables were excluded because significant relationships (Pearson's $r > 0.7$) were not detected between any of the predictor variables (Table 5.4) (Dormann et al. 2013). A step-wise sequential selection procedure was then used on the variables included in the full model to show the cumulative variance accounted for by each after mud was fitted. The predictor variables in the full DistLM model were grouped into sedimentary and macrofaunal variables, and a variance partitioning analysis was performed to determine how much of the variability was attributed to each group individually, and how much was shared (Borcard et al. 1992, Anderson & Cribble 1998).

Table 5.4 Pearson's correlation coefficients between environmental variables and DEA (n=118). Significance levels are **p ≤ 0.01, and * p ≤ 0.05.

	OC	**Mud**	**GSM**	**Chl *a***	**Phaeo**	**S**	**N**	**LB**
OC								
Mud	0.69**							
GSM	-0.18	-0.51**						
Chl *a*	0.20*	0.12	-0.04					
Phaeo	0.00	0.05	0.05	0.63**				
S	-0.31**	-0.31**	0.17	-0.17	0.23*			
N	0.14	0.20*	0.24**	0.60**	0.42**	0.10		
LB	-0.22*	-0.36**	0.55**	0.05	0.08	0.27**	0.19*	
DEA	0.64**	0.78**	-0.42**	0.36**	0.10	-0.39**	0.32**	-0.18

OC: sediment organic content, Mud: sediment mud content, GSM: grain size median, Chl *a*: chlorophyll *a*, Phaeo: phaeophytin, S: number of species, N: number of individuals, LB: number of large bivalves *A. stutchburyi* and *M. liliana.*

5.3 Results

In the full dataset, the sampled plots encompassed a wide range in sediment properties (Table 5.2). Specifically, mud content varied from 0 to 52% and with increasing mud content there were increases in OC and decreases in GSM (Table 5.4). Macrofaunal community characteristics were weakly correlated with mud content; total abundance (N) increased with increasing mud, whereas taxonomic richness (S) decreased (Table 5.4, Figure 5.3a, b). Numbers of large bivalves also decreased with increasing mud content (Table 5.4), with an apparent threshold at 30% mud content above which large bivalves completely disappeared (Figure 5.3c).

Both mud and OC were strong individual predictors of DEA (Table 5.4, Figure 5.4), and they co-varied (Pearon's r = 0.69). DEA was positively correlated with mud, and the relationship was best described by a non-linear 2nd order polynomial (DistLM R^2 = 0.71, AIC_c = 1404, $p < 0.0001$) rather than a linear model (DistLM R^2 = 0.63, AIC_c = 1433, $p < 0.0001$). The better fitting non-linear model indicates a threshold above 30% mud content where DEA no longer increased (Figure 5.4a). DEA and OC were also positively correlated although but there was no evidence of a threshold response; a non-linear model (DistLM R^2 = 0.46, AIC_c = 1485, $p < 0.0001$) did not account for more variation than a linear model (DistLM R^2 = 0.46, AIC_c = 1484, $p < 0.0001$) (Table 5.4b).

DistLM marginal tests showed that individually mud accounted for the largest proportion of the variability in DEA (61%), and therefore was fitted first in the full DistLM model (Table 5.5). After mud was fitted, the other variables accounted for less than 5% each of the total explained variation in DEA (Table 5.5). The model cumulatively explained 74% of the variability and included three sedimentary variables (mud, OC, and GSM) and three macrofaunal variables (N, S and large bivalves) (Table 5.5). Variance partitioning analysis showed that 44% of the variability in DEA was accounted for by the sedimentary environment alone, 10% by macrofaunal community characteristics, and 20% was shared due to co-variation between mud and macrofauna.

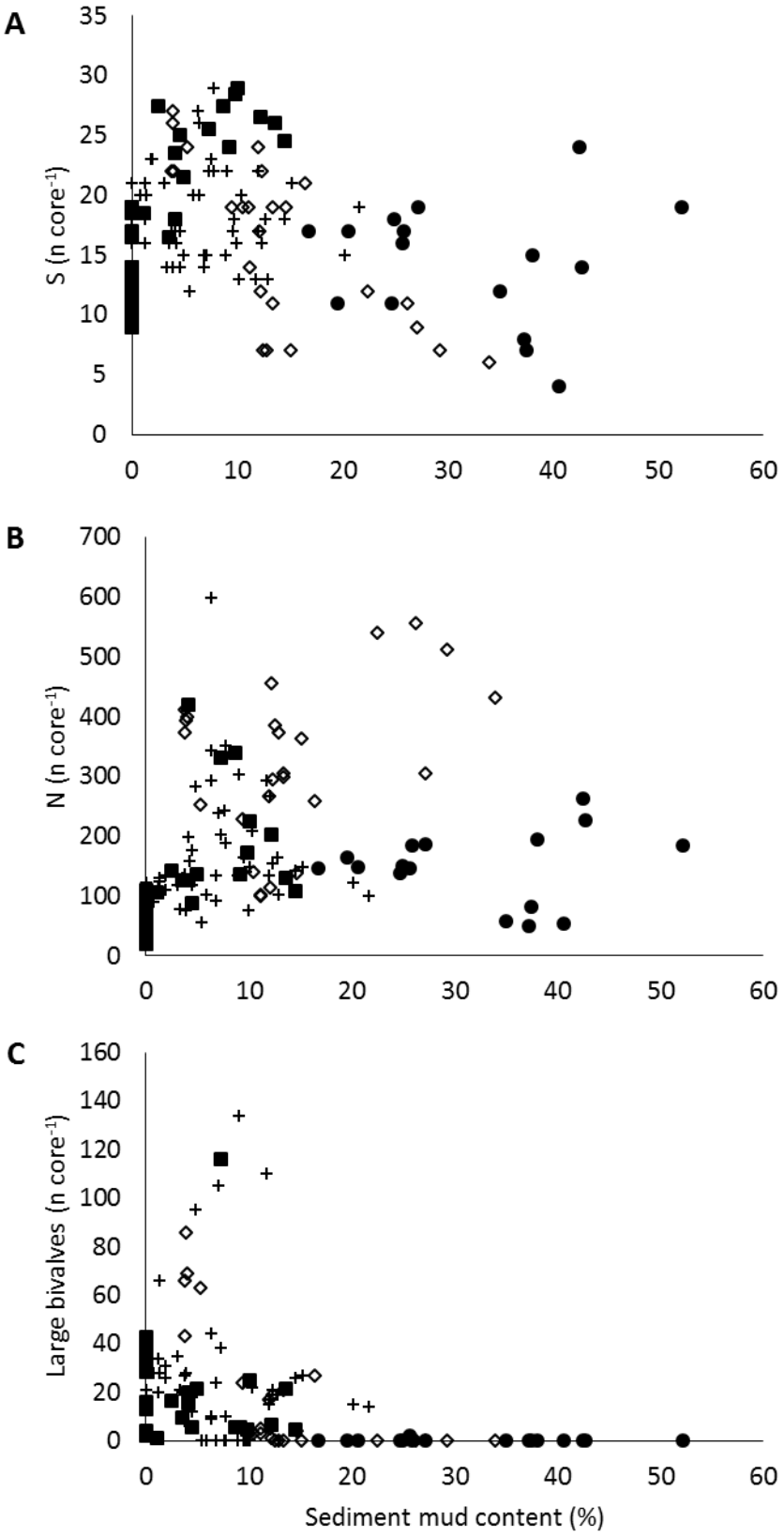

Figure 5.3 Macrofaunal community charachteristics as a function of sediment mud content; number of (a) species (S), (b) individuals (N), and (c) large bivalves (*M. liliana* and *A. stutchburyi*) in Waikareao (◊), Tuapiro (+), Kaipara (■), and Mahurangi (●) estuaries.

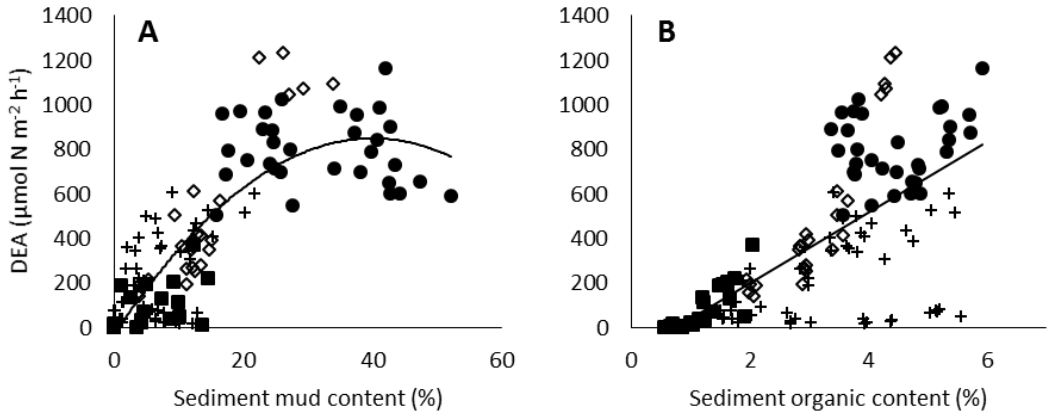

Figure 5.4 Relationship between DEA and a) sediment mud content and b) organic content from Waikareao (◊), Tuapiro (+), Kaipara (■), and Mahurangi (●) estuaries. Fitted lines are (a) 2nd order polynomial (DistLM R^2 = 0. 71, AIC_c = 1404, p = 0.0001), and (b) linear (DistLM R^2 = 0.46, AIC_c = 1484, p = 0.0001), which provide the best description of the data (see text for details).

Table 5.5 Results of stepwise sequential DistLM test showing the combination of predictors that best explain variability in DEA

		Variation explained		
Variable	**AIC_c**	**Prop.**	**Cumul. R^2**	**p**
Mud	1253	0.61	0.61	0.0001
N	1246	0.03	0.64	0.004
S	1236	0.04	0.68	0.0005
OC	1233	0.01	0.69	0.03
GSM	1226	0.02	0.71	0.003
LB	1217	0.03	0.74	0.002

OC: sediment organic content, Mud: sediment mud content, GSM: grain size median, Chl *a*: chlorophyll *a*, Phaeo: phaeophytin, S: number of species, N: number of individuals, LB: number of large bivalves *A. stutchburyi* and *M. liliana.*

5.4 Discussion

In this study I used a large dataset to identify the abiotic and biotic controls on denitrification activity across multiple estuaries. Within and among the sampled estuaries there was a range of sediment types from sandy (0% mud) with little organic content, to very muddy (52% mud) organic rich sediments. The main variables influencing DEA were sedimentary variables; proportion of fine sediment (% mud) was the strongest predictor of DEA across all the estuaries, but organic content (which co-varies with mud) was also strongly positively correlated with DEA. In different sediment and habitat types there will be different selective pressures which will influence the standing stock, community composition and performance (i.e. rates) of nitrifying and denitrifying bacteria (Groffman & Tiedje 1989, Cavigelli & Robertson 2000, Cavigelli & Robertson 2001, Wallenstein et al. 2006).

Optimal conditions for denitrifiers include a source of nitrate, and in most northern New Zealand estuaries, water column and pore water nitrate concentrations are generally low (pore water < 100 µM, water column < 50 µM) (Lohrer et al. 2004a, Thrush et al. 2006, Tay et al. 2012, Santos et al. 2014, Gongol & Savage 2016), therefore most denitrification is probably coupled to, and limited by nitrification (microbial conversion of ammonium to nitrate) in the sediments (Seitzinger et al. 2006, Gongol & Savage 2016). The controls on coupled nitrification-denitrification may differ to those of denitrification fuelled by water column nitrate (D_w), but the diffusive properties of sediments will influence both coupled and D_w (Cornwell et al. 1999). If an estuary shifts to a eutrophic state, D_w would likely become dominant over coupled nitrification-denitrification, meaning less reliance on conditions that facilitate nitrification. However, denitrification would still be limited by factors that influence the delivery of nitrate to the denitrification zone. Specifically, nitrogen removal would be restricted by sediment conditions that reduce solute movement and diffusion (i.e. more mud, less animals).

Data showed a threshold in DEA at about 30% mud, an important finding considering the substantial and ongoing increases in fine sediment inputs to coastal ecosystems (Thrush et al. 2004). This study clearly identified sediment

grain size as a key controller of DEA, but despite increasing mud inputs being a key stressor in aquatic systems, variation in grain size is not often explored as driver of denitrification (reviewed by Cornwell et al. 1999). Sediment mud content influences the physical movement of pore water throughout the sediment profile and therefore the availability of nitrogen and carbon for denitrifiers, as well as the oxygen profiles in the sediment. 30% mud content may be the point where further increases in mud inhibits factors that contribute to the coupling of nitrification and denitrification. Muddy sediments may reduce movement of oxygenated water due to lower advective exchange from the water column to the pore spaces (Glud 2008). The extent of the oxygenated zone in the sediments determines the region where nitrification, and therefore where coupled nitrification-denitrification can occur. Sediment size was an important predictor of denitrification in a study of four South Island New Zealand estuaries measured using the isotope pairing technique. Similar to my study, higher denitrification rates were found in sites with finer sediments (Gongol & Savage 2016). Furthermore, this study showed that coupled nitrification-denitrification was greater at sites with finer sediments and deeper oxygen penetration depth. In a study of wetland soils where most of the denitrification was of nitrate produced within the soil, soil properties associated with high denitrification rates were those that facilitated nitrification (Palta et al. 2014).

Sediment organic content was the second strongest individual predictor of DEA after mud content, and mud and organic content always co-varied. Sediment organic matter can provide both a nitrogen source (through mineralisation to ammonium), and a source of organic carbon which heterotrophic denitrifying bacteria require for energy (Wallenstein et al. 2006). Many studies have measured higher denitrification rates at sites with high organic matter loading (e.g. Barnes & Owens 1998, Bruesewitz et al. 2012, Eyre et al. 2013). Also, decomposition of organic matter in the sediments consumes oxygen (Glud 2008) which may reduce the nitrification zone (Eyre & Ferguson 2009). Not only the quantity, but the quality of organic carbon available to denitrifiers can determine denitrification rates (Dodla et al. 2008, Eyre et al. 2013). Although not measured, differences in the quality of organic matter in the sediments may be the reason I did not find OC

to be the best predictor of denitrification. Even though sediment OC was positively correlated with DEA across all estuaries, I found in amendment trials that availability of organic carbon was not limiting DEA in surveys of two of these estuaries. From this I can assume that higher DEA in muddy organic rich sediments (compared with sandy organic poor sediments) is associated with the physical sediment properties and/or the supply of ammonium (from organic matter mineralisation) for coupled nitrification-denitrification, rather than a higher supply of organic carbon for denitrifiers.

Benthic macrofauna were important, but weak co-variance with mud content meant that effects were secondary to sediment properties for explaining DEA in the statistical model. Correlations show that higher DEA was seen in areas with high abundances but low diversity of benthic macrofauna. The threshold response of increasing DEA up to 30% mud content is also reflected in the sediment mud-macrofaunal relationships; above 30% mud content, abundances of large bivalves reduce to zero (Figure 5.3, Figure 5.4a). Similar responses of macrofaunal communities to sediment mud content (>20% mud) have been demonstrated in other studies as well as their impacts on ecosystem functions (Thrush et al. 2003b, Anderson 2008, Pratt et al. 2013). Decreases in ecosystem function (DEA) at the same level of sediment mud content where macrofaunal diversity and key macrofaunal species appear to decline, imply there may be important biodiversity-ecosystem function relationships at play, and that the importance of these relationships may change as mud content increases (Thrush et al. 2017).

Macrofaunal bioturbation and burrowing can increase the oxygen penetration depth as well as the heterogeneity of the interface between the oxic and anoxic layers in the sediments, thereby increasing sites for coupled nitrification-denitrificaton (Aller 1988, Kristensen 1988). However, this positive influence may cease at about 30% mud content, due to loss of macrofaunal diversity and large species, or reductions in macrofaunal activities such as burrowing (Needham et al. 2011). Similarly, other studies have shown that with increasing environmental stress ecosystem functions decrease (Pratt et al. 2013, Douglas et al. 2017). Different elements of macrofaunal diversity are important for maintaining ecosystem functions at different levels of stress (Norkko et al. 2015, Douglas et al.

2017, Gammal et al. 2017, Thrush et al. 2017), but this study shows that there are limits to the amount of stress that can be endured whilst maintaining important ecosystem functions such as denitrification.

Estuaries are the interface between the land and the sea, and they are the receiving environment of land use intensification and increased human activity. Inputs of stressors to estuarine soft sediment ecosystems, especially sediments and nutrients, will continue to increase, and a broader knowledge of how these systems will respond is needed. Using this study encompassing environmental gradients and spatial variability in DEA measurements I can make some generalisations about denitrification in estuaries and how it might respond to environmental change. It is fitting that I have measured DEA, as it does not represent an actual denitrification rate, but an integrator of the duration a site experienced conditions that supported the production of denitrification enzymes and microbes. Despite sampling across substantial spatial and temporal variability, I have seen a distinct relationship between DEA and sedimentary environment; higher fine sediment and organic matter loading is associated with higher denitrification activity, but only up to 30% mud. Identification of factors that can be used as proxies for denitrification aids in the development of tools such as mapping and modelling ecosystem services. Just six easily measured biotic and abiotic variables were able to explain 74% of the variation in denitrification activity across 4 different estuaries. Furthermore, sediment grain size, which has been absent from many denitrification studies, explained 61% of the variability on its own. Extension of this study to include more estuaries encompassing broader spatial scales and environmental gradients while measuring multiple biotic and abiotic co-variables will help to enhance habitat and regional-scale understanding of estuary denitrification, as well as increase the utility for scaling up measurements and creating models. However, cross-comparison of DEA with direct measurements of in situ denitrification rates is needed (e.g. using membrane inlet mass spectrometry techniques). Future studies should also consider measurement of other microbial pathways that lead to gaseous nitrogen losses, such as anammox (Dalsgaard et al. 2005, Hulth et al. 2005, Burgin & Hamilton 2007), to understand the full potential of estuaries to serve as nitrogen

sinks prior to discharge to the ocean. Identifying tipping points and drivers of estuarine nitrogen removal across broader environmental gradients will significantly enhance the ability to manage and predict changes in ecosystem service delivery with impending environmental change.

Chapter Six

General Discussion

This thesis investigates denitrification in New Zealand estuaries using a combination of literature review and method development, observational studies, and manipulative field experiments. I used denitrification enzyme activity (DEA) as a proxy for denitrification, a measure that represents the maximum denitrification that can occur under optimal conditions. It was suitable for the large sampling programs of this thesis because it is cheap and easy to sample, and it is a proven tool for spatial comparisons of denitrification (Barnes & Owens 1998, Groffman et al. 1999, Livingstone et al. 2000, Bernot et al. 2003, Wigand et al. 2004). Collectively, the research chapters show that denitrification is a highly variable ecosystem function that is sensitive to stressors, and is important for maintaining resilience of coastal ecosystems to nutrient enrichment.

6.1 Summary

Before this thesis, there have been few studies that have attempted to measure denitrification response to experimental nutrient or organic matter enrichment beyond the laboratory (e.g. Caffrey et al. 1993, Koop-Jakobsen & Giblin 2010, Oakes et al. 2011). Nutrient enrichment experiments in situ have been variable in both their methodologies and aims (see Chapter 2), and to the best of my knowledge, this thesis contains the first in situ sediment nutrient enrichment experiments that have measured denitrification, or a proxy for denitrification. Furthermore, simultaneous investigation of response to nutrient and sediment stressors in situ, has not been demonstrated in the literature before this work. The manipulative experiments in Chapters 3 and 4 both show that increasing stressors cause increased variability in DEA, and higher unexplained variability. Another attribute of this thesis is that I have measured multiple co-variables alongside DEA. This is unique, and has pinpointed that there are important local and habitat level controls on denitrification that may have been overlooked in previous studies. Identification of biotic and abiotic controls on denitrification can

help us to understand feedbacks between co-variables and denitrification activity, and how they change with increasing stressors, or combinations of stressors.

One of the main thesis objectives was to investigate the response of denitrification and other ecosystem functions to nutrient enrichment. To achieve this, field experiments were conducted to assess the effects of increased sediment pore water nitrogen on ecosystem function. In **Chapter 2** I assessed and compared existing nutrient enrichment experiment literature, and found that an appropriate technique for artificially increasing sediment pore water nitrogen concentration was absent. This chapter therefore described and evaluated a new field method, and assessed the environmental controls on the enrichment effect (pore water ammonium concentration). Sedimentary and macrofaunal variables were important factors governing the enrichment effect; pore water ammonium concentrations in enriched plots were negatively correlated with sediment mud content and macrofaunal community richness. This showed that sedimentary and macrofaunal variables are necessary to consider when designing nutrient enrichment experiments.

Chapter 3 investigated how sedimentary environment influenced ecosystem function response to nutrient enrichment. Using the technique described in Chapter 2, I conducted a field experiment where plots were enriched along a sand to mud gradient on an intertidal flat. Ecosystem functions including DEA, nutrient processing, primary productivity and community metabolism, were all influenced by sediment mud content. Where nutrient treatment effects occurred, they were dependent on mud content, showing that increasing sedimentation can influence the response of ecosystem functions to enrichment. Factors that increase sediment oxygenation (e.g. abundance of benthic macrofauna) helped to maintain DEA under enriched conditions. Results of this chapter show that ultimately, increasing the muddiness of estuaries will reduce their ability to be resilient to nutrient stress. Furthermore, the muddying of estuaries may reduce nitrogen removal capacity of soft sediment ecosystems resulting in shifts toward, and feedbacks that facilitate eutrophication. This study is unique to the global literature because it demonstrates multiple and cumulative stressor effects of sediments and nutrients on estuarine soft sediment ecosystem functioning.

The purpose of **Chapter 4** was to investigate the influence of macrofaunal community on DEA and the response of DEA to nutrient enrichment. This was done with a focus on variability in the abundance and diversity of macrofauna species within a functional trait group associated with nutrient processing. A manipulative field experiment with 2 levels of enrichment (medium and high), was conducted across a landscape of benthic macrofaunal community composition (using the technique described in Chapter 2). This study demonstrated that key species as well as the diversity of functional species are both important for maintaining denitrification under nutrient enriched conditions, and that as nutrient stress increased, different elements of diversity were important. The findings of this chapter are significant ecologically because they support the hypothesis that biodiversity enhances ecosystem resilience to stress.

Chapter 5 aimed to elucidate the variability in, and drivers of denitrification in New Zealand estuaries which are relatively un-impacted compared with those dominating the existing estuarine denitrification literature. Combining measurements of DEA in ambient conditions in a range of estuaries showed that DEA is highly variable and is influenced by multiple and interacting biotic and abiotic factors. Most importantly, I found that DEA was influenced primarily by the local sedimentary environment, particularly the proportion of fine sediment ('mud'). DEA increased with increasing sediment mud content, but only up to a threshold (30% mud), after which there were no further increases. These findings have important implications for management considering sedimentation is an increasing stressor for coastal ecosystems globally; if muddiness increases too much, the ability of soft sediment ecosystems to remove excess nitrogen may decrease and resilience to nutrient enrichment will be lost.

Collectively the research chapters of this thesis comprehensively demonstrate the factors that control sediment denitrification activity in northern New Zealand estuaries, and that these controlling factors can change with increasing stressors.

6.2 Consequences of increasing sediment and nutrient loads for denitrification and estuary resilience

Environmental change is likely to bring increasing stressors to coastal ecosystems, especially increased sediment and nutrient loads from land (Hewitt et al. 2016). This thesis has shown that these stressors independently, and cumulatively influence ecosystem functioning and denitrification through direct and indirect mechanisms. Globally denitrification is reported to be strongly associated with water column nitrate concentrations (Seitzinger 1988), but water column nitrate concentrations in New Zealand estuaries are generally very low (Thrush et al. 2006, Lohrer et al. 2010, Tay et al. 2012, Santos et al. 2014). Therefore most of the denitrification is probably coupled to nitrification in the sediments (Gongol & Savage 2016), and environmental variables may have a different influence than estuaries where denitrification of water column nitrate is the dominant process of nitrogen removal. The variables that explained DEA in Chapters 3, 4 and 5 were those that would enhance coupled nitrification denitrification by; supplying nitrate (sediment organic content), or providing sediment mixing and oxygenation which stimulates nitrification and coupling to denitrification (e.g. macrofaunal community variables). If these factors that enhance the coupling of nitrification and denitrification are reduced with increasing stressors, so too will denitrification and estuary resilience to nutrient enrichment.

6.2.1 Sedimentary environment

Sedimentary environment was the main factor controlling nutrient enrichment level, or DEA in the research chapters of this thesis. In general, DEA increased with increasing sediment mud and organic content. Sediment mud and organic content controlled the level of elevation in pore water ammonium concentration after enrichment (Chapter 2). The proportion of fine sediments influenced DEA directly by influencing pore water and solute movement, and indirectly by influencing macrofaunal communities (Chapter 3). Furthermore, the proportion of fine sediments influenced the response of ecosystem functions to nutrient enrichment (Chapter 3). Spatial variability (driven largely by differences in sediment organic and mud content) was responsible for most of the variability in DEA in Chapter 4, and masked any nutrient enrichment effects. Conclusively, Chapter 5

demonstrated the importance of sediment mud content by showing that it accounted for most of the explained variability in DEA in ambient sediments from five separate studies across four different estuaries. These results are significant since sedimentation of coastal environments is likely to increase due to intensifying catchment land use and increased frequency of storms (Thrush et al. 2004, Hewitt et al. 2016).

6.2.2 The role of macrofauna

Macrofaunal community variables were important for explaining variability in DEA in the different studies of this thesis. This is because of their role in mediating sediment mixing through bio-irrigation which controls oxygen and nutrient profiles in the sediments (Henriksen et al. 1983, Aller 1988, Braeckman et al. 2010, Stief 2013). Macrofaunal effects on DEA were masked due to natural (and expected) covariance of community characteristics with the sedimentary environment. However, when this overriding influence of background variability (sedimentary environment) on DEA was removed by control normalising enrichment plot data, the importance of the macrofaunal community and its diversity was very clear (Chapter 4). Macrofauna played a crucial role in mediating nutrient enrichment effects on denitrification (by facilitating coupled nitrification denitrification through bioturbation), and their role was dependent on the level of nutrient stress. On average, enrichment caused reductions in DEA, but these reductions were lessened by the presence of large bioturbating macrofauna (medium enrichment) or abundance of individuals with nutrient processing traits (high enrichment) (Chapter 4). This showed that in a stressed system, macrofaunal communities contribute to resilience of ecosystem functioning (in this case denitrification), and that a more biodiverse ecosystem may have better insurance against loss of ecosystem function. The study highlights the importance of conserving the necessary aspects of biodiversity to maintain ecosystem functioning and resilience to stressors.

To fully understand the importance of macrofaunal communities for ecosystem resilience to nutrient and sediment stressors, further exploration of denitrification and community characteristics and structure across stressors gradients is needed. Investigation of the role of macrofauna in a multiple stressor experiment

(nutrients and sediments) like that in Chapter 3 would be an obvious next step for further research. Furthermore, using interaction networks may help answer some of these questions and provide insight into how ecosystem resilience to nutrients may change under different stress regimes (Thrush et al. 2012). For example, interactions between the variables identified as important for denitrification will change with increasing stressors, but we do not know how this will influence denitrification. Interaction network analyses can do this, and help forecast ecosystem stability with environmental change (Thrush et al. 2014).

6.2.3 Multiple stressors and tipping points

In real world ecosystems stressors never occur independent of others, therefore it is important to investigate effects together. Complicating the effects of multiple stressors is the interacting effects of local stressors with global stressors such as climate change. For example, effects of climate change on communities may be greater in places with higher levels of existing stressors (Folke et al. 2004, Harley et al. 2006, Hewitt et al. 2016), and this may have knock-on effects for ecosystem functions (and their resilience) that are highly connected to macrofaunal community attributes. Tipping points or threshold changes occur when the level of a stressor, or combination of stressors, reaches a point where an ecosystem changes, often irreversibly, to an alternate state (Holling 1973, Thrush et al. 2014). Furthermore, tipping points and thresholds are more likely to be met in ecosystems where resilience has been reduced (Scheffer et al. 2001, Folke et al. 2004).

My research suggests that the factors controlling denitrification may be different depending on the state of estuary degradation. Addition of nutrients caused changes in both the relationships among co-variables, as well as between co-variables and DEA. There were changes in the sediment-macrofauna relationships between ambient and enriched sediments; when nutrients were added, many of the mud-macrofauna relationships changed, but these changes were different in the different experiments and with the different levels of nutrient stress (Chapters 3 & 4). Increased variability can be a symptom of stress (Warwick & Clarke 1993), and the changes in sediment-macrofauna relationships reflects this. Predicting and understanding ecosystem resilience to nutrient enrichment requires more

rigorous testing of the factors controlling denitrification under different stress regimes. One way to do this would be to conduct denitrification surveys of, or enrichment experiments in, estuaries that have been subject to higher levels of nutrient enrichment, or have shifted closer to a eutrophic state (i.e. higher ambient water column and pore water nutrient concentrations). Such research may show context dependency of thresholds, and differences in ecosystem function response and resilience to nutrient and sediment stressors.

Defining thresholds and limits in ecosystem variables where tipping points may occur under different stress or climate change scenarios is integral to predicting and preventing ecosystem degradation and ecosystem service loss. For estuaries, knowing where thresholds in factors that will influence denitrification occur (e.g. sediment mud content), will be important for managing nutrients and preventing eutrophication. DEA in ambient nutrient conditions increased with increasing mud content up to 30%. Beyond this point, DEA was variable but no longer increasing, suggesting that there is a threshold at which DEA rate is maintained below 30% mud content (Figure 6.1, Chapter 5). Chapter 3 showed that under nutrient enriched conditions this threshold may occur at a lower mud content (10%), and combining all fertilised plots from Chapters 3 and 4 further supports this (Figure 6.1). These findings demonstrate that when stressors are combined (in this case nutrients and sediments) they can act cumulatively on ecosystem functions, and their impacts are greater together than they are alone. Investigating the spread of ambient and enriched DEA data in plots with less than 25% mud content (the maximum of enriched plots) supports this (especially the modelled line for enriched sediments in Figure 6.1), and shows that enrichment lowered DEA (Figure 6.2). To be more confident in this conclusion, however, measurements of DEA under nutrient enriched conditions are needed over a greater range of sediment mud content.

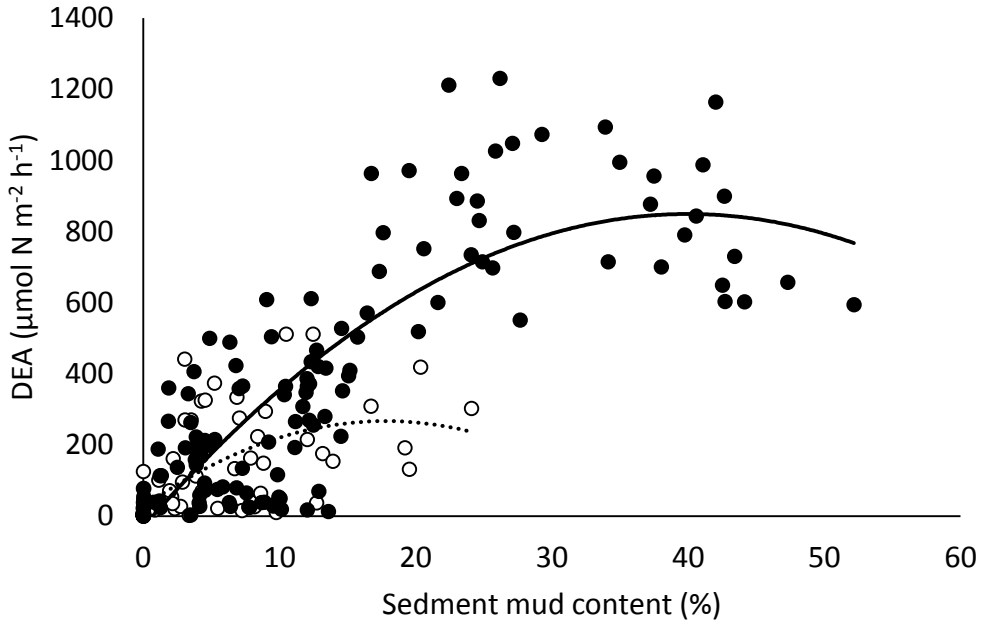

Figure 6.1 Relationship between sediment mud content and DEA in ambient (black circles) and enriched (white circles, 150 & 600 g N m^{-2}) sediments. 2nd order polynomial regressions provided the best fit for both ambient (DistLM R2 = 0.71, AIC = 1404, p = 0.0001) and enriched datasets (DistLM R^2 = 0.39, AIC = 749, p = 0.0003).

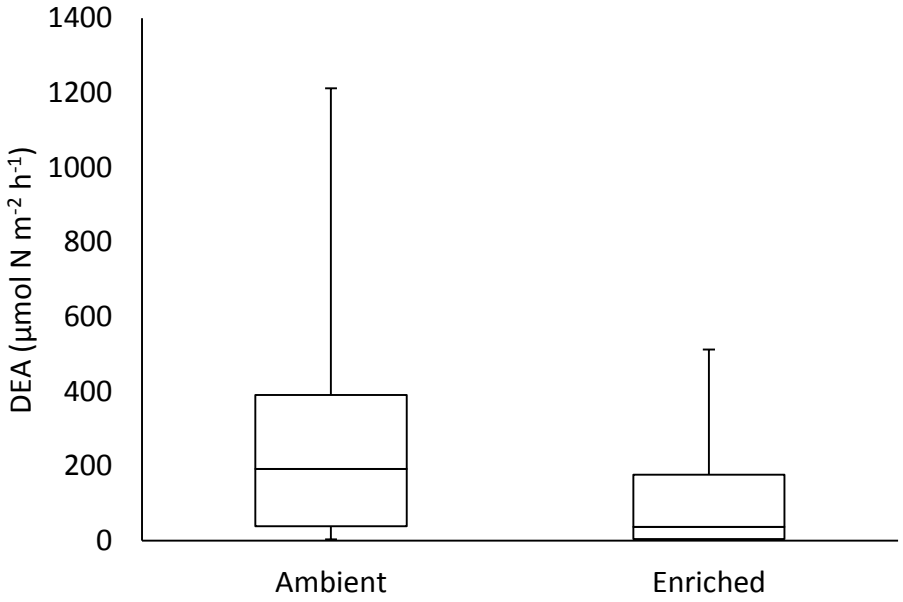

Figure 6.2 Variability in DEA in ambient (n = 107) and enriched plots (n = 80, application rates of 150 and 600 g N m^{-2}) with <25% mud content. Boxes represent 25%, median and 75% distributions, with whiskers the non-outlier minimum and maximum.

To fully understand nitrogen cycling in estuaries, and especially tipping points, other pathways of nitrogen cycling need to be investigated (e.g. dissimilatory nitrate reduction to ammonium (DNRA), anaerobic ammonium oxidation (anammox), iron-driven denitrification, sulfur-driven denitrification, biomass assimilation). As discussed in Chapter 3, the threshold in DEA may be reflecting a switch to other nitrogen cycling pathways, such as DNRA or anammox (Burgin & Hamilton 2007, Giblin et al. 2013). DNRA may have become dominant over denitrification in enriched sediments if there was a high Carbon:Nitrogen ratio, or if conditions became sulfidic (Burgin & Hamilton 2007). Sulfidic conditions may have occurred if macrofaunal die-off occurred in plots with the high enrichment treatment, although I do not have the data to speculate on whether this happened. Anammox is unlikely to be a dominant pathway of nitrogen removal in New Zealand estuaries because anammox bacteria are slow-growing autotrophs that require both nitrite and ammonium (Burgin & Hamilton 2007). New Zealand estuaries generally have well oxygenated water columns with low nitrite and nitrate concentrations, so it is improbable that anammox bacteria would be competitive against heterotrophic denitrifiers. However, future work needs to incorporate and investigate the importance of other nitrogen cycling pathways in New Zealand estuaries, as well as across stressor gradients. Identification of tipping points where other nitrogen cycling processes may become dominant over denitrification is highly relevant for nitrogen management with environmental change.

6.3 Thesis synthesis and future directions

Context dependent effects were evident throughout the chapters of this thesis, and further support the notion that ecological experiments should encompass broad environmental gradients to increase the generality of findings (Thrush et al. 2000, Snelgrove et al. 2014). Nutrient enrichment was expected to instigate increases in DEA, however this only happened in some instances, and without some further carefully designed experiments it is difficult to speculate on which abiotic and biotic factors can be attributed to increases versus decreases in DEA at larger scales (i.e. among estuaries). DEA rates were expected to be higher in estuaries that had experienced higher levels of background nutrient loading.

Although I did not assess catchment nitrogen loading in this thesis, it was clear that DEA responded positively to ambient (natural) sediment nutrient loading (Chapters 3 & 5). DEA was always significantly positively correlated with sediment organic content, and this is likely the main supply of ammonium for coupled nitrification denitrification (as shown in Chapter 5). How estuary denitrification relates to catchment nitrogen loading requires further research; this could be done by extending this study to include more estuaries with a greater range of degradation status or catchment land use, and combining this with indicators of anthropogenic land use pressures and nutrient loading (e.g. Savage et al. 2010, Bruesewitz et al. 2011, Bierschenk et al. 2012).

This study only used DEA, a proxy for denitrification, and I therefore cannot make conclusions about absolute denitrification rates or quantities of nitrogen removal in the studied estuaries. However, use of this method has enabled investigation of relative denitrification at scales and replication levels that are not possible with other techniques. DEA provides a measure of the resident denitrifier population and its nitrogen removal capability under optimum conditions. It provides an indication of the history of denitrification in the sediments, and therefore what an ecosystem has been exposed to and what it might be resilient to. Extending the duration of a nutrient enrichment study and measuring DEA throughout a period of ongoing long-term enrichment may provide insight into how denitrifying populations change in response to nutrient enrichment both short- and long-term. Or better yet, combining DEA measurements with measurements of the microbial community (e.g. Abell et al. 2013, Yazdani Foshtomi et al. 2015), over a course of sediment enrichment. This may reveal further how denitrification as an ecosystem service will be resilient to environmental change and increasing stressors. Monitoring DEA more frequently and earlier following experimental enrichment would also provide a better picture of how denitrifier populations respond to enrichment.

DEA can be used as an indicator of 'denitrification potential' to show the maximum nitrogen removal capacity of a sediment sample (Sorensen 1978, Ogilvie et al. 1997, Livingstone et al. 2000, Magalhães et al. 2005, Zhong et al. 2010). Scaling these values up can therefore be a tool for (coarsely) estimating maximum whole

ecosystem nitrogen removal capacity. There are caveats to this however, because assays are conducted under optimal and unrealistic conditions (ideal temperature, constant anoxia, constant mixing, and unlimited carbon and nitrate supply). Also, DEA values do not account for enzymatic and microbial growth and change that would be constantly occurring in the real world.

Understanding nitrification in oligotrophic (or non-eutrophic) estuaries is arguably as important as understanding denitrification, since most of the bioavailable nitrogen in New Zealand estuaries is in the form of ammonium (Lohrer et al. 2010). The DEA method blocks the nitrification process so does not provide an indication of coupled nitrification-denitrification (Groffman et al. 2006). Therefore, I needed to draw assumptions about the nitrification process, and factors that were possibly reducing or restricting it, and in turn reducing DEA. Measuring both nitrification and denitrification in future studies would remove the need for such assumptions. Direct methods of measuring denitrification such as Membrane Inlet Mass Spectrometry using chamber incubations of the sediment water interface would enable this (Kana et al. 1998), and alongside DEA would provide better ability to scale-up and generalise DEA studies (Bernot et al. 2003).

Laboratory denitrification experiments enable precise monitoring of oxygen and solute concentrations, however, broad scale field experiments provide generality in conclusions that far outweigh the benefits of more 'accurate' laboratory studies (Thrush & Lohrer 2012). Studies of denitrification with more intensive monitoring of pore water conditions in the field would increase the understanding of smaller scale controls on denitrification. The eutrophic conditions simulated in the enrichment experiments of this thesis showed elevations of pore water ammonium for a duration of up to 7 weeks. In reality, eutrophication and its effects on benthic communities and ecosystem functioning would happen gradually and over much longer timescales. Future research would therefore also benefit from longer term (e.g. months to years) monitoring of such enrichment experiments, and subsequent changes in DEA.

The studies in this thesis have direct relevance and applications for environmental managers. Data has been used to inform the development of ecosystem principles for denitrification as an ecosystem service, and these will be used to develop

ecosystem service maps for denitrification in New Zealand coastal ecosystems as part of the Valuable Seas component of the National Science Challenges. As part of this work, the Whitford embayment (an area with a long history of estuarine intertidal monitoring) in the Auckland Region, will be surveyed in March 2018. This study will measure; direct denitrification using chamber incubations and MIMS, DEA, and macrofaunal community and environmental variables, with the aim to further understand the relationships between nutrient processing ecosystem functions and local drivers.

Estuary habitat type can have a strong influence on denitrification (Eyre et al. 2011), but in New Zealand the value of different habitats for nitrogen removal is not known. Investigation of where hotspots of denitrification occur in coastal ecosystems according to habitat type (e.g. mangrove forests, seagrass beds, mudflats, sandflats) will further enhance the ability to map and model denitrification. This research will be carried out in conjunction with analyses of catchment land use and nitrogen loading, developing much needed knowledge of the connectivity between terrestrial and marine systems. Additionally, data from this thesis could also be used as a starting point for developing monitoring programs for denitrification in northern New Zealand estuaries.

6.4 Concluding remarks

The effects of nitrogen enrichment in aquatic ecosystems are well established, but the value of receiving environments in processing nitrogen is yet to be realised. Collectively the studies that comprise this thesis have underscored the importance of healthy soft sediment ecosystems for ecosystem service delivery, and resilience to environmental stressors in the face of environmental change. This thesis combined studies of denitrification at habitat, estuary, and regional scales and therefore encompasses a large degree of environmental variability, providing a significant contribution to the literature. I have shown that the effects of nutrients and sedimentation on denitrification are multifaceted; responses of DEA to nutrients were context dependent, and all four research chapters showed that multiple co-variables were needed to explain the high degree of DEA variability in both ambient and nutrient enriched sediments. New Zealand estuaries are going to be subjected to a rapid rise in nitrogen enrichment in the coming years, as the

effects of intensifying land use, and nutrients stored in groundwater make their way to the sea. Understanding the drivers of nitrogen removal in estuaries, and being able to foresee ecosystem response and potential for resilience will be paramount for environmental management, sustainability and restoration of healthy estuary ecosystems.

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Appendices

Appendix 1

Summary of published literature of in situ sediment fertiliser enrichment studies (Chapter 2).

	Experimental design					Fertiliser application				Pore water enrichment	
Source	**Purpose of study**	**Substrate**	**Sites**	**Treatment replicates**	**Spatial scale (km)**	**Type**	**Diffuser device**	**Depth (cm)**	**Rate (g N m^{-2})**	**Effect detected**	**Effect size**
Orth (1977)	SG	Veg	2	40	> 5	SR	N	0-1	64-128	NR	-
Bulthuis and Woelkerling (1981)	SG	Veg	1	3	< 1	Inorg	Y	10	100	↑	4.8-30
Pulich Jr (1985)	SG	Veg	2	12	> 5	SR	N	0-1	20	NR	-
Dennison et al. (1987)	SG	Veg	2	12	< 1	Inorg	Y	0-10	NR	↑	-
Williams (1987)	SG	Veg	1	8	< 1	SR	Y	0-5	140	↑	209-352
Powell et al. (1989)	SG	Veg	5	5	< 1	Org	N	0	NR	↑	-
Short et al. (1990)	SG	Veg	1	6	< 1	SR	N	20-25	NR	NR	-
Williams (1990)	SG	Veg	1	4	< 1	SR	Y	0-5	604	↑	0.19-5.6
Perez et al. (1991)	SG	Veg	1	1	< 1	SR	N	0-1	2150	NR	-
Bulthuis et al. (1992)	SG	Veg	5	15	> 5	SR	Y	10	100	↑	1.0-52.6
Flothmann and Werner (1992)	EU	Un-veg	1	6	< 1	Inorg	Y	8	NR	↑	-
Kenworthy and Fonseca (1992)	SG	Veg	3	9	1-5	SR	Y	?	3.2-53	NR	-
Murray et al. (1992)	SG	Veg	1	?	< 1	Inorg	Y	15	100-200	↑	1.3-2.0
Williams and Ruckelshaus (1993)	SG	Veg	1	7	< 1	Inorg	Y	0-5	54	↑	5.0-9.3
Erftemeijer et al. (1994)	SG	Veg	3	18	> 5	SR	N	10-15	4.9	↑	1.2
Fonseca et al. (1994)	SG	Veg	2	2	> 5	SR	Y	7.6	694	NR	-
Feller (1995)	M	Veg	1	3	< 1	SR	Y	0-10	30-135	↑	6.8-61.0
McGlathery (1995)	SG	Veg	2	2	1-5	SR	Y	?	NR	?	-
Pedersen (1995)	A+SG	Veg	1	1	< 1	Inorg	N	?	NR	NR	-
Posey et al. (1995)	EU+FW	Un-veg	1	15	< 1	SR/Inorg	Y	0, 0-7.5	2.3	NR	-
van Lent et al. (1995)	SG	Veg	2	8	> 5	SR	N	10	190	↑	1.4-3.0
Vetter (1996)	EU	Un-veg	1	4	< 1	Org	N	0	NR	?	-
Ceccherelli and Cinelli (1997)	EU+A+SG	Veg	1	6	< 1	SR	Y	1-6	10.4	NS	10.5
Udy and Dennison (1997)	SG	Veg	1	3	< 1	SR	N	0.5-1.0	88	↑	139
Posey et al. (1999)	EU	Un-veg	2	14	> 5	SR	Y	0-7.5	69	NR	-

	Experimental design					Fertiliser application				Pore water enrichment	
Source	**Purpose of study**	**Substrate**	**Sites**	**Treatment replicates**	**Spatial scale (km)**	**Type**	**Diffuser device**	**Depth (cm)**	**Rate (g N m^{-2})**	**Effect detected**	**Effect size**
Piceno and Lovell (2000)	EU, B	Veg	1	1	< 1	Inorg	N	0	16.3	NS	0.74-1.44
Worm et al. (2000)	Method review	Un-veg	1	8	< 1	SR	N	0-10	150	↑	17.5
Posey et al. (2002)	EU, FW	Un-veg	2	14	> 5	SR	Y	0-7.5	NR	↑	-
Morris and Keough (2003a)	EU	Un-veg	1	8	< 1	SR	Y	0-1	1579-3158	NS	-
Morris and Keough (2003b)	EU	Un-veg	2	12	> 5	SR	Y	1-2	123-2467	↑	-
Ferdie and Fourqurean (2004)	SG	Veg	6	24	> 5	SR	N	0	NR	NR	-
Armitage et al. (2005)	SG, FW	Veg	6	36	> 5	SR	N	0	NR	NR	-
Lever and Valiela (2005)	EU	Un-veg	3	15	1-5	SR	Y	1	196	↑	20.4-34.6
Armitage et al. (2006)	EU	Veg	4	24	> 5	SR	N	0	NR	NR	-
Gil et al. (2006)	EU	Veg	2	12	> 5	SR	N	0-1	NR	NR	-
Posey et al. (2006)	EU, FW	Un-veg	4	36	> 5	SR	Y	0-7.5	NR	NR	2.2
Stutes et al. (2006)	EU	Un-veg	2	20	1-5	QR	Y	10	3.2-4.5	↑	1.3-100
O'Brien et al. (2009)	EU	Un-veg	1	24	< 1	SR	N	4	389	↑	14.9-51.9
Santos et al. (2009)	EU	Un-veg	1	6	< 1	QR	N	0	NR	NS	-
O'Brien et al. (2010)	EU	Veg + Un-veg	1	5	> 5	SR	N	5	750	↑	7.0-16.0
Olsen and Valiela (2010)	SG	Veg	1	6	< 1	SR	N	0-20	306	↑	289
Piehler et al. (2010)	EU	Un-veg	1	4	< 1	Inorg	N	0	NR	NS	-
Cebrian et al. (2012)	EU	Un-veg	2	20	1-5	QR	Y	10	NR	↑	-
Fitch and Crowe (2012)	EU	Un-veg	1	8	< 1	SR	Y	0-6	10-20	↑	4.8-7.6
O'Gorman et al. (2012)	EU	Un-veg	1	8	< 1	SR	Y	0-6	10-20	NR	-
Botter-Carvalho et al. (2014)	EU	Un-veg	1	6	< 1	QR	N	0	1200-2400	NR	-
Guevara et al. (2014)	EU, B	Veg	6	36	> 5	SR	N	0	NR	NR	-
Current study		Veg + Un-veg	1	28	< 1	SR	N	0-15	150 & 600	↑	1-580

Abbreviations: Purpose of study: EU; eutrophication/nutrient effects, SG; seagrass growth and nutrient limitation, FW; foodweb/community structure, M; mangrove growth, A; macroalgae growth, B; bacterial community response. Fertiliser type: SR; slow release, QR; quick release, Inorg; inorganic salts or solutes, Org; organic nutrients. Rate: NR; application rate not reported, or not reported in a comparable way. Pore water enrichment: ↑; pore water nutrient concentration increase, NS; no significant increase in pore water nutrient concentration detected, NR; pore water concentration not reported, or not reported in a comparable way. Effect size: treatment concentration/ambient concentration.

Appendix 2

Location of study site on Tapora Bank, Kaipara Harbour, 36° 39’ S, 174° 29’ E (Chapters 2 & 4).

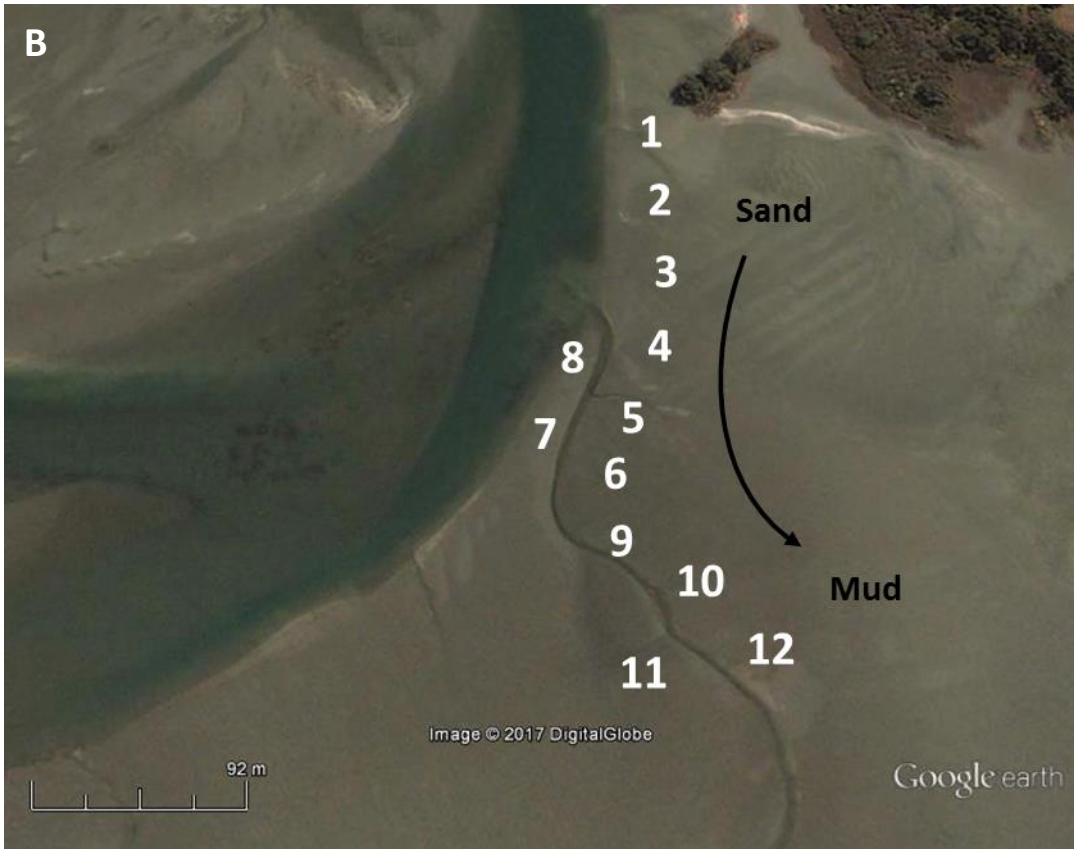
Appendix 3

Examples of sediment pore water NH_4^+ concentrations from estuaries with developed (anthropogenically modified) catchments sampled from a range of sediment depths (0-100 cm), compared to those observed during this study (Chapter 2).

Source	Estuary	Country	NH_4^+ (µM)
Santos et al. (2014)	Tauranga	New Zealand	6-52
Cabrita and Brotas (2000)	Tagus Estuary	Portugal	18-40
Percuoco et al. (2015)	Great Bay Estuary	USA	50-1400
De Vittor et al. (2012)	Marano-Grado Lagoon	Italy	52-900
Zhang et al. (2013)	Pearl River Estuary	China	64-321
Vidal and Morgui (1995)	Alfacs Bay	Spain	100-600
Magni et al. (2014)	Shinkawa-Kasugawa Estuary	Japan	200-500
Lohrer et al. (2010)	Mahurangi Estuary	New Zealand	257-1542
Pérez-Villalona et al. (2015)	San Juan Bay Estuary	Puerto Rico	461-572
Cook et al. (2004b)	Huon Estuary	Australia	500
Clavero et al. (2000)	Palmones River Estuary	Spain	500-3500
Bally et al. (2004)	Seine Estuary	France	1940
Gonçalves et al. (2012)	Santos-Cubatao Estuarine System	Brazil	2495-4989
This study	Application rate 150 g N m^{-2}		64-10275
	Application rate 600 g N m^{-2}		11-18842

Appendix 4

Tuapiro Estuary, Tauranga Harbour (37° 29’ S 175° 57' E) showing (A) locations of survey sites (1-5, Chapter 5), and experimental site (white box, Chapter 3), and (B) Locations of 12 sites across a sedimentary gradient within the experimental site (Chapter 3).



Appendix 5 Pearson's correlation coefficients between environmental and community variables, and ecosystem functions for control plots (n=24) (Chapter 3).

	OC	Mud	GSM	Chl *a*	Phaeo	pH	aRPD	NO_2^-	NO_3^-	NH_4^+	S	N	*A. stu* (<10 mm)	*A. stu* (<10 mm)	*A. stu* (<10 mm)	*A. stu* (<10 mm)
OC																
Mud	0.92															
GSM	-0.91	-0.77														
Chl *a*	0.63	0.43	-0.61													
Phaeo	0.93	0.90	-0.84	0.68												
pH	-0.48	-0.52	0.23	-0.03	-0.35											
aRPD	-0.19	-0.32	0.15	0.47	-0.12	0.41										
NO_2^-	-0.66	-0.52	0.65	-0.48	-0.65	0.23	0.13									
NO_3^-	-0.67	-0.64	0.60	-0.40	-0.71	0.24	0.36	0.87								
NH_4^+	0.37	0.20	-0.40	0.53	0.39	0.08	0.31	-0.14	-0.11							
S	-0.23	-0.17	0.19	-0.01	-0.10	0.11	0.11	0.20	0.18	-0.38						
N	0.42	0.38	-0.36	0.19	0.43	-0.30	-0.13	-0.13	-0.06	0.11	0.34					
A. stu (<10 mm)	-0.49	-0.47	0.46	-0.05	-0.42	0.43	0.45	0.65	0.68	-0.09	0.38	0.01				
A. stu (≥10 mm)	0.39	0.35	-0.29	0.42	0.44	-0.24	0.12	0.02	-0.07	0.04	0.29	0.48	0.07			
M. lil (<10 mm)	-0.25	-0.23	0.20	-0.12	-0.14	0.24	0.04	0.49	0.40	0.12	0.18	0.13	0.14	0.12		
M. lil (≥10 mm)	-0.36	-0.43	0.22	-0.01	-0.35	0.39	0.39	0.24	0.31	-0.15	0.21	-0.06	0.49	0.05	-0.10	
GPP	-0.51	-0.68	0.41	0.10	-0.51	0.49	0.54	0.25	0.45	0.05	0.12	-0.15	0.56	-0.13	0.08	0.57
$GPP_{Chl\ a}$	-0.91	-0.80	0.85	-0.79	-0.91	0.38	-0.03	0.66	0.66	-0.46	0.13	-0.39	0.40	-0.47	0.26	0.28
SOC	0.33	0.39	-0.21	0.36	0.44	-0.12	0.04	-0.26	-0.31	-0.14	0.42	0.44	-0.04	0.40	0.02	0.20
Dark NH_4^+ flux	0.62	0.72	-0.45	0.19	0.52	-0.47	-0.34	-0.12	-0.21	0.16	-0.17	0.26	-0.14	0.05	-0.12	-0.43
DEA	0.95	0.86	-0.92	0.59	0.89	-0.43	-0.19	-0.62	-0.62	0.37	-0.15	0.40	-0.42	0.44	-0.31	-0.30

Appendix 6 Pearson's correlation coefficients between environmental and community variables, and ecosystem functions for enrichment plots (n=24) (Chapter 3).

	OC	Mud	GSM	Chl *a*	Phaeo	pH	aRPD	NO_2^-	NO_3^-	NH_4^+	S	N	*A. stu* (<10 mm)	*A. stu* (<10 mm)	*A. stu* (<10 mm)	*A. stu* (<10 mm)
OC																
Mud	0.90															
GSM	-0.89	-0.78														
Chl *a*	0.65	0.49	-0.61													
Phaeo	0.94	0.88	-0.87	0.72												
pH	-0.11	-0.02	0.05	-0.18	-0.16											
aRPD	-0.06	-0.13	0.09	0.53	0.05	-0.18										
NO_2^-	-0.42	-0.28	0.28	-0.43	-0.39	0.06	-0.05									
NO_3^-	-0.73	-0.66	0.53	-0.56	-0.67	0.12	-0.01	0.74								
NH_4^+	-0.25	-0.16	0.14	-0.50	-0.26	0.06	-0.18	0.73	0.40							
S	0.18	0.09	-0.34	0.60	0.28	-0.13	0.58	-0.08	-0.26	-0.11						
N	0.52	0.46	-0.64	0.47	0.52	0.00	0.31	-0.17	-0.41	-0.05	0.70					
A. stu (<10 mm)	-0.39	-0.42	0.26	-0.20	-0.38	0.42	-0.07	0.03	0.14	-0.09	0.13	0.00				
A. stu (≥10 mm)	0.37	0.24	-0.35	0.39	0.34	0.07	0.06	-0.32	-0.30	-0.20	0.23	0.43	-0.15			
M. lil (<10 mm)	0.34	0.20	-0.46	0.59	0.39	-0.01	0.30	-0.36	-0.48	-0.25	0.59	0.41	0.17	0.09		
M. lil (≥10 mm)	-0.15	-0.04	-0.05	0.13	0.02	0.07	0.20	0.14	-0.03	0.10	0.55	0.36	0.40	-0.17	0.53	
GPP	-0.25	-0.31	0.08	0.12	-0.16	-0.05	0.43	0.16	0.15	0.07	0.36	0.12	0.31	0.03	0.47	0.55
$GPP_{Chl\ a}$	-0.75	-0.60	0.63	-0.81	-0.70	-0.01	-0.21	0.51	0.67	0.52	-0.37	-0.44	0.20	-0.35	-0.36	0.19
SOC	0.29	0.16	-0.18	0.28	0.32	-0.34	0.03	-0.45	-0.56	-0.17	0.20	0.27	-0.15	0.51	0.38	0.19
Dark NH_4^+ flux	-0.48	-0.42	0.56	-0.28	-0.53	0.26	0.04	0.00	0.13	-0.02	-0.06	-0.36	0.26	-0.04	-0.17	-0.15
DEA	0.59	0.41	-0.63	0.61	0.60	-0.12	0.08	-0.48	-0.50	-0.38	0.40	0.60	-0.05	0.51	0.43	0.20

Abbreviations (Appendices 5-6): Sediment organic content (OC), sediment mud content (Mud), Grain size median (GSM), Chlorophyll *a* content (Chl *a*), phaeophytin content (Phaeo), apparent Redox Potential Discontinuity (aRPD), pore water concentrations of nitrite, nitrate and ammonium (NO_2^-, NO_3^- and NH_4^+), macrofaunal taxonomic richness (S), macrofaunal abundance (N), juvenile *A. stutchburyi* (*A. stu* (<10 mm)), adult *A. stutchburyi* (*A. stu* (≥10 mm)), juvenile *M. liliana* (*M. lil* (<10 mm)), adult *M. liliana* (*M. lil* (≥10 mm)), gross primary productivity (GPP), gross primary productivity normalised to chlorophyll *a* biomass ($GPP_{Chl\ a}$), sediment oxygen consumption (SOC), nutrient regeneration (Dark NH_4^+ flux), and denitrification enzyme activity (DEA).

Appendix 7 Site locations and raw data (Chapter 4).

	Location				Sediment properties				Microphytobenthic biomass		Pore water NH_4^+		Macrofaunal community						
Site	**Latitude**	**Longitude**	**Treatment**	**DFS**	**Seagrass**	**OC**	**Mud**	**GSM**	**Chl *a***	**Phaeo**	**0-2 cm**	**5-7 cm**	**S**	**N**	***A. stutchburyi***		***M. liliana***		**DEA**
															< 10 mm	≥ 10 mm	< 10 mm	≥ 10 mm	
	NZTM	NZTM	g N m^{-2}	m	% cover	%	%	µm	µg g^{-1}	µg g^{-1}	µM	µM	n $core^{-1}$	n $core^{-1}$	n $core^{-1}$	n $core^{-1}$	n $core^{-1}$	n $core^{-1}$	µmol N m^{-2} h^{-1}
1	1715904	5971943	0	100	56	1.89	10.04	210	13.16	9.67	0.59	5.28	13	83	10	12	2	2	50.6
			150		75	1.62	8.40	226	17.40	8.98	5.27	22.12	17	38	1	5	5	2	41.2
			600		23	1.29	9.76	210	12.07	9.11	173.50	125.89	11	38	7	9	1	0	10.9
2	1715908	5971771	0	270	17	0.86	0	216	12.74	1.59	0.17	4.56	11	57	18	6	18	2	3.6
			150		0	0.71	0	220	8.82	3.60	1.86	1.78	9	39	14	6	11	1	1.6
			600		12	0.76	0	218	9.76	4.43	67.39	83.92	10	30	8	7	7	2	1.6
3	1715908	5971577	0	463	48	1.65	7.25	207	14.63	11.25	0.57	0.00	13	147	91	22	3	1	134.7
			150		77	1.83	7.24	211	16.30	4.30	8.65	152.29	13	185	99	14	1	2	16.2
			600		41	1.77	8.61	203	31.91	9.87	71.33	166.86	14	118	64	21	2	2	65.3
4	1715904	5971494	0	546	84	1.48	1.07	209	12.47	9.34	1.29	4.15	11	32	0	1	1	0	189.0
			150		92	1.53	1.92	196	8.72	21.52	4.20	6.02	16	35	1	1	1	0	72.0
			600		72	1.55	4.53	201	25.84	7.74	8.23	57.08	16	34	2	1	1	0	181.7
5	1715921	5971296	0	743	0	1.21	9.86	187	5.96	5.20	1.67	3.10	18	140	1	0	3	1	116.8
			150		0	1.27	3.84	190	5.52	9.21	n.d.	76.57	18	160	1	0	2	2	113.4
			600		0	1.19	4.09	194	6.22	3.98	6.68	36.56	21	133	1	0	4	1	177.2
6	1715921	5971196	0	843	19	1.54	4.93	191	7.85	6.38	2.35	5.80	16	108	9	0	11	3	198.8
			150		47	1.38	2.73	209	6.75	3.57	2.99	10.80	16	52	13	1	11	1	27.9
			600		32	1.62	3.35	220	8.81	3.43	21.25	10.20	17	48	7	0	7	2	2.4
7	1715922	5971171	0	868	0	0.55	0	237	6.09	1.47	0.43	0.91	10	25	1	0	1	0	4.1
			150		0	0.59	0	229	5.66	1.58	6.60	53.01	9	21	3	0	2	1	3.1
			600		0	0.64	0	237	5.60	1.83	174.24	111.48	8	10	2	0	1	0	1.2
8	1715923	5971091	0	946	19	0.82	0	223	5.50	2.11	1.28	1.47	13	49	7	1	4	3	6.3
			150		0	0.59	0	240	5.28	1.61	4.30	23.54	9	28	4	0	0	1	6.6
			600		21	0.64	0	250	4.63	1.68	9.22	38.40	9	12	3	1	1	0	2.4
9	1715922	5971015	0	1021	47	1.05	13.58	196	7.00	4.87	0.24	0.80	20	87	9	1	8	4	14.0
			150		48	0.95	1.24	233	5.87	6.37	15.01	9.40	16	77	3	0	9	3	27.3
			600		32	0.88	0.84	213	9.93	3.30	65.35	196.91	14	34	12	1	2	2	17.9
10	1716025	5970999	0	1023	48	1.14	8.66	195	23.24	16.88	3.30	1.14	17	249	1	0	3	2	38.8

	Location				Sediment properties				Microphytobenthic biomass		Pore water NH_4^+		Macrofaunal community						
Site	**Latitude**	**Longitude**	**Treatment**	**DFS**	**Seagrass**	**OC**	**Mud**	**GSM**	**Chl *a***	**Phaeo**	**0-2 cm**	**5-7 cm**	**S**	**N**	***A. stutchburyi***		***M. liliana***		**DEA**
															< 10 mm	≥ 10 mm	< 10 mm	≥ 10 mm	
	NZTM	NZTM	g N m^{-2}	m	% cover	%	%	µm	µg g^{-1}	µg g^{-1}	µM	µM	n $core^{-1}$	n $core^{-1}$	n $core^{-1}$	n $core^{-1}$	n $core^{-1}$	n $core^{-1}$	µmol N m^{-2} h^{-1}
			150		72	1.05	8.16	201	16.77	21.61	2.92	9.52	17	155	0	0	1	2	27.2
			600		25	1.12	8.39	193	31.18	12.11	7.90	147.92	21	301	1	2	3	1	223.9
11	1716004	5971315	0	711	4	1.01	4.13	218	3.57	3.84	2.65	0.90	16	376	1	0	14	1	27.6
			150		20	1.05	7.89	210	9.59	10.29	2.44	9.18	18	519	1	0	2	3	39.9
			600		32	1.12	7.88	206	9.33	8.32	2.00	17.71	16	59	1	0	4	0	163.7
12	1715991	5971518	0	515	40	1.24	4.09	211	7.83	5.81	1.51	1.27	11	53	14	5	1	1	37.7
			150		70	1.49	2.15	221	9.93	7.65	9.82	10.27	14	96	32	3	6	0	36.3
			600		75	1.66	4.31	221	9.22	7.67	45.26	82.22	18	101	30	13	1	2	70.5
13	1715985	5971672	0	367	0	0.81	0	223	9.79	4.88	0.27	1.47	13	66	10	9	14	3	9.9
			150		0	0.79	0	215	15.46	18.39	6.08	9.50	10	39	9	5	9	2	4.4
			600		0	0.78	0	227	14.93	4.82	n.d.	n.d.	11	24	6	2	5	1	6.9
14	1715978	5971755	0	284	0	0.77	0	225	12.15	3.83	3.58	0.28	9	30	6	5	5	1	6.0
			150		0	0.73	0	225	13.95	4.51	2.70	5.85	7	23	6	4	4	2	3.2
			600		0	0.70	0	221	11.04	3.16	184.70	254.04	6	14	4	4	0	1	2.2
15	1715977	5971813	0	226	0	0.79	0	216	11.66	3.24	1.54	2.10	9	61	8	13	14	0	6.5
			150		0	0.71	0	213	13.08	3.18	21.52	226.58	8	16	2	4	3	1	1.4
			600		0	0.71	0	219	10.10	2.86	158.27	167.46	5	13	4	2	1	0	0.0
16	1716072	5971935	0	91	0	0.66	0	241	5.34	2.20	0.37	20.32	7	60	2	1	24	3	6.5
			150		0	0.73	0	242	6.86	2.57	39.87	80.56	10	44	1	0	8	1	6.8
			600		0	0.68	0	235	4.85	2.67	14.56	200.15	9	34	1	0	9	1	2.4
17	1716072	5971835	0	188	0	0.76	3.48	227	8.57	3.18	0.32	1.44	8	53	0	4	5	1	3.5
			150		9	0.74	0	223	9.01	3.90	100.35	185.34	7	33	1	2	1	0	3.0
			600		0	0.72	0	239	9.66	3.04	99.71	225.41	4	17	1	1	1	1	2.5
18	1716075	5971656	0	364	9	0.77	0	214	10.33	3.76	0.30	8.73	11	61	11	3	25	3	6.1
			150		19	0.70	0	231	9.38	10.65	81.18	156.81	11	45	15	3	14	2	23.2
			600		0	1.03	0	221	25.88	3.12	n.d.	n.d.	9	18	4	1	4	0	4.4
19	1716079	5971559	0	460	8	0.73	0	223	5.93	2.15	0.37	2.57	9	45	6	2	24	1	9.9
			150		12	0.68	0	225	28.32	3.05	3.74	13.39	13	25	4	1	6	3	9.0
			600		15	0.70	0	218	5.02	1.84	69.91	229.67	10	13	1	0	0	1	2.9

	Location				Sediment properties				Microphytobenthic biomass		Pore water NH_4^+		Macrofaunal community						
Site	**Latitude**	**Longitude**	**Treatment**	**DFS**	**Seagrass**	**OC**	**Mud**	**GSM**	**Chl *a***	**Phaeo**	**0-2 cm**	**5-7 cm**	**S**	**N**	***A. stutchburyi***		***M. liliana***		**DEA**
															< 10 mm	≥ 10 mm	< 10 mm	≥ 10 mm	
	NZTM	NZTM	g N m^{-2}	m	% cover	%	%	µm	µg g^{-1}	µg g^{-1}	µM	µM	n $core^{-1}$	n $core^{-1}$	n $core^{-1}$	n $core^{-1}$	n $core^{-1}$	n $core^{-1}$	µmol N m^{-2} h^{-1}
20	1716111	5971224	0	794	47	1.74	14.50	177	14.50	13.72	0.29	0.84	14	60	0	0	2	3	224.9
			150		97	2.06	5.22	200	12.10	14.33	2.66	15.82	18	132	0	0	3	1	374.1
			600		53	1.67	8.81	194	16.37	12.53	27.11	45.95	20	204	1	0	3	3	149.6
21	1716120	5971055	0	963	29	1.20	2.49	189	7.16	4.37	0.37	16.22	17	78	6	0	9	2	138.1
			150		39	1.43	7.04	194	9.27	10.82	21.01	52.55	18	147	3	0	6	1	276.7
			600		23	1.29	2.19	190	14.21	9.85	144.31	105.45	14	106	1	0	4	4	162.2
22	1716225	5971094	0	928	29	1.62	9.19	194	13.72	17.90	1.84	1.33	13	61	0	0	4	2	208.5
			150		21	1.42	13.17	182	12.23	10.59	12.85	45.00	15	49	1	0	3	1	176.5
			600		45	1.50	6.69	195	11.31	9.92	4.28	29.09	15	118	0	0	2	2	134.4
23	1716216	5971177	0	845	32	2.04	12.20	195	12.85	15.25	1.92	0.64	17	124	0	0	5	2	372.9
			150		29	1.43	13.91	194	15.00	20.16	3.87	28.67	14	40	0	0	1	1	154.9
			600		36	1.84	12.03	190	12.79	18.75	14.61	65.25	18	69	1	1	6	6	216.2
24	1716205	5971253	0	768	43	1.36	4.50	182	20.90	17.47	0.05	0.47	17	56	0	0	3	3	72.6
			150		87	1.49	2.86	200	10.12	12.82	1.15	6.06	15	23	0	0	6	2	96.3
			600		49	1.32	5.44	191	18.08	6.92	17.15	36.40	13	23	0	0	4	3	22.6
25	1716172	5971446	0	573	15	0.67	0	222	5.68	2.28	0.29	0.42	13	73	14	2	21	3	9.7
			150		27	0.63	0	230	5.68	2.01	5.41	35.90	13	52	13	5	6	1	3.5
			600		20	0.73	0	230	5.82	1.86	18.91	40.72	7	15	7	1	1	1	3.9
26	1716167	5971463	0	556	0	0.60	0	227	4.17	2.22	0.00	6.61	9	30	6	3	4	3	4.8
			150		3	0.64	0	226	8.47	2.37	3.20	21.80	10	19	4	2	2	1	4.2
			600		0	0.59	0	224	7.13	1.06	39.59	n.d.	5	16	5	4	1	0	1.6
27	1716143	5971774	0	244	8	0.69	0	232	9.64	1.99	0.00	0.26	7	38	3	0	10	1	3.9
			150		11	0.65	0	232	8.77	3.96	13.64	10.23	7	31	2	1	5	1	4.2
			600		1	0.69	0	232	8.19	2.87	6.18	148.90	4	16	2	2	2	1	1.9
28	1716127	5971955	0	63	0	0.70	0	239	8.89	2.33	0.03	0.50	8	15	1	1	3	0	20.1
			150		0	0.72	0	231	10.77	3.03	4.83	35.24	5	12	0	0	4	0	25.0
			600		0	0.83	0	230	11.25	2.41	61.59	n.d.	5	7	0	1	2	0	38.5

Abbreviations (Appendices 7-11): DFS = Distance from Shore, OC = sediment organic content, Mud = sediment mud content, GSM = Grain size median, Chl-*a* = chlorophyll *a* content, Phaeo = phaeophytin content, S = number functional group species, N = number of functional group individuals, *AS* = *A. stutchburyi, ML = M. liliana*, DEA = denitrification enzyme activity.

Appendix 8

Pearson's correlation coefficients (*r*) between control and treatment plot DEA and measures of macrofauna community diversity. Significance levels are *p ≤ 0.05, and **p ≤ 0.01 (Chapter 4).

	Control (0 g N m^{-2})						
		Community		*A. stutchburyi*		*M. liliana*	
	DEA	S	N	Juvenile (<10 mm)	Adult (≥ 10 mm)	Juvenile (<10 mm)	Adult (≥ 10 mm)
Medium (150 g N m^{-2})	0.69**	0.79**	0.89**	0.97**	0.91**	0.65**	0.30
High (600 g N m^{-2})	0.67**	0.75**	0.48	0.94**	0.80**	0.37	0.23

Appendix 9. Pearson's correlation coefficient matrix for control plots (n= 28) (Chapter 4).

	OC	Mud	GSM	Seagrass	Chl *a*	Phaeophytin	DFS	NH_4^+ (5-7 cm)	NH_4^+ (0-2 cm)	S	N	*AS* juvenile	*AS* adult	*ML* juvenile	*ML* adult
Mud	0.77														
GSM	-0.75	-0.75													
Seagrass	0.72	0.53	-0.58												
Chl *a*	0.48	0.36	-0.49	0.55											
Phaeophytin	0.79	0.68	-0.72	0.65	0.79										
DFS	0.33	0.48	-0.64	0.34	0.10	0.44									
NH_4^+ (5-7 cm)	-0.11	-0.23	0.09	-0.11	-0.27	-0.24	-0.15								
NH_4^+ (0-2 cm)	0.25	0.20	-0.23	0.06	0.22	0.30	0.30	-0.21							
S	0.56	0.69	-0.80	0.43	0.23	0.51	0.76	-0.12	0.27						
N	0.24	0.36	-0.28	0.08	0.08	0.25	0.33	-0.11	0.53	0.53					
AS juvenile	0.19	0.05	-0.01	0.20	0.13	0.05	-0.12	-0.11	-0.13	0.01	0.11				
AS adult	0.15	-0.03	0.12	0.10	0.19	0.00	-0.45	-0.13	-0.09	-0.18	-0.01	0.79			
ML juvenile	-0.46	-0.44	0.32	-0.44	-0.33	-0.46	-0.32	0.41	-0.22	-0.23	0.03	0.00	-0.02		
ML adult	0.00	0.21	-0.26	0.05	-0.07	0.08	0.35	0.30	-0.18	0.41	0.02	-0.11	-0.28	0.32	
DEA	0.84	0.61	-0.67	0.48	0.29	0.67	0.44	-0.04	0.25	0.46	0.12	0.02	-0.14	-0.36	0.00

Appendix 10. Pearson's correlation coefficient matrix for medium treatment plots (n= 28) (Chapter 4).

	OC	Mud	GSM	Seagrass	Chl *a*	Phaeophytin	DFS	NH_4^+ (5-7 cm)	NH_4^+ (0-2 cm)	S	N	*AS* juvenile	*AS* adult	*ML* juvenile	*ML* adult	DEA	DEA_{CN}
Mud	0.67																
GSM	-0.68	-0.70															
Seagrass	0.82	0.41	-0.41														
Chl *a*	0.17	0.27	-0.15	0.16													
Phaeophytin	0.51	0.55	-0.66	0.52	0.24												
DFS	0.31	0.45	-0.49	0.30	-0.19	0.34											
NH_4^+ (5-7 cm)	-0.12	-0.11	0.06	-0.22	-0.03	-0.25	-0.33										
NH_4^+ (0-2 cm)	-0.25	-0.22	0.26	-0.20	-0.15	-0.13	-0.33	0.68									
S	0.72	0.63	-0.63	0.66	0.07	0.54	0.61	-0.30	-0.31								
N	0.28	0.41	-0.29	0.17	-0.03	0.20	0.28	-0.07	-0.14	0.53							
AS juvenile	0.31	0.08	0.03	0.26	0.15	-0.18	-0.10	0.29	-0.02	-0.01	0.17						
AS adult	0.14	-0.02	0.12	0.11	0.25	-0.20	-0.40	0.34	-0.01	-0.17	0.01	0.83					
ML juvenile	-0.19	-0.35	0.29	-0.10	-0.09	-0.14	-0.22	-0.08	0.22	-0.04	-0.20	-0.01	0.07				
ML adult	-0.04	0.10	-0.01	-0.02	0.33	0.12	0.15	-0.17	-0.15	0.35	0.48	0.04	0.10	0.23			
DEA	0.69	0.55	-0.66	0.43	0.02	0.45	0.45	-0.14	-0.13	0.57	0.19	-0.17	-0.30	-0.15	-0.09		
DEA_{CN}	-0.05	-0.07	0.15	0.02	-0.18	0.08	0.10	0.11	0.47	0.13	0.15	-0.19	-0.33	0.41	0.30	0.26	
Control DEA	0.74	0.73	-0.74	0.45	0.09	0.54	0.44	-0.12	-0.23	0.53	0.08	0.06	-0.12	-0.24	-0.21	0.69	0.16

Appendix 11 Pearson's correlation coefficient matrix for high treatment plots (n= 28) (Chapter 4).

	OC	Mud	GSM	Seagrass	Chl *a*	Phaeophytin	DFS	NH_4^+ (5-7 cm)	NH_4^+ (0-2 cm)	S	N	*AS* juvenile	*AS* adult	*ML* juvenile	*ML* adult	DEA	DEA_{CN}
Mud	0.82																
GSM	-0.72	-0.75															
Seagrass	0.79	0.62	-0.53														
Chl *a*	0.49	0.46	-0.51	0.36													
Phaeophytin	0.80	0.90	-0.77	0.59	0.50												
DFS	0.37	0.35	-0.45	0.43	0.10	0.37											
NH_4^+ (5-7 cm)	-0.49	-0.39	0.32	-0.49	0.01	-0.30	-0.51										
NH_4^+ (0-2 cm)	-0.27	-0.24	0.17	-0.37	-0.09	-0.18	-0.32	0.54									
S	0.78	0.73	-0.75	0.66	0.37	0.69	0.63	-0.52	-0.42								
N	0.53	0.63	-0.63	0.38	0.50	0.65	0.48	-0.18	-0.28	0.75							
AS juvenile	0.36	0.21	-0.04	0.31	0.38	0.13	-0.09	0.14	0.07	0.13	0.13						
AS adult	0.31	0.26	-0.03	0.26	0.34	0.17	-0.28	0.19	0.21	0.04	0.12	0.92					
ML juvenile	0.22	0.16	-0.19	-0.05	0.06	0.25	0.03	-0.27	-0.37	0.33	0.13	-0.10	-0.15				
ML adult	0.59	0.46	-0.56	0.37	0.07	0.67	0.42	-0.18	-0.11	0.46	0.28	0.03	-0.04	0.41			
DEA	0.63	0.71	-0.77	0.49	0.41	0.79	0.52	-0.40	-0.38	0.78	0.73	-0.09	-0.11	0.16	0.44		
DEA_{CN}	0.10	0.35	-0.29	0.16	0.29	0.31	0.28	-0.14	-0.30	0.43	0.54	-0.09	-0.08	0.05	-0.14	0.58	
Control DEA	0.83	0.71	-0.67	0.56	0.27	0.77	0.44	-0.48	-0.30	0.64	0.40	0.03	-0.05	0.30	0.74	0.67	-0.08

Appendix 12 Site information and raw data (Chapter 5).

				Location			Sediment properties			Microphytobenthic biomass		Macrofaunal community			
Estuary study	Site	Plot	Sample date	Latitude	Longitude	Water depth	OC	Mud	GSM	Chl *a*	Phaeo	S	N	LB	DEA
			dd/mm/yyyy	NZTM	NZTM	m	%	%	µm	µg g^{-1}	µg g^{-1}	n $core^{-1}$	n $core^{-1}$	n $core^{-1}$	µmol N m^{-2} h^{-1}
Waikareao survey	1	Q1	13/01/2013	5823722	1878093	Intertidal	4.3	29.3	149	27.7	19.2	7	513	0	1073.9
	1	Q2	13/01/2013	5823722	1878093	Intertidal	4.4	22.4	208	21.8	4.0	12	540	0	1212.4
	1	Q3	13/01/2013	5823722	1878093	Intertidal	4.4	26.2	176	37.2	7.3	11	557	0	1231.5
	1	Q4	13/01/2013	5823722	1878093	Intertidal	4.2	27.1	167	34.9	12.9	9	305	0	1048.8
	1	Q5	13/01/2013	5823722	1878093	Intertidal	4.3	33.9	130	34.2	8.8	6	432	0	1094.3
	2	Q1	13/01/2013	5824126	1878123	Intertidal	2.9	10.4	149	10.7	4.2	19	141	3	365.8
	2	Q2	13/01/2013	5824126	1878123	Intertidal	2.8	12.0	208	6.0	5.3	17	115	5	365.3
	2	Q3	13/01/2013	5824126	1878123	Intertidal	2.9	11.2	176	12.6	6.4	14	100	3	266.4
	2	Q4	13/01/2013	5824126	1878123	Intertidal	2.8	14.6	167	11.6	7.0	19	138	4	352.6
	2	Q5	13/01/2013	5824126	1878123	Intertidal	2.9	11.1	130	10.5	6.3	19	102	5	193.7
	3	Q1	13/01/2013	5824432	1877789	Intertidal	3.0	15.1	157	25.7	5.7	7	364	0	394.7
	3	Q2	13/01/2013	5824432	1877789	Intertidal	2.9	13.3	181	25.7	8.1	11	299	0	280.8
	3	Q3	13/01/2013	5824432	1877789	Intertidal	3.0	12.8	188	22.8	9.1	7	374	0	421.1
	3	Q4	13/01/2013	5824432	1877789	Intertidal	2.9	12.2	188	26.4	8.4	12	457	1	270.2
	3	Q5	13/01/2013	5824432	1877789	Intertidal	2.9	12.5	186	26.3	7.8	7	386	0	256.6
	4	Q1	13/01/2013	5824939	1878310	Intertidal	3.5	12.3	238	17.1	11.1	22	296	19	612.3
	4	Q2	13/01/2013	5824939	1878310	Intertidal	3.5	9.4	273	19.3	6.2	19	228	24	504.5
	4	Q3	13/01/2013	5824939	1878310	Intertidal	3.7	16.4	203	17.8	7.2	21	260	27	571.2
	4	Q4	13/01/2013	5824939	1878310	Intertidal	3.4	11.9	259	14.8	12.5	24	267	17	348.7
	4	Q5	13/01/2013	5824939	1878310	Intertidal	3.6	13.4	236	21.7	10.1	19	306	21	416.4
	5	Q1	13/01/2013	5824771	1878741	Intertidal	2.0	4.0	445	11.4	4.8	22	400	69	195.7
	5	Q2	13/01/2013	5824771	1878741	Intertidal	2.0	3.8	392	13.6	6.1	26	411	43	159.3
	5	Q3	13/01/2013	5824771	1878741	Intertidal	2.1	3.9	393	12.6	7.9	27	394	86	145.1
	5	Q4	13/01/2013	5824771	1878741	Intertidal	1.9	5.2	385	13.8	4.5	24	252	63	216.0
	5	Q5	13/01/2013	5824771	1878741	Intertidal	2.1	3.7	404	17.1	8.0	22	374	66	191.5
Tuapiro survey	1	Q1	11/02/2013	5846357	1859717	Intertidal	5.2	5.8	391	7.9	2.8	20	103	0	82.7

				Location			Sediment properties			Microphytobenthic biomass		Macrofaunal community			
Estuary study	Site	Plot	Sample date	Latitude	Longitude	Water depth	OC	Mud	GSM	Chl *a*	Phaeo	S	N	LB	DEA
			dd/mm/yyyy	NZTM	NZTM	m	%	%	µm	µg g^{-1}	µg g^{-1}	n $core^{-1}$	n $core^{-1}$	n $core^{-1}$	µmol N m^{-2} h^{-1}
Tuapiro survey	1	Q2	11/02/2013	5846357	1859717	Intertidal	5.1	6.8	370	7.8	3.5	15	93	0	80.0
	1	Q3	11/02/2013	5846357	1859717	Intertidal	5.2	5.4	379	6.7	3.6	12	55	0	75.5
	1	Q4	11/02/2013	5846357	1859717	Intertidal	5.0	12.9	310	7.1	3.2	13	103	0	70.2
	1	Q5	11/02/2013	5846357	1859717	Intertidal	5.6	9.9	358	7.9	3.6	16	76	0	54.1
	2	Q1	11/02/2013	5846597	1860032	Intertidal	4.4	9.5	242	6.4	2.9	17	165	0	29.2
	2	Q2	11/02/2013	5846597	1860032	Intertidal	4.0	7.8	305	9.3	4.7	22	188	0	25.6
	2	Q3	11/02/2013	5846597	1860032	Intertidal	3.9	8.9	254	8.7	3.0	15	134	0	39.6
	2	Q4	11/02/2013	5846597	1860032	Intertidal	4.4	9.7	251	9.7	4.0	18	141	0	34.4
	2	Q5	11/02/2013	5846597	1860032	Intertidal	3.9	10.1	266	9.5	2.9	13	149	0	20.2
	3	Q1	11/02/2013	5846485	1860748	Intertidal	3.0	7.7	201	10.2	7.1	29	351	10	25.9
	3	Q2	11/02/2013	5846485	1860748	Intertidal	2.8	6.3	202	11.2	5.2	27	293	10	39.1
	3	Q3	11/02/2013	5846485	1860748	Intertidal	2.6	7.6	196	12.1	4.5	23	242	0	66.1
	3	Q4	11/02/2013	5846485	1860748	Intertidal	2.7	6.4	204	11.2	4.0	26	343	9	28.2
	3	Q5	11/02/2013	5846485	1860748	Intertidal	2.7	12.0	207	12.4	5.8	22	267	17	18.0
	4	Q1	11/02/2013	5847046	1860495	Intertidal	3.7	7.0	435	14.7	6.5	15	239	105	358.8
	4	Q2	11/02/2013	5847046	1860495	Intertidal	4.3	11.7	415	15.7	8.1	13	294	110	308.3
	4	Q3	11/02/2013	5847046	1860495	Intertidal	3.4	9.0	414	13.8	5.2	22	303	134	608.9
	4	Q4	11/02/2013	5847046	1860495	Intertidal	3.8	4.9	462	16.5	9.7	15	283	95	500.7
	4	Q5	11/02/2013	5847046	1860495	Intertidal	3.6	6.3	416	16.1	5.8	20	599	44	489.6
	5	Q1	11/02/2013	5847350	1860678	Intertidal	1.9	4.5	268	8.8	3.3	14	119	12	213.4
	5	Q2	11/02/2013	5847350	1860678	Intertidal	1.6	4.3	269	8.5	3.1	16	158	6	169.5
	5	Q3	11/02/2013	5847350	1860678	Intertidal	2.2	4.5	272	10.7	4.0	17	176	18	93.6
	5	Q4	11/02/2013	5847350	1860678	Intertidal	2.0	3.5	278	10.0	3.3	16	131	20	263.8
	5	Q5	11/02/2013	5847350	1860678	Intertidal	2.0	4.1	269	10.7	3.9	17	198	15	59.7
Tuapiro experiment	1	2	27/11/2014	5846678	1860839	Intertidal	1.5	0.8	243	9.1	1.7	20	91	28	40.1
	1	4	27/11/2014	5846678	1860839	Intertidal	1.6	0.0	223	9.0	1.6	21	122	33	46.9
	2	6	27/11/2014	5846660	1860848	Intertidal	1.7	1.2	214	6.1	2.1	16	115	20	43.2
	2	8	27/11/2014	5846660	1860848	Intertidal	1.8	1.3	212	6.6	2.2	18	131	28	24.7

				Location			Sediment properties			Microphytobenthic biomass		Macrofaunal community			
Estuary study	Site	Plot	Sample date	Latitude	Longitude	Water depth	OC	Mud	GSM	Chl *a*	Phaeo	S	N	LB	DEA
			dd/mm/yyyy	NZTM	NZTM	m	%	%	µm	µg g^{-1}	µg g^{-1}	n $core^{-1}$	n $core^{-1}$	n $core^{-1}$	µmol N m^{-2} h^{-1}
Tuapiro experiment	3	10	27/11/2014	5846620	1860828	Intertidal	3.0	3.1	161	20.3	3.8	21	118	35	192.6
	3	12	27/11/2014	5846620	1860828	Intertidal	3.0	3.9	158	21.1	5.3	14	76	28	223.7
	4	14	27/11/2014	5846603	1860818	Intertidal	2.8	1.8	156	14.8	4.2	23	135	26	267.2
	4	16	27/11/2014	5846603	1860818	Intertidal	2.9	1.9	154	16.1	4.2	23	111	31	360.6
	5	18	27/11/2014	5846589	1860812	Intertidal	3.4	3.7	147	14.3	3.6	17	137	27	406.4
	5	20	27/11/2014	5846589	1860812	Intertidal	3.4	3.3	150	13.0	3.3	14	79	21	345.0
	6	22	27/11/2014	5846578	1860808	Intertidal	3.6	7.3	138	15.6	5.1	22	202	38	366.1
	6	24	27/11/2014	5846578	1860808	Intertidal	3.9	6.8	140	16.5	5.0	14	134	24	424.2
	7	26	27/11/2014	5846609	1860800	Intertidal	1.5	0.0	177	6.0	1.6	16	53	16	78.7
	7	28	27/11/2014	5846609	1860800	Intertidal	1.7	0.0	175	7.4	1.7	18	75	21	78.5
	8	30	27/11/2014	5846631	1860811	Intertidal	1.7	1.2	191	6.7	1.7	21	114	34	114.5
	8	32	27/11/2014	5846631	1860811	Intertidal	1.8	1.3	196	11.0	2.1	20	124	66	114.8
	9	34	27/11/2014	5846560	1860817	Intertidal	3.8	10.3	132	14.2	5.4	20	208	22	342.1
	9	36	27/11/2014	5846560	1860817	Intertidal	4.7	12.0	130	17.4	5.8	17	134	15	387.0
	10	38	27/11/2014	5846549	1860828	Intertidal	5.1	14.5	125	18.1	8.3	18	142	26	528.2
	10	40	27/11/2014	5846549	1860828	Intertidal	4.6	12.3	130	15.3	5.7	16	154	21	434.9
	11	42	27/11/2014	5846538	1860828	Intertidal	5.4	21.6	112	20.0	8.5	19	100	14	601.3
	11	44	27/11/2014	5846538	1860828	Intertidal	5.5	20.2	114	19.8	8.2	15	123	15	519.1
	12	46	27/11/2014	5846536	1860850	Intertidal	3.9	15.2	142	12.9	6.5	21	149	27	410.7
	12	48	27/11/2014	5846536	1860850	Intertidal	4.1	12.7	148	13.3	5.6	18	164	19	466.8
Kaipara	1	C	17/03/2014	5971943	1715904	Intertidal	1.9	10.0	210	13.2	9.7	29	225	25	50.6
	2	C	17/03/2014	5971771	1715908	Intertidal	0.9	0.0	216	12.7	1.6	19	92	42.5	3.6
	3	C	17/03/2014	5971577	1715908	Intertidal	1.7	7.3	207	14.6	11.2	26	332	116	134.7
	4	C	17/03/2014	5971494	1715904	Intertidal	1.5	1.1	209	12.5	9.3	19	107	1	189.0
	5	C	17/03/2014	5971296	1715921	Intertidal	1.2	9.9	187	6.0	5.2	29	174	4.5	116.8
	6	C	17/03/2014	5971196	1715921	Intertidal	1.5	4.9	191	7.9	6.4	22	137	21.5	198.8
	7	C	17/03/2014	5971171	1715922	Intertidal	0.6	0.0	237	6.1	1.5	13	47	2	4.1
	8	C	17/03/2014	5971091	1715923	Intertidal	0.8	0.0	223	5.5	2.1	19	72	14.5	6.3

				Location			Sediment properties			Microphytobenthic biomass		Macrofaunal community			
Estuary study	Site	Plot	Sample date	Latitude	Longitude	Water depth	OC	Mud	GSM	Chl *a*	Phaeo	S	N	LB	DEA
			dd/mm/yyyy	NZTM	NZTM	m	%	%	µm	µg g^{-1}	µg g^{-1}	n $core^{-1}$	n $core^{-1}$	n $core^{-1}$	µmol N m^{-2} h^{-1}
Kaipara	9	C	17/03/2014	5971015	1715922	Intertidal	1.0	13.6	196	7.0	4.9	26	130	21.5	14.0
	10	C	17/03/2014	5970999	1716025	Intertidal	1.1	8.7	195	23.2	16.9	28	340	5.5	38.8
	11	C	17/03/2014	5971315	1716004	Intertidal	1.0	4.1	218	3.6	3.8	24	419	15.5	27.6
	12	C	17/03/2014	5971518	1715991	Intertidal	1.2	4.1	211	7.8	5.8	18	126	20	37.7
	13	C	17/03/2014	5971672	1715985	Intertidal	0.8	0.0	223	9.8	4.9	19	111	35	9.9
	14	C	17/03/2014	5971755	1715978	Intertidal	0.8	0.0	225	12.1	3.8	14	53	16	6.0
	15	C	17/03/2014	5971813	1715977	Intertidal	0.8	0.0	216	11.7	3.2	14	90	34	6.5
	16	C	17/03/2014	5971935	1716072	Intertidal	0.7	0.0	241	5.3	2.2	10	79	28.5	6.5
	17	C	17/03/2014	5971835	1716072	Intertidal	0.8	3.5	227	8.6	3.2	17	127	9.5	3.5
	18	C	17/03/2014	5971656	1716075	Intertidal	0.8	0.0	214	10.3	3.8	17	87	41	6.1
	19	C	17/03/2014	5971559	1716079	Intertidal	0.7	0.0	223	5.9	2.2	14	54	32	9.9
	20	C	17/03/2014	5971224	1716111	Intertidal	1.7	14.5	177	14.5	13.7	25	108	4.5	224.9
	21	C	17/03/2014	5971055	1716120	Intertidal	1.2	2.5	189	7.2	4.4	28	142	16.5	138.1
	22	C	17/03/2014	5971094	1716225	Intertidal	1.6	9.2	194	13.7	17.9	24	136	5.5	208.5
	23	C	17/03/2014	5971177	1716216	Intertidal	2.0	12.2	195	12.8	15.2	27	203	6.5	372.9
	24	C	17/03/2014	5971253	1716205	Intertidal	1.4	4.5	182	20.9	17.5	25	88	5.5	72.6
	25	C	17/03/2014	5971446	1716172	Intertidal	0.7	0.0	222	5.7	2.3	17	95	38.5	9.7
	26	C	17/03/2014	5971463	1716167	Intertidal	0.6	0.0	227	4.2	2.2	12	38	15.5	4.8
	27	C	17/03/2014	5971774	1716143	Intertidal	0.7	0.0	232	9.6	2.0	11	50	13	3.9
	28	C	17/03/2014	5971955	1716127	Intertidal	0.7	0.0	239	8.9	2.3	9	19	4	20.1
Mahurangi	1	A	5/03/2015	5958958	1755223	4.9	5.7	37.2	103	6.6	2.4	8	50	0	877.0
	1	B	5/03/2015	5958958	1755223	4.9	5.2	35.0	104	7.1	1.8	12	58	0	995.1
	1	C	5/03/2015	5958958	1755223	4.9	5.4	40.6	83	2.6	1.6	4	53	0	844.4
	1	D	5/03/2015	5958958	1755223	4.9	5.7	37.5	87	6.8	4.7	7	82	0	956.5
	1	E	5/03/2015	5958958	1755223	4.9	5.9	42.0	80						1164.5
	1	F	5/03/2015	5958958	1755223	4.9	5.3	39.8	84						791.3
	1	G	5/03/2015	5958958	1755223	4.9	5.2	41.1	82						988.3
	1	H	5/03/2015	5958958	1755223	4.9	5.4	42.6	79						900.3

				Location			Sediment properties			Microphytobenthic biomass		Macrofaunal community			
Estuary study	Site	Plot	Sample date	Latitude	Longitude	Water depth	OC	Mud	GSM	Chl *a*	Phaeo	S	N	LB	DEA
			dd/mm/yyyy	NZTM	NZTM	m	%	%	µm	µg g^{-1}	µg g^{-1}	n $core^{-1}$	n $core^{-1}$	n $core^{-1}$	µmol N m^{-2} h^{-1}
Mahurangi	2	A	5/03/2015	5960410	1754366	5.3	4.4	52.2	65	8.3	3.3	19	184	0	594.5
	2	B	5/03/2015	5960410	1754366	5.3	4.9	42.7	111	7.8	2.1	14	226	0	603.7
	2	C	5/03/2015	5960410	1754366	5.3	4.5	38.0	151	6.0	1.3	15	195	0	701.4
	2	D	5/03/2015	5960410	1754366	5.3	4.8	42.5	88	4.3	2.1	24	263	0	649.6
	2	E	5/03/2015	5960410	1754366	5.3	4.8	43.4	120						730.6
	2	F	5/03/2015	5960410	1754366	5.3	4.7	47.3	71						658.1
	2	G	5/03/2015	5960410	1754366	5.3	4.9	34.1	126						715.7
	2	H	5/03/2015	5960410	1754366	5.3	4.7	44.1	109						602.9
	3	A	5/03/2015	5962275	1754645	4.5	3.7	19.5	115	6.7	1.8	11	164	0	971.7
	3	B	5/03/2015	5962275	1754645	4.5	3.8	25.7	103	7.4	2.8	16	147	2	698.4
	3	C	5/03/2015	5962275	1754645	4.5	3.8	27.2	99	7.5	2.2	19	186	0	798.4
	3	D	5/03/2015	5962275	1754645	4.5	3.8	25.9	100	6.5	1.4	17	184	0	1027.0
	3	E	5/03/2015	5962275	1754645	4.5	3.5	23.4	107						964.0
	3	F	5/03/2015	5962275	1754645	4.5	3.5	17.6	116						797.2
	3	G	5/03/2015	5962275	1754645	4.5	3.7	24.5	105						886.4
	3	H	5/03/2015	5962275	1754645	4.5	3.4	23.0	108						893.3
	4	A	5/03/2015	5964378	1753855	3.8	4.1	20.6	127	9.9	2.1	17	148	0	752.3
	4	B	5/03/2015	5964378	1753855	3.8	4.2	24.9	118	9.2	2.0	18	151	0	715.5
	4	C	5/03/2015	5964378	1753855	3.8	4.5	24.7	118	9.8	1.8	11	138	0	831.5
	4	D	5/03/2015	5964378	1753855	3.8	3.9	16.7	133	8.2	1.4	17	146	0	963.4
	4	E	5/03/2015	5964378	1753855	3.8	4.0	27.7	115						551.9
	4	F	5/03/2015	5964378	1753855	3.8	3.6	15.7	133						504.2
	4	G	5/03/2015	5964378	1753855	3.8	3.8	24.1	120						735.3
	4	H	5/03/2015	5964378	1753855	3.8	3.8	17.3	132						688.7

Abbreviations: OC: sediment organic content, Mud: sediment mud content, GSM: grain size median, Chl-*a*: chlorophyll *a* content, Phaeo: phaeophytin content, S: number of species, N: number of individuals, LB: number of large bivalves *A. stutchburyi* and *M. liliana*, DEA: denitrification enzyme activity.

Appendix 13 Locations of survey sites in (A) Waikareao Estuary, Tauranga Harbour (37° 41’ S 176° 9’E), and (B) Mahurangi Estuary (36° 27' S 174° 43' E) (Chapter 5).