Ecogeochemistry of oligotrophic, soft sediment estuarine systems

Amanda Marie Vieillard

ORCID ID: 0000-0002-8397-7110

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Institute of Marine Science

Faculty of Science

University of Auckland

Auckland, New Zealand

"We must believe that our actions have power and that results will be obtained. Without hope there is little reason to change and, in fact, a message of hopelessness breeds inaction… It is a question of what kind of marine and coastal environment we wish to have and leave for future generations."

Fulweiler, Rabalais, & Heiskanaen (2012) *Marine Pollution Bulletin*

Abstract

Estuaries are some of our most economically and ecologically valuable natural systems; however, human propensity to settle along coasts makes estuaries vulnerable to anthropogenic stress, and nutrient pollution has caused wide-spread eutrophication in many of the world's estuaries. While many eutrophication mitigation strategies are being investigated and deployed in many of these chronically impacted systems, New Zealand's estuaries have remained largely oligotrophic. However, nitrogen inputs have increased more in New Zealand than anywhere else in the world, and an estimated 27 % of New Zealand estuaries are now considered susceptible to eutrophication. In addition to this increasing threat, many New Zealand estuaries are also under the threat of increasing sediment deposition as a result of increasing population and land-use change.

In this dissertation research, I sought to understand how this increasing terrestrial sediment deposition is impacting the ecology and biogeochemistry of these low-nutrient ecosystems, and to investigate how they intersect to influence the delivery of the key ecosystem service of denitrification. Due to its ability to mitigate nitrogen pollution, denitrification will be of particular importance to New Zealand ecosystems as nitrogen pollution continues to rise.

Using a combination of literature analysis, *in situ* field studies, controlled laboratory experiments, and multi-variate modelling approaches, I have demonstrated the importance of these understudied, low-nutrient systems, showing that they function differently than their more eutrophic counterparts. I found that the macrofaunal community is inexorably linked to the biogeochemical processing in these systems, exhibiting direct control on rates of net denitrification. Embracing more complex and non-linear stress responses, I have shown that even small changes in anthropogenic impact

can induce regime shifts within these systems. Overall, results from this work show that terrestrial sediment deposition is directly affecting the macrofaunal community and decreasing denitrification rates, thus impairing the ability of these important ecosystems to mitigate ever increasing nitrogen pollution. It is my hope that interdisciplinary studies like the ones in this dissertation can be used to inform more wholistic and effective management of estuarine systems in the future, hopefully keeping eutrophication from becoming an inevitability in New Zealand.

Preface

This dissertation is made up of one perspective chapter (Chapter 2) and three research chapters (Chapters 3-5) in addition to the general introduction (Chapter 1) and the general discussion (Chapter 6). Each of the chapters in the body of the work (Chapters 2-5) are either published or in preparation for publication in peer-reviewed journals. I was the primary author and contributor on all chapters of this dissertation including experimental design, field work, sample collection, laboratory analysis, data analysis, and writing. This work was conducted under the supervision of my primary supervisor, Professor Simon Thrush, who provided direction and advice though out my PhD process. Professor Judi Hewitt, of the National Institute of Water and Atmospheric Research Ltd. (NIWA) and Department of Statistics, University of Auckland was my co-supervisor who provided support for data analysis and statistics, particularly for Chapter 5.

Chapter 2 was written in collaboration with Simon Thrush and Silvia Newell of Wright State University. Its focus was originally solely on low-nutrient estuaries but was expanded to include a broader range of under-studied estuaries including tropical as well as low-nutrient systems. This chapter has been published in the *Journal of Geophysical Research: Biogeoscience*, volume 125 under the title 'Recovering from bias: A call for further study of underrepresented tropical and low-nutrient estuaries' by AM Vieillard, SE Newell, and SF Thrush (DOI: 10.1029/2020JG005766).

Chapter 3 has been published in *Estuaries and Coasts* under the title 'Ecogeochemistry and denitrification in non-eutrophic coastal sediments' by AM Vieillard & SF Thrush (DOI: 10.1007/s12237-021-00912-7).

Chapter 4 is in review at *Marine Ecology Progress Series* under the title ‘Simultaneous nitrogen fixation and denitrification in oligotrophic sediments’ by AM Vieillard & SF Thrush (submission number: MEPS-2020-09-052).

Chapter 5 is in review at *Ecosystems* under the title “Reaching the tipping point: Terrestrial sediment deposition in oligotrophic sediments” by AM Vieillard, JE Hewitt, and SF Thrush.

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I've had a tremendous amount of help throughout this incredible journey, and there are so many I wish to thank. First and foremost, I'd like to thank my advisor, Professor Simon Thrush, for taking a chance on me and convincing me that my academic journey wasn't quite done. I met Simon at a time when I was uncertain whether I wanted to pursue a PhD, or even be a scientist anymore, and under his supervision I was given space to re-discover my love of science and my passion for protecting coastal systems. His guidance has certainly challenged my preconceptions and forced me to confront my own biases, but he has also pushed me to be a better and more independent scientist. I sincerely thank Simon for the opportunity to move to this amazing country, and for making me feel safe enough to remember my true calling.

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I need to extend two special thank-yous to Samantha Ladewig and Dr. Julie Hope. I want to thank Sam for being the most sincere friend and wonderfully supportive flat-mate I ever could have asked for. I'm sorry that our time together in New Zealand was cut short by COVID-19, but her strength, positivity, and grace in the face of the most incredible adversity inspires me every single day. And to Julie, I want to say the biggest "thank you," for being such a wonderful colleague, confidant, and friend. Not only is she a kick-ass scientist, but also her seemingly endless generosity both in and out of work is incredible. She helped make New Zealand feel like home and I feel privileged to have learned and worked alongside her.

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List of Abbreviations

%	percent
[ ]	concentration
°C	degrees Celsius
°N	degrees North
°S	degrees South
±	plus or minus standard error
^{14}N	lighter nitrogen isotope
^{15}N	heavier nitrogen isotope
$^{28}N_2$	di-nitrogen gas composed of two light isotopes
$^{29}N_2$	di-nitrogen gas composed of one light isotope + one heavy
$^{30}N_2$	di-nitrogen gas composed of two heavy isotopes
Anammox	anaerobic ammonium oxidation
ANOVA	analysis of variance
Ar	argon
C	carbon
chl a	chlorophyll a
cm	centimetre
C-PC	phycocyanin
DIN	dissolved inorganic nitrogen

DIP	dissolved inorganic phosphorus
DislM	distance-based linear model
DNA	deoxyribonucleic acid
DNF	denitrification
DNRA	dissimilatory nitrate reduction to ammonium
DO	dissolved oxygen
DON	dissolved organic nitrogen
DOP	dissolved organic phosphorus
e.g.	exempli grata [Latin: "for example"]
EMB	ecosystem-based management
et al.	et alia [Latin: “and others”]
Fig.	figure
g	gram
h	hour
i.e.	id est [Latin: "that is:]
IEA	integrated ecosystem assessment
IPT	isotope pairing technique
IRMS	isotope ratio mass spectrometer
km	kilometre
LECZ	low elevation coastal zone
LOI	loss on ignition

m	meter
M	molar
m^2	square metre
Mili-Q	distilled and sterilized water
MIMS	membrane inlet mass spectrometer
min	minute
mL	millilitre
MLR	multiple linear regression
N	nitrogen
n	number of samples
N_2	di-nitrogen gas
N_2-N	di-nitrogen gas in terms of units of nitrogen
N_2/Ar	di-nitrogen to argon ratio
N_2O	nitrous oxide
Nfix	nitrogen fixation
NH_4^+	ammonium
NIWA	National Institute of Water and Atmospheric Research, New Zealand
NO	nitric oxide
NO_2^-	nitrite
NO_3^-	nitrate
NO_x	nitrate + nitrite

O_2	oxygen
OECD	Organization of Economic Co-operation and Development
OM	organic matter
p	p-value
P	phosphorus
r	correlation coefficient (directional)
R^2	correlation coefficient
RNA	ribonucleic acid
RPM	revolutions per minute
SOD	sediment oxygen demand
TN	total nitrogen
TP	total phosphorus
UK	United Kingdom
US, USA	United States of America
vs	versus
α	alpha, significance level
µg	microgram
µL	microliter
µM	micromolar or micromoles per litre
µm	micrometre
µmol	micromole

Chapter 1

General Introduction

Amanda M Vieillard

1.1 | Context and Introduction

1.1.1 | Estuarine Ecology & Ecosystem Services

Estuaries are dynamic ecosystems linking the terrestrial and marine environments. They are characterized by steep physical, biological, and geochemical gradients resulting in very high rates of nutrient cycling as well as primary and secondary production. These characteristics make estuarine habitats some of the most ecologically, recreationally, and economically valuable ecosystems in the world. In fact, though the coastal zone makes up only 8 % of Earth's surface area, it has been estimated to contribute more than 60 % of the total economic value of the biosphere (Liquete *et al.*, 2013). This value is allocated based on the delivery of numerous ecosystem services by coastal systems. Ecosystem services are defined as the benefits that humans derive from nature, and our survival is dependent on them (Costanza *et al.*, 1997). Estuaries can deliver a wide variety of ecosystem services, from highly productive fisheries, to water and air quality regulation, nutrient cycling, essential nursery habitats, and providing cultural, recreational, and aesthetic value (Millennium Ecosystem Assessment, 2005). However, human propensity to settle on and exploit coastal ecosystems makes estuaries particularly vulnerable to a wide array of anthropogenic stressors (Cooper and Brush, 1993). Examples of these stressors include fishing pressures, habitat degradation, and numerous forms of pollution and runoff, all of which hinder the delivery of many vital estuarine ecosystem services (Nichols *et al.*, 1986). Therefore, understanding the complex way in which these systems respond to stress is key in preserving their functionality and ensuring their continued provisioning of these vital services.

The delivery of ecosystem services is inexorably linked to the ecology of an ecosystem. Estuaries experience strong spatial and temporal gradients resulting from both terrestrial and marine processes, and their subsequent interactions. Additionally, tidal

forcing constantly changes the conditions of the estuarine environment, this is especially true for the shallow and intertidal areas. As a result, these systems have been dubbed "consistently variable" in that variability is a hallmark condition of the estuarine environment (Elliott and Whitfield 2011). Due to their variability, estuaries are generally classified as species poor, by marine standards, as relatively few species are thought to be able handle the ever-changing conditions (Elliott and Whitfield 2011; Thrush *et al*., 2013). However, estuarine conditions also result in very high rates of nutrient cycling and primary productivity, therefore faunal communities generally have higher productivity rates and greater biomass than those in other aquatic environments (Saiz-Salinas and González-Oreja, 2000; McLusky and Elliott, 2004). This productivity coupled with their ability to cope with variability results in high ecological resistance. Resistance is a component of resilience, and in this case refers to the ability of a species to survive and carry out its functions under stress (Gladstone-Gallagher *et al.*, 2019). Estuarine species have therefore been shown to have an enhanced ability to tolerate and recover from a diverse array of stressors at individual, population, and community scales compared to species in other aquatic ecosystems (Elliott and Whitfield 2011).

In heavily impacted systems however, chronic exposure to various stressors can break down community resistance. Additionally, being relatively species-poor, drops in species diversity due to stress in estuaries diminishes community redundancy, causing the loss of any additional species to have a seemingly disproportionate impact on ecosystem function (Hughes *et al.*, 2005). This phenomenon is commonly seen in the chronically eutrophic estuarine systems of the North Atlantic (e.g., Nyström *et al.*, 2012). However, many more pristine estuarine communities have been shown to be complex and diverse with high resistance and redundancy, which ultimately result in greater ecological resilience (e.g., Thrush, Hewitt and Lohrer, 2012; Douglas *et al.*, 2017). Ecological

resilience refers to the magnitude of a disturbance that can be tolerated before an ecosystem shifts to a different functional state (Gunderson, 2000; Carpenter *et al.*, 2001). Resilience does not denote the directionality of this shift (i.e., the shift can be to a more degraded state, a more beneficial state, or simply a different state that is governed by a different set of processes), however, in this work I will primarily be focused on the shift to a more degraded functional state.

As a result of its climate and topography, New Zealand's estuaries are generally characterized by short, steep streams and rivers resulting in flashy freshwater inflows; therefore, except in heavy rain events, estuarine salinity very closely mimics that of the coastal ocean and the vast majority of estuarine residents are marine species (Thrush *et al.*, 2013). On the extensive intertidal sand flats of Northern New Zealand, the macrofaunal community has been very well characterized, has been found to represent a wide range of taxa, and be functionally diverse (e.g., Greenfield *et al.*, 2016; Thrush *et al.*, 2017). Additionally, macrofaunal diversity has been shown to buffer against anthropogenic stress (e.g., Thrush *et al.*, 2020), and has been linked to a wide variety of ecosystem services. However, the interconnected nature of estuarine ecosystem functions can make identifying the relative contributions of specific processes or species to the delivery of specific services challenging (Thrush *et al.*, 2013).

Overall, the high degree of variability in estuarine conditions corresponds to ecosystem functions that are also inherently variable, making stress responses difficult to quantify (Elliott and Quintino, 2007; Dauvin and Ruellet, 2009). This is exacerbated by the fact that these systems can have very complex, often non-linear responses to stress. While functional diversity increases estuarine resilience, the decreased redundancy with increased anthropogenic impact makes estuaries vulnerable to often unpredicted threshold responses and tipping points (Lotze *et al.*, 2006; Barbier *et al.*, 2008; Cloern *et al.*, 2016).

A tipping point denotes a point at which an ecosystem rapidly shifts from one functional state to another, typically more degraded, state when certain environmental thresholds are crossed (Nyström *et al.*, 2012; Dakos *et al.*, 2019). Therefore, tipping points and ecological resilience are very closely linked. These shifting states often cause large scale losses of ecological and economic resources, resulting in regime shifts that are often very difficult, if not impossible, to reverse (Mäler, 2000; Duarte *et al.*, 2009; Petraitis *et al.*, 2009). Hysteresis and positive feedbacks within the system can cause a "runaway" scenario where change is exacerbated and returning to the original state may not be possible (Duarte *et al.*, 2009). The effect of turbidity on seagrass is a classic example of this. Increase in turbidity as a result of anthropogenic activities, whether it be increased sediment run off or eutrophic algal blooms, shades benthic primary producers such as seagrasses causing them to decrease in productivity and die off. As the extent of seagrass coverage decreases, there is no redundant benthic species to take its place, and sediments become exposed to currents. Exposed sediment is more easily resuspended and further contributes to increasing turbidity, resulting in further seagrass die off until the seagrass is gone entirely and only bare sediments remain (McGlathery, 2001; Nyström *et al.*, 2012; Dakos *et al.*, 2019).

Many crucial ecosystem parameters such as resilience are "invisible" making observation or quantification of changes to them incredibly challenging; therefore tipping points often appear to happen very suddenly (Scheffer *et al.*, 2001). Due to their complexity and variability, estuaries are particularly prone to tipping points and other non-linear stress responses, which are difficult to predict (Thrush *et al.*, 2020). It has been suggested that efforts to reduce unwanted tipping points should address the smaller and more gradual perturbations that impact ecosystem resilience and redundancy (Scheffer *et al.*, 2001; Dakos *et al.*, 2019). However, this is further complicated by the fact that

variability occurs in the estuarine environment on a variety of scales, and there is also disparity in the scales of stressors disturbing these systems (Elliott and Whitfield, 2011). Estuaries are impacted by global scale stressors such as altered rainfall patterns, increasing temperatures, and sea level rise as a result of global climate change, but they are also subject to regional scale stressors such as storm events or nation/state-specific resource management, more local stressors such as land use or land cover changes, and estuary specific issues such as dredging or the construction of sea walls. The scales of ecosystem response also vary from chemical reactions to the microbial, individual, community, population, and ecosystem levels. As a result there is potential for tipping points to be reached at each of these scales, with smaller, more local regime shifts potentially going unnoticed, while larger scale tipping points often have catastrophic consequences.

Non-linear stress responses, like tipping points, are the result of complex interactions among the physical, chemical, biological, and ecological components of the estuarine ecosystem. All of these components create a network of processes which occur from the microbial to ecosystem scale. These processes within the system, then, combine into ecosystem functions, and it is these functions which make up ecosystem services. In order to understand this complex network of interactions, processes, and functions, and how they affect the delivery of ecosystem services, we need a more wholistic and inclusive means of considering ecosystems. I have chosen to name this wholistic study "ecogeochemistry" as it is the intersection and combination of the biogeochemistry and ecology of a system.

Ecogeochemistry *– The study of the relationships between organisms and the microbial, geophysical, and chemical processes within an ecosystem, and how they govern the natural environment.*

This is an expanded definition of an existing term. The study of ecogeochemistry, as I have proposed it, takes the interconnected, mechanistic nature of the study of biogeochemistry and expands it into a broader ecological context, encompassing multiple scales and functional consequences, as is more common in ecological studies. This intersection of ecology and biogeochemistry allows us to think beyond the core, so to speak, expanding the impact of traditional biogeochemical techniques.

In this dissertation, I seek to broaden our understanding of ecogeochemistry in low-nutrient estuaries and to elucidate how anthropogenic stressors shift an important element of ecosystem function in estuaries: the nitrogen cycle. I wish to emphasize the importance of these under-studied ecosystems and to understand the consequences of multiple stressors on their functionality. In this work, I primarily focus on two of these anthropogenic stressors: nutrient pollution and terrestrial sediment deposition. By teasing apart the complex interaction networks in these systems from mechanisms to processes and functions, I seek to further understand the interconnections between the physical, biogeochemical, and ecological elements of the environment and how they respond to human-derived stress. It is my hope that this work will help pave the way for not only more wholistic understanding of all estuarine systems, but also for more effective management and protection of estuaries and the vital ecosystem services they provide.

1.1.2 | Nitrogen: Too much of a good thing

Nitrogen (N) is an essential nutrient for all life on Earth. In living tissues it is a key component of enzymes and amino acids as well as genetic material, including DNA and RNA (Schlesinger, 1997). On geologic time scales, fluctuation in the availability of N, as well as phosphorus (P), are likely to have controlled the size and activity of the biosphere. This is because the largest pool of N on the surface of the earth is in the atmosphere, which is 78 % nitrogen, in the form of dinitrogen gas (N_2). However, N_2 is

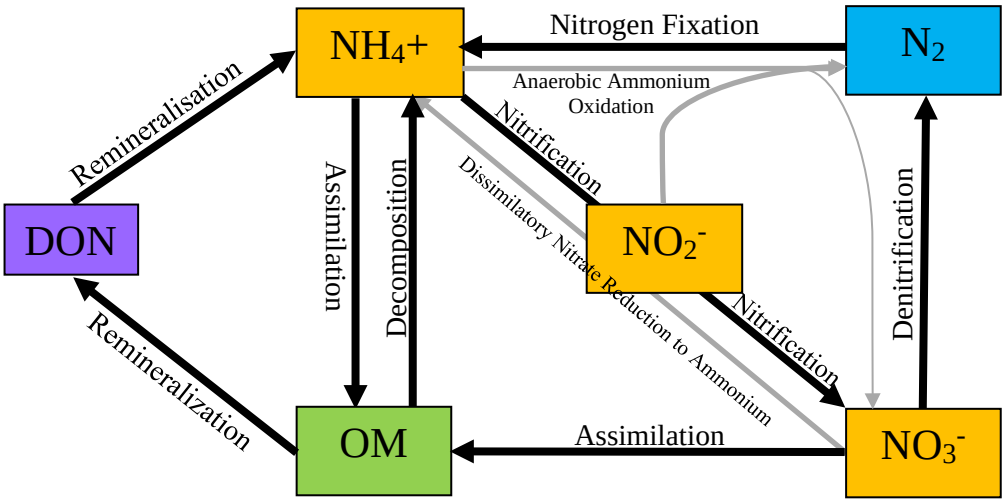
not bio-available to most species, as its triple bond requires large energy inputs to break. Ultimately, all the N that is available to biota was originally fixed from the atmosphere either by lightning or by N fixing microbes. After passing through the N cycle, N is returned to the atmospheric pool by the process of denitrification (DNF). Prior to life on earth, when all N fixation was abiotic, the residence time of N_2 in the atmosphere was approximately 1.3 billion years, but when biological N fixation was added, the residence time dropped to ~ 20 million years (Schlesinger, 1997). This residence time is much shorter than the 3.5 billion years life has existed on this planet, and therefore highlights the importance of the biological components of the N cycle, not just N fixation, but also DNF, which recycles N back into the atmosphere. Without DNF, the vast majority of N on earth would be in solution as nitrate (NO_3^-) in the oceans, making them more acidic (Schlesinger, 1997). The global cycle of N, then, is biologically mediated from start to finish. Because bio-available N species exist at an uncommonly wide range of valent energies, many microbial organisms can take advantage of their transformation potential, and use the energy released by the transitions of N redox states to fuel their life processes (Rosswall, 1982). These microbially mediated, N transformation processes are, collectively, the N cycle; this cycle, in conjunction with other elemental cycles allow for life on earth.

On a global scale, N is the limiting nutrient for marine primary productivity, making the cycling of N of critical importance for the functioning of marine systems and the delivery of ecosystem services (Paerl, 1997). Because the vast majority of N on earth is bound in relatively inert pools, such as atmospheric N_2, biological N fixation has historically been the primary source of N to marine ecosystems (Knapp, 2012). However, with increasing human population, agricultural demand, fossil fuel combustion, and the invention of the Haber-Bosch processes to synthesize explosives and chemical fertilizers,

anthropogenic N fixation has surpassed natural fixation and, as a result, humans have more than doubled the amount of reactive N available on Earth in the last century or so (Millennium Ecosystem Assessment, 2005; Fowler *et al.*, 2013). As these anthropogenic sources of N increased, excess N ran off in to streams and ultimately into estuarine systems (Nixon *et al.*, 1996; Vitousek *et al.*, 1997). Such a massive increase in the primary limiting nutrient had disastrous consequences for many costal systems, namely extensive eutrophication, particularly throughout the Northern Hemisphere (e.g., Smith, 2003; Brush, 2009; Carstensen *et al.*, 2014). Eutrophication is defined as "an increase in the rate of supply of organic matter to an ecosystem" (Nixon, 1995). Increased N delivery to coasts caused widespread eutrophication in the form of massive phytoplankton and macroalgal blooms. These blooms shade the benthos, inhibiting the growth of benthic primary producers such as microphytobenthos (MPB) and seagrass, causing shifts from primarily benthic primary production, to phytoplankton dominated systems (Hauxwell *et al.*, 2001; Burkholder, Tomasko and Touchette, 2007). The subsequent decomposition of blooms rapidly consumed bottom water oxygen, resulting in hypoxic conditions (Rabalais, Turner and Wiseman, 2001; Diaz and Rosenberg, 2008). Eutrophication became more wide-spread as N delivery to coasts continued to rise, becoming an acute pollution problem from ~1950 (Nixon, 1995; Brush, 2009). With this increasing eutrophication came extensive hypoxic "dead zones" around the world (Diaz and Rosenberg, 2008; Howarth *et al.*, 2011), resulting in massive fin and shellfish kills (Fleischer and Stibe, 1989; Conley *et al.*, 2011). Additional consequences of increasing eutrophication included benthic habitat degradation, shifts in phytoplankton community composition, extensive harmful algal blooms (Paerl, 1997; Sellner, Doucette and Kirkpatrick, 2003), as well as an overall reduction in water clarity and quality (Smith, 2003; Howarth and Marino, 2006). The threat of eutrophication was so severe that nearly every aspect of

estuarine research became linked with N pollution and eutrophication in one way or another, and the marine nitrogen cycle became an area of focused research interest (Fig. 1.1). In particular, DNF was found to be an important estuarine ecosystem service due to its ability to mitigate anthropogenic nitrogen pollution by transforming bio-available NO_3^- into inert, N_2 gas, effectively removing it from the system and returning it to the atmosphere (Seitzinger, 1988). As a result, coastal marine N cycling and denitrification have been widely studied, particularly in the North Atlantic (e.g., Seitzinger, 1988; Galloway *et al.*, 2004; Burgin and Hamilton, 2007; Fowler *et al.*, 2015). To this day, eutrophication is one of the greatest threats facing estuaries world-wide (Desmit *et al.*, 2018), to the extent that the biogeochemical flows of N and P have been designated as beyond safe operating zones, and some of the highest risk planetary boundaries by the Stockholm Resilience Centre (Steffen *et al.*, 2015).

The eutrophication of coastal systems has led many nations to make considerable efforts to reduce N runoff to coasts in an attempt reverse it (Boesch, 2019). These mitigation strategies range from reducing agricultural fertilizer use, to managing urban storm water runoff, and investing in tertiary wastewater treatment, all involving a wide array of stakeholders with differing interests (Bernhardt *et al.*, 2008; Detenbeck, You and Torre, 2019; Le Moal *et al.*, 2019). While N reductions have been accomplished in many areas, the resulting reversal of eutrophication has been unpredictable and slow to change (Boesch, 2019). There are signs that eutrophication is beginning to reverse as a result of abatement efforts in systems such as the Chesapeake Bay (Harding *et al.*, 2020) and the Baltic Sea (Reusch *et al.*, 2018), but many systems such as the Adriatic (Viaroli *et al.*, 2018) and various Australian systems (Archambault, Banwell and Underwood, 2001; Larsson *et al.*, 2017) have not seen the desired recovery, despite nutrient reduction efforts.



Process	Formula	Description
Assimilation	DIN → Organic Matter	Autotrophic (part of the photosynthetic process), produces oxygen
Decomposition	Organic matter → DIN	Heterotrophic, aerobic or anaerobic
Remineralization	Organic matter → DON → NH_4^+	Heterotrophic, aerobic or anaerobic
Denitrification	NO_3^- → NO_2^- → NO → N_2O → N_2	Transformation of bio-available NO_3^- to inert N_2 gas. Heterotrophic, anaerobic
Nitrogen Fixation	N_2 → OM → NH_4^+	Transformation of inert N_2 gas into organic matter, then bio-available NH_4^+. Autotrophic or heterotrophic, aerobic or anaerobic
Nitrification	NH_4^+ → NH_2OH → NO_2^- → NO_3^-	Transformation of bio-available NH_4^+ into bio-available NO_3^-. Autotrophic, requires molecular oxygen
Dissimilatory Nitrate Reduction to Ammonium (*DNRA*)	NO_3^- → NO_2^- → NH_4^+	Transformation of bio-available NO_3^- into bio-available NH_4^+. Heterotrophic (fermentive) or autotrophic, anaerobic
Anaerobic Ammonium Oxidation (*Anammox*)	$NH_4^+ + NO_2^- \rightarrow N_2 + NO_3^-$	Transformation of bio-available NH_4^+ and NO_2^- into inert N_2 gas (~90%) and bio-available NO_3^- (~10%). Autotrophic, anaerobic

Figure 1.1 Simplified schematic of the nitrogen cycle with formulas and descriptions. Colours denote different N pools while arrows represent N transformation processes. Orange boxes show dissolved inorganic nitrogen (DIN) species, specifically nitrate (NO_3^-), nitrite (NO_2^-) and ammonium (NH_4^+), the purple box shows dissolved organic nitrogen (DON), the blue box shows di-nitrogen gas (N_2), and the green box shows organic matter (OM). Bold arrows and larger text indicate processes that were quantified in some way in this dissertation. Anammox and DNRA were not specifically measured. Anammox is more commonly an important N cycle process in the water columns and subtidal sediments of deep estuaries and open ocean systems (Dalsgaard, Thamdrup and Canfield, 2005; Devol, 2015), and DNRA is more commonly dominant in N-polluted systems with high organic matter inputs and high sulphide concentrations (Burgin and Hamilton, 2007; Giblin *et al.*, 2013), therefore neither were hypothesized to be key factors in the N cycling of the oligotrophic, intertidal sediments of New Zealand.

Still other systems have experienced a “new eutrophication phenomenon” where eutrophication was temporarily curtailed by the reduction of intensive point sources of nutrient pollution, only to return again fuelled by more diffuse sources (Le Moal *et al.*, 2019). The overall consensus is that reducing nutrient loads alone is not sufficient to fully restore chronically eutrophied systems (Boesch, 2019). As a result there is still great uncertainty as to whether reduction efforts to eutrophic systems will have the desired effect (Desmit *et al.*, 2018; Boesch, 2019), and what kinds of regime shifts might be observed (Duarte *et al.*, 2009).

As estuarine N dynamics are not uniform worldwide, differences in N loading, pollution, and processing present important opportunities to further understand the N cycle and how it impacts the ecogeochemistry of coastal systems. While much of the world has been fighting coastal eutrophication, New Zealand’s estuaries have, until recently, largely been spared. The subtropical estuaries of Northern New Zealand, in which this dissertation research was conducted, have fringing mangrove forests which give way to extensive intertidal sand flats. The unique combination of the climate, geomorphology, and hydrodynamics in the region, coupled with the low population density mean that the majority of these systems are oligotrophic. Unlike their North Atlantic counterparts, Northern New Zealand estuaries have water column NO_3^- concentrations hovering at or below detection limits with NH_4^+ as the dominant DIN species (Lohrer *et al.*, 2010; O’Meara *et al.*, 2020). These DIN dynamics are a result of relatively low anthropogenic N runoff in the form of NO_3^-, and the dominance of N retention and recycling processes, characteristic of low-nutrient estuaries. However, increases in population, land clearing, and agriculture, particularly dairy farming, in the past twenty years or so have increased N run off in New Zealand more than in any other Organization for Economic Co-operation and Development (OECD) member country (OECD, 2017; Snelder, Larned and

McDowell, 2018; Fig. 1.2). This puts New Zealand in a key global position to understand how relatively un-impacted systems function, as well as to learn from countries that have experienced coastal eutrophication and prevent it from being an inevitability. However, recent models suggest that approximately 27 % of New Zealand estuaries are now highly or very highly susceptible to eutrophication (Plew *et al.*, 2020). While these models are based solely on the physical and chemical characteristics of estuaries and do not consider biological or ecological parameters, it is clear the nutrient pollution is an ever-increasing threat in New Zealand and that time is running out to stay ahead of this issue.

1.1.3 | Terrestrial Sediment Deposition

While N pollution is certainly an important threat facing estuaries it is by no means the only one. Another pressing stressor to New Zealand coastal systems is the increasing deposition of terrestrial sediment. Various human activities such as logging, land clearing, agriculture, and urbanization have increased erosion rates in coastal catchments resulting in massive increases in terrigenous sediment delivery to coasts (Lohrer, Hewitt and Thrush, 2006). This phenomenon is common world-wide, particularly throughout the Pacific Rim, and sediment deposition is widely recognized as an estuarine disturbance agent (GESAMP *et al.*, 1994). In New Zealand it is estimated that the coastal sedimentation rate has increased by ten-fold in the last ~150 years (Green, 2006). This sediment threatens biodiversity and estuarine ecosystem function. While suspended, the sediment elevates turbidity, shades benthic primary producers, and inhibits the filter feeding mechanisms of many benthic macrofauna; once it settles, the layer of sediment can also smother the benthos leading to mass die-offs of benthic communities (Thrush *et al.*, 2004). This sedimentation also alters sediment grain size, porosity, permeability, and changes the organic matter quantity and quality.

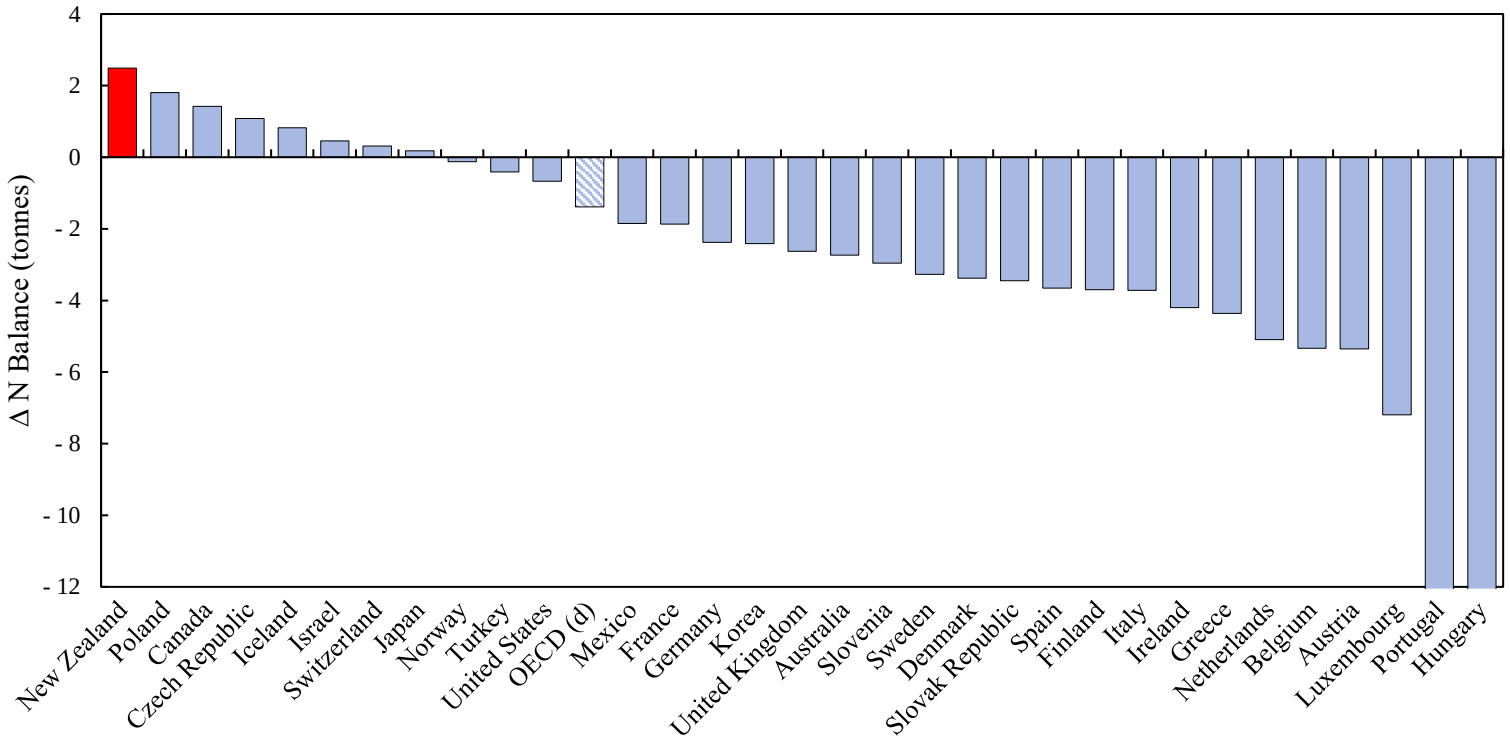


Figure 1.2. Changes in N balance for the Organization for Economic Co-operation and Development (OECD) member countries from 1998 to 2009. New Zealand (in red) has seen the largest increase in N. Figure modified from OECD Environmental Performance Reviews: New Zealand (OECD, 2017).

These perturbations lead to changes in oxygen penetration and redox conditions (Billerbeck *et al.*, 2007), both of which result in alterations to the N cycle (Pratt *et al.*, 2015; Douglas *et al.*, 2018), including reductions in DNF (Gongol and Savage, 2016; O'Meara *et al.*, 2020).

These effects of increased terrestrial sediment deposition can often overlap with or exacerbate the effects of increasing N pollution and eutrophication. For example, both eutrophication as a result of nutrient pollution and terrestrial sediment deposition cause an increase in turbidity, which has been shown to impact nutrient processing, primary production, and shellfish densities, with more turbid estuaries having diminished complexity and resilience against increased nutrient loading (Thrush *et al.*, 2020), but the specific mechanisms of how this stress impacts the interconnected biogeochemical and ecological processes is still not well constrained. It is therefore crucial that we understand how estuarine ecosystem functions change as a result of increased sediment deposition in an ecogeochemical context in order to help assess how these ecosystems might respond to the ever-increasing threat of eutrophication.

1.2 | Thesis Organization & Objectives

Overall, the goal of my thesis was to highlight the importance of and understand the ecogeochemistry in low-nutrient estuari3es, such as those found in Northern New Zealand, and to clarify how anthropogenic stressors impact the intersection of the ecological and biogeochemical processes regulating ecosystem function and services in these key environments. This dissertation is comprised of one perspective chapter (Chapter 2), and three research chapters (Chapters 3-5) which collectively investigate how low-nutrient estuaries function differently from their more eutrophic counterparts and identify how the

anthropogenic increases in terrestrial sediment deposition hinder the resilience of these important systems against the ever-increasing threats of nutrient pollution and eutrophication. Each chapter is designed to be a stand-alone research paper as well as a cohesive section of the dissertation, with this general introduction chapter providing context for the subsequent chapters, and a general discussion chapter to synthesize results and provide directions for future research. The individual aims and objectives of each chapter are described below:

In Chapter 2, I analysed the most cited, primary research articles on N processing in estuaries, highlighting that a significant bias has developed in the literature toward chronically eutrophic estuaries of the Northern Hemisphere, particularly the North Atlantic. I emphasize that the paucity of data from underrepresented systems (such as tropical and low-nutrient estuaries) results in a poor understanding of how these ecosystems function, and how chronically eutrophic estuaries behaved pre-nutrification. I demonstrate that what we do know suggests that low-nutrient and tropical systems function very differently from their eutrophic and temperate counterparts, and that there is value in further study of these underrepresented systems. This chapter then provides context for the rest of my dissertation research, which was conducted entirely in subtropical, oligotrophic estuaries.

In Chapter 3, I seek to establish a baseline of N cycling in the low-nutrient estuaries of North Eastern New Zealand, and to understand how the ecology and biogeochemistry of these systems intersect to influence ecosystem function, particularly the key ecosystem service of denitrification. This goal was accomplished by deploying a series of *in situ* benthic incubation chambers across three different estuaries. This chapter identifies the specific controls on denitrification in these low-nutrient estuaries, including

the contribution of the macrofaunal community to denitrification rates, and creates interaction networks of ecogeochemical processes within these systems.

In Chapter 4, I address some common methodological issues in the quantification of denitrification and nitrogen fixation. As these two processes are often quantified as a net change in dissolved N_2 gas, their relative contributions to N cycling and overall ecosystem function are not well constrained, this is especially true for N fixation. Therefore, I executed the first simultaneous measurements of these two processes in oligotrophic estuarine sediments. Using intact sediment cores and the addition of isotopically labelled $^{30}N_2$ gas, I was able to quantify the individual contributions of each of these important processes to the total N_2 flux. I was also able to perform a methodological comparison to the more commonly used N_2/Ar method, and address issues of quantifying N_2 fluxes, specifically nitrogen fixation, under light conditions.

In Chapter 5, I build on the results of Chapters 3 & 4 and identify a specific mud content threshold that induces a tipping point in the macrofaunal community and ecosystem function of an oligotrophic estuary. A high resolution of *in situ* benthic incubations were deployed across a mud gradient in the Okura Marine Reserve, which has experienced a substantial increase in terrestrial sediment deposition in recent years. The results of this chapter show that even small changes in overall sediment mud content can induce a regime shift in these systems to a state with diminished functionality and resilience against future pollution and eutrophication.

In Chapter 6, I synthesize the research I conducted throughout my dissertation, outline conclusions, discuss limitations, and offer suggestions for future research directions.

1.3 | Methodological Overview

There are several methodologies that are common between my dissertation chapters, some of which are modified versions of methods that are widely used throughout the biogeochemical and ecological study of estuarine systems. Outlines and justifications for key methods are below.

1.3.1 | Sediment Incubations

Incubations of marine sediments have been integral to, particularly the biogeochemical study of estuaries for decades (e.g., Seitzinger, Nixon and Pilson, 1984; Nielsen and Glud, 1996), and are still widely used to this day (e.g., Kellogg *et al.*, 2013; Foster and Fulweiler, 2014; Song *et al.*, 2020). The data chapters of my dissertation (Chapters 3-5) all employed the use of various forms of sediment incubations. Incubation chambers allow for the measurement of fluxes of dissolved gases and nutrients across the sediment water interface. Various mechanisms and ecosystem functions such as, primary productivity, sediment oxygen demand, DNF, N fixation, and DIN and DON production or consumption etc. can then be quantified using this method. All incubation methods encase a specific area of sediment and volume of water in a closed system for a period of time ranging from 4-48 hours depending on the chamber (Fig. 1.3). The flux of all solutes across the sediment water interface is assumed to be linear (Nielsen and Glud, 1996; Kana *et al.*, 1998), and is calculated from the slope of the change in concentration over time, normalized to enclosed water volume and sediment surface area, giving fluxes in μmol m^{-2} h^{-1}. Regardless of type, all of these sediment chamber incubations yield *net* fluxes. In the case of net N_2 flux for example, a positive flux indicates net DNF, while a negative flux indicates net N fixation, though both processes could be occurring simultaneously.

In Chapter 3, I used the large, *in situ* incubation chambers (Fig 1.3A), which allow for the incubation of large areas of sediment, volumes of water, and infauna over the entire inundated tidal cycle. These are *in situ* measurements which cause very little disturbance to the sediment or macrofaunal community, making them the method that most closely mimics the natural environment (Lohrer, Thrush and Gibbs, 2004; Lohrer *et al.*, 2016). However, their large size requires extensive person-power to move and deploy, limiting the amount of possible replication.

In Chapter 5, I used the small, *in situ* incubation chambers (Fig 1.3B), which share many of the same non-invasive benefits of the larger chambers, but are much smaller, lighter, and quicker to deploy, making them better suited for scenarios that require higher replication (Thrush *et al.*, 2006; Gladstone-Gallagher *et al.*, 2016), such as the quantification of the effects of a sediment gradient in this chapter. Because this chapter had 36 chamber incubation sites, only dark chambers were used.

As Chapter 4 was a laboratory based experiment, I used the more traditional whole core incubation (e.g., Fulweiler *et al.*, 2007; Vieillard and Fulweiler, 2012). Cores can be taken from a variety of environments. While they keep the sediment water interface and sediment column intact, cores encompass the smallest surface area, and therefore a more limited sampling of the macrofauna community, which can be problematic in systems as patchy as estuarine sediments. They also remove sediments from the environment and bring them into a more controlled lab setting. While this can make core incubations less representative of the natural ecosystem, it also allows for more controlled manipulation of conditions than is tractable *in situ,* as was required in Chapter 4. In order to mimic the natural environment as closely as possible, these where whole core, not slurry, incubations.

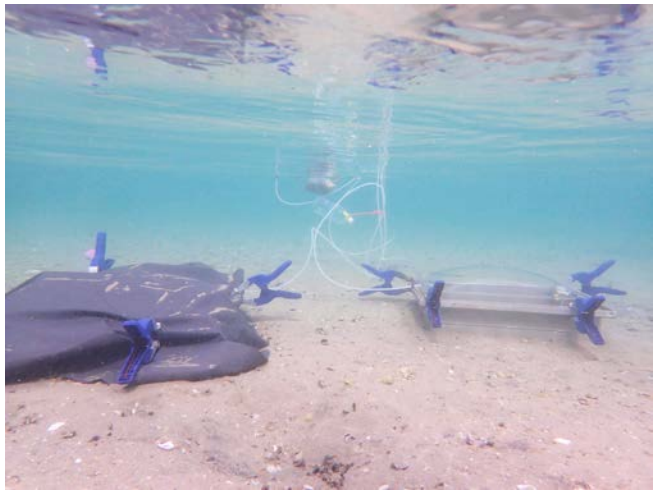


Figure 1.3A Large, *in situ* sediment incubation chambers. Aluminum bases are inserted 5 cm into the sediment at low tide, enclosing 0.25 m^2 (0.5 m x 0.5 m) of intact, undisturbed, intertidal benthic habitat. Once the tide is in with a tidal height of ~0.5 m, acrylic lids are rid of bubbles and clamped to bases, enclosing ~41 L of overlying water. Chambers are deployed in pairs with opaque, black plastic sheeting clamped on to one, leaving the other open to ambient light. Foam seals on the inside lip of both lid and base ensure a gas tight seal while underwater. Approximately 2 m of sample tubing is attached to a port on each chamber lid with a luer lock on the other end, allowing for sampling of the water within the chambers at the surface. One small port on the other side of the chamber lid (~3 mm diameter) is left open so that sampling does not create negative pressure and pull out sediment pore waters. Light and dark bottles filled with ambient site water are also incubated (without bubbles) to account for any water column activity. Dissolved oxygen and temperature sensors as well as small pumps (to prevent stratification) are deployed in each chamber, and the light chambers are also equipped with light loggers. The large footprint of the cambers allows for capture of a broader range of animal activity than other methods.

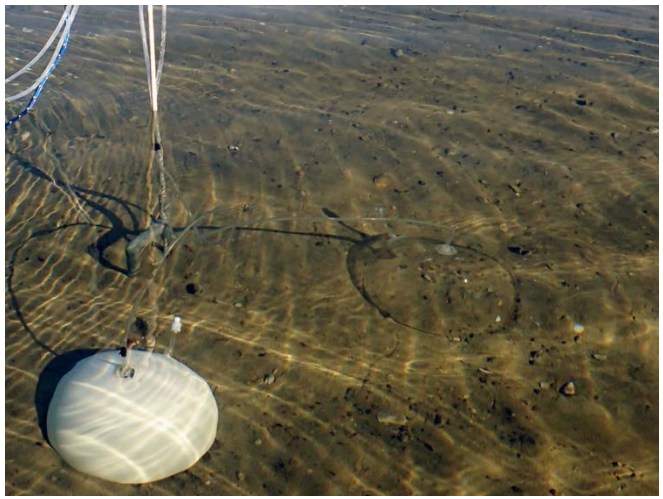


Figure 1.3B Small, *in situ* sediment incubation chambers. Half-sphere chambers are inserted 2 cm into the sediment once it is inundated to a tidal height of ~ 20 cm, enclosing 0.025 m^2 (17 cm diameter) of intact benthic habitat and 1.2 L of overlying water. Circular, lead weights sit about half way down the chamber to hold it in place (not pictured). Light chambers are clear while dark chambers are black with the outsides painted white to help keep the temperature uniform on sunny days. Approximately 2 m of sample tubing is attached to a port at the top of each chamber with a luer lock on the other end, allowing for sampling of the water within the chambers at the surface. One small port on the other side of the chamber lid (~3 mm diameter) is left open so that sampling does not create negative pressure and pull out sediment pore waters. Light and dark bottles filled with ambient site water are also incubated (without bubbles) to account for any water column activity. Temperature and light loggers are staked to the sediment outside the chambers. While the surface area of these chambers is less than that of the large benthic chambers, it is larger than cores, thus likely capturing greater animal activity than core incubations.

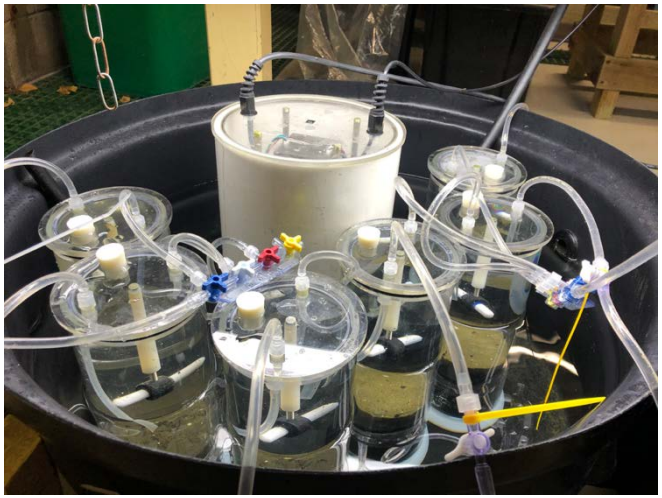


Figure 1.3C Intact core incubation chambers. Cores are inserted into the sediment in the field at low tide, removing a section of sediment with a surface area of 0.01 m^2, approximately 15 cm deep. Cores are then gently filled with 0.2 μm filtered site water and placed in constant temperature, flow through water baths. Core lids are equipped with magnetic stirring bars, which are moved by a magnetic carousel in the middle of the water bath. The carousels turn at ~50 RPM, slow enough to prevent sediment resuspension, but fast enough to prevent stratification. Inflow ports are attached to gas-tight bags of filtered site water on a table above, resulting in a gravity fed system that replaces any water that is removed for sampling without introducing any air, and creating sufficient head pressure to fill sample vials without suction. Silicone stoppers plug a hole in each lid which allows for removal of air bubbles in the capping process and for the deployment of various sensors if needed. Water baths can be kept open under lights to mimic light conditions, or covered with opaque sheeting for darkness. Temperature and light sensors are deployed at sediment height within each water bath. While cores capture the smallest surface area, they are ideal for performing more technical experiments were a controlled environment is needed.

1.3.2 | Denitrification

The three most common methods for measuring DNF in aquatic systems are the acetylene block technique (Tiedje, Simkins and Groffman, 1989), the N_2/Ar method (Kana *et al.*, 1994), and the isotope pairing technique (Nielsen and Nielsen, 1992). Acetylene block measures the enzyme activity of the population of denitrifying bacteria within a sediment sample. This method is carried out at ideal conditions for DNF, namely, anoxic with continuous mixing and unlimited carbon and NO_3^- sources. Additionally, the acetylene used in this method inhibits nitrification and therefore the coupled nitrification-denitrification process that is common in most estuarine sediments, therefore it is not an actual measure of DNF rates, but of the potential for direct DNF to occur under optimal conditions (Tiedje, Simkins and Groffman, 1989; Groffman *et al.*, 2006). Before the development of more direct methods of quantifying DNF, acetylene block was widely used in estuaries (e.g., Kaspar, 1983; Kieskamp *et al.*, 1991), and its inexpensive and easily accessible analysis makes it valuable in remote and understudied systems (e.g., Douglas *et al.*, 2017); however the use of this technique to measure DNF rates has largely been phased out in favour of other methods.

The N_2/Ar technique uses the ratio of the gasses N_2 and argon (Ar) to measure very small changes in the concentration of the very large background N_2 pool from water samples. The idea being that Ar is biologically inert and so, provided the temperature remains constant, its concentration should not change over the course of an incubation, therefore changes in the N_2 to Ar ratio are proportional to the change in N_2, but larger and easier to measure. As the N_2 concentration changes over time a flux (rate) can be calculated. This measurement is generally carried out on a membrane inlet mass spectrometer (MIMS) which can detect these small changes in concentration with very high precision (<0.03 %, Kana *et al.*, 1994). The advantages of the N_2/Ar method are that

it is a direct measurement, is fast (1-2 minutes per sample), precise, and is done on water samples so intact sediments can be used with minimal disturbance. The primary disadvantages are that the rate from this method is a net N_2 flux rate, or the sum of the DNF rate (positive N_2 flux) and the N fixation rate (negative flux), and their individual contributions cannot be determined. Another disadvantage of the N_2/Ar method is that it is incredibly sensitive to air bubbles; because of the high concentration of N_2 in air, bubbles introduced at any point of the incubation or sampling process can alter the concentration of N_2 and skew the resulting rate. Therefore the preparation of N_2/Ar samples has to be gas tight and air free. Despite these complications, the N_2/Ar method is a widely accepted and used method to quantify denitrification in aquatic systems (e.g., Eyre and Ferguson, 2002; Fulweiler *et al.*, 2007).

The Isotope Pairing Technique takes advantage of the differing concentrations of N's stable isotopes, ^{14}N (99.64 %) and ^{15}N (0.36 %) . Heavy, labelled ^{15}N-NO_3^- is added to the overlying water of a sediment incubation at a concentration that will ensure that NO_3^- will not become limiting. The heavy isotope that was added can then be traced into the N_2 pool over time yielding DNF rates. The heavier isotopes of N_2 ($^{29,30}N_2$) are measured in this method on either a MIMS or isotope ratio mass spectrometer (IRMS) with very high precision. IPT is a direct measure of DNF and yields 3 rates: the direct DNF rate, the coupled nitrification-denitrification rate, and the total DNF rate (Steingruber, Friedrich, Gächter, *et al.*, 2001). Direct DNF refers to the direct denitrification of NO_3^- that diffuses into the sediment from the water column, while the coupled nitrification-denitrification rate refers the rate of denitrification of NO_3^- that was first nitrified from NH_4^+, with the total rate being the sum of the two. The advantages of this method are that, unlike the N_2/Ar method, the rates are total, not net rates, and the partitioning into different forms of DNF allow for more nuanced analysis of the

biogeochemical mechanisms at play in a system. Like N_2/Ar this measurement can be done on water samples quickly (in the case of the MIMS) or on equilibrated gas samples which take more time (~12 minutes per sample in the case of the IRMS). The primary disadvantage of IPT is that NO_3^- has to be added to the system (as the label) and remain non-limiting throughout the incubation, this changes the natural conditions in the system, which can be problematic especially for oligotrophic sediments. This limitation makes the results of IPT potential DNF rates; however, IPT is still widely used in quantifying marine DNF and has informed many additional N isotopic methods (e.g., An and Joye, 2001). Additionally, labelled stable isotopes can be cost prohibitive, particularly in more remote environments.

In this dissertation, I primarily used the N_2/Ar method to quantify DNF. I chose this method mainly because it is a direct measure that is non-damaging to the sediment and includes information about DNF and N fixation together. I used the N_2/Ar method in chapters 3 and 5. In Chapter 4, I specifically wanted to quantify DNF and N fixation separately, therefore I used an isotopic tracer technique to accomplish this. Like IPT, this technique used a stable N isotope label, but to trace N fixation instead of DNF and the DNF rate was calculated by measuring the production of the more abundant $^{28}N_2$ isotope, which is not measured in IPT.

1.3.3 | Nitrogen Fixation

There are also three primary methods for the quantification of N fixation in the marine environment, one of which is the N_2/Ar method described above. The oldest, and most commonly used method is the Acetylene Reduction Assay (ARA), which takes advantage of the fact that *nitrogenase*, the enzyme required to fix N can also reduce acetylene to ethylene. Therefore, acetylene is added to the headspace of sediment slurries and the resulting ethylene production is measured and converted to an N fixation rate.

ARA is affordable, and very widely employed, however, it has well documented impacts on nearly every N cycle process, including a suppression of N fixation itself, and is therefore considered to vastly underestimate rates of N fixation in the environment (Fulweiler *et al.*, 2015).

The more recently developed method for measuring N fixation is via the addition of isotopically labelled $^{30}N_2$ gas. This heavy nitrogen gas is made into an aqueous standard by equilibrating water with a $^{30}N_2$ headspace for at least 24 hours. The standard is then diluted to the desired concentration and added to the overlying water of a sediment incubation system. Mass spectrometry is then used to track the consumption of the heavy isotope. After accounting for diffusion, any the only process that can consume N_2 is N fixation, and so the rate of consumption of the $^{30}N_2$ becomes the rate of N fixation. This is a direct measure that can be used on intact sediments, making it a very robust measurement. Additionally, the heavy isotope can be further tracked into other N pools such as dissolved NH_4^+ and sediment organic matter in order to shed light on the fate of the fixed N within the system. This method also does not impact DNF rates, and therefore allows for the simultaneous quantification of DNF either through the addition of another label (as in IPT) or through the production of unlabelled $^{28}N_2$. The primary drawbacks of this method are that $^{30}N_2$ is much more difficult to measure accurately than $^{28}N_2$, and that the labelled $^{30}N_2$ needed for the addition is very expensive, particularly in countries where it is not produced, limiting its more widespread use.

While N fixation is accounted for by using the N_2/Ar method in Chapters 3 and 5, I chose to use the addition of isotopically labelled $^{30}N_2$ to specifically quantify N fixation in Chapter 4. As it was carried out in a controlled, laboratory setting, and I was looking to measure N fixation and DNF simultaneously, this method was a good fit for that

experiment, and I was able to strategically use the $^{30}N_2$ label to keep it from being completely cost-prohibitive.

Chapter 2

Recovering from Bias: A call for further study of under-represented tropical and low-nutrient estuaries*

A.M. Vieillard, S.E. Newell, S.F. Thrush

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2.1 | Summary

Estuaries are some of the most dynamic of Earth's ecosystems and are also some of the most heavily impacted by anthropogenic activities. Coastal nutrient pollution has been identified as one of the greatest threats facing these valuable systems worldwide. As a result, a strong literature bias has developed toward heavily eutrophied estuaries in the Northern Hemisphere, with relatively little data available from tropical and low-nutrient systems. Here we discuss the implications of this bias and argue for a need for further work in a larger variety of systems. Further understanding of these underrepresented systems is vital to future estuarine management.

2.2 | Introduction

At the interfaces of the terrestrial and marine environments, estuaries are some of the most dynamic and reactive ecosystems on earth. Many of these systems are on the front lines of human-environment interactions and have been central to the development of human civilization. As a result, estuaries are also some of the most heavily impacted and threatened natural systems (Kennish, 2002; Barbier *et al.*, 2011). Estuarine ecosystems are comprised of several diverse habitats which transition over relatively small spatial scales, and therefore provide many valuable ecosystem services (Piehler and Smyth, 2011). These systems also have incredibly high rates of both benthic (MacIntyre, Geider and Miller, 1996; Sundbäck, Miles and Göransson, 2000) and pelagic primary productivity (Underwood and Kromkamp, 1999; Barbier *et al.*, 2011), supporting some of the world's most productive fisheries (Conley, Schelske and Stoermer, 1993). They are also very biogeochemically active habitats, serving as filters and processors of terrestrially derived inputs (Sundbäck, Miles and Göransson, 2000). Thus, maintaining and recovering functionality of estuaries is crucial from both an economic and ecological perspective. Further, a recent article by Oczkowski *et al.* (2020) highlights the need to turn our

collective attention to understudied, tropical, urban estuaries. We seek to broaden that call to action to all tropical estuaries as well as non-eutrophic, low-nutrient systems around the globe, in order to achieve a truly representative understanding of estuarine ecosystem functions.

As human populations increase and estuarine systems become increasingly stressed, effective estuarine management and recovery plans are critical, particularly in developing countries (Le Moal *et al.*, 2019). Approximately one third of the global population lives in low-elevation coastal zones (LECZ), over 75% of which reside in developing nations (Neumann *et al.*, 2015). The combination of densely populated LECZ's, tropical locales, low socio-economic standing, and lower quality infrastructure make tropical, and particularly tropical urban estuaries especially vulnerable (McGranahan, Balk and Anderson, 2007; Oczkowski *et al.*, 2020). However, without good benchmarks for comparison, estuarine restoration and recovery is likely to be compromised. In this commentary, we will discuss how the research focus on estuaries in developed nations has resulted in a bias toward temperate and eutrophic systems in the North Atlantic. Both low-nutrient and tropical systems have generally been poorly represented in the literature, and as a result, we understand very little about the corresponding differences in biogeochemical cycling, or how management approaches must differ in these different environments.

Here, we review the contrasting functionality of N cycling in temperate, heavily eutrophied estuaries with that of lesser-studied tropical and low-nutrient estuaries. Tropical estuaries are defined as estuaries within the tropics of Cancer and Capricorn (between 23.4° North and South latitudes). These systems behave quite differently from temperate estuaries in terms of nutrient cycling; where N loss via DNF tends to dominate in temperate, eutrophic estuaries, tropical systems tend to see higher rates of N fixation

and retention, even when heavily nutrified (Eyre and Balls, 1999; Bhavya *et al.*, 2016; Oczkowski *et al.*, 2020). Indeed, the results presented by Oczkowski *et al*. (2020) show that in an urban, tropical estuary with restricted flow, the traditional view of estuaries as nutrient filters no longer applies. The high organic matter and sulphide levels result in an ecosystem where nitrogen-fixing, sulphate-reducing microbes produce as much N as the load from urban runoff and sewage (Oczkowski *et al.*, 2020). Meanwhile, temperate, low-nutrient estuaries (annual N load of less than 20 g N m^{-2} of estuarine area) maintain the functions that have been lost in eutrophic systems and may therefore hold the keys to preserving or restoring the variety of vital ecosystem services that estuaries world-wide provide. By comparing these systems, we will be able to better identify targets for recovery, and to better inform the management of all estuarine systems in the future.

2.3 | The Eutrophication Problem

Coastal nutrient pollution has long been identified as one of the greatest threats facing estuaries world-wide (Le Moal *et al.*, 2019). By 2000, global riverine nitrogen inputs were, on average, double pre-industrial levels (Galloway et al., 2004; Howarth, 2008). As coastal N loads increased, there was a push to understand and document the widespread effects of anthropogenic nutrification on critical coastal ecosystem functions including: eutrophication (Martin et al., 2008; Nixon, 1995), changes to phytoplankton community composition (Ryther, 1969; Schelske and Stoermer, 1971), harmful algal blooms (Paerl, 1997), hypoxia and dead zones (Conley *et al.*, 2007; Diaz and Rosenberg, 2008), seagrass die off (McGlathery, 2001; Burkholder, Tomasko and Touchette, 2007), and overall loss of biodiversity (Smith *et al.*, 2000). Today, nutrification remains a major challenge, to the extent that the Stockholm Resilience Centre has classified the biogeochemical flows of N and P to coastal systems as beyond safe planetary operating zones (Steffen *et al.*, 2015). This raises fundamental questions of how much these

ecosystems have changed, and the degree to which their capacity to process nutrients and provide ecosystem services has been compromised.

Extensive monitoring and laboratory-based studies have thus been conducted on estuarine nitrogen dynamics (e.g., Nixon, 1981; Burgin and Hamilton, 2007; Howarth, 2008; Le Moal *et al.*, 2019). DNF, nitrification, DNRA, and N fixation are the primary N transforming processes in estuarine sediments, all of which transform N into various reactive or bioavailable forms, with the exception of DNF, which converts reactive nitrogen into the largely inert N_2 gas, effectively removing N from the system. DNF has therefore received particular attention due to its potential to mitigate anthropogenic N pollution (Seitzinger, Nixon and Pilson, 1984). As a result, the controls on DNF and inorganic N cycling in temperate, eutrophic systems are well studied (Burgin and Hamilton, 2007), and it is tempting to think that we have a good handle on estuarine nutrient dynamics.

However, a strong bias has developed in this work toward Northern, temperate estuaries which are heavily, and chronically nutrified (Kroeze & Seitzinger, 1998, Fig. 2.1). A survey of the 45 most cited, primary research studies of N in estuaries since 1990 highlight the literature bias towards chronically eutrophic, or hypereutrophic estuaries of the North Atlantic (Fig. 2.1, see Appendix I for methodology). From these 45 studies, 140 individual study sites were identified, 92 % of which were above 30 °N latitude and none were below 30 °S (Fig 2.1). Additionally, 83 % were located in Northern Hemisphere, Atlantic systems, and a massive 95 % of the study sites were located in eutrophic or hyper-eutrophic systems (annual N load > 20 g N m^{-2} y^{-1}, Fig.2.1). By comparison, the data available from tropical and low-nutrient or oligotrophic estuaries (annual N load < 20 g N m^{-2} y^{-1}) are incredibly limited (e.g., Cook *et al.*, 2004; Eyre *et al.*, 2011; Thomson *et al.*, 2012; O'Meara, Hillman and Thrush, 2017; Oczkowski *et al.*, 2020). Additionally, a

large proportion of studies in low-nutrient systems are now nearly 30 to 40 years old, and employed out-of-date methods such as the acetylene block technique (e.g., Kaspar, 1983; Kieskamp et al., 1991; Koch et al., 1992), making direct comparisons to modern studies difficult (Groffman *et al.*, 2006). Even common, modern methodologies for quantifying DNF are inherently biased toward systems with a high N load. For example, IPT requires NO_3^- to be non-limiting, meaning a surplus has to be added to the overlying water column, pushing low-nutrient sediments outside their natural state (Steingruber, Friedrich, Gächter, *et al.*, 2001). As a result, the current understanding and modelling of nitrogen cycling in estuaries, which has been gleaned largely from eutrophic, temperate North Atlantic systems, may not be applicable to many, more low-nutrient or tropical systems world-wide (Harris, 2001; Cook, Revill, Butler, *et al.*, 2004; Ferguson, Eyre and Gay, 2004; Oczkowski *et al.*, 2020). This bias has broad implications, not only for DNF, but for all ecosystem services that interact with or rely on N processing.

2.4 | Ghosts of Functions Lost

It has been suggested that, pre-industrial revolution and pre-eutrophication, European estuaries were net importers of N from the sea, with tight recycling and storage of N, enabling the support of high secondary production (Malcolm and Sivyer, 1997). There are similar claims for estuaries along the east coast of the US, with an estimated 75-90 % of pre-historic nitrogen inputs to Narragansett Bay coming from the coastal ocean (Nixon, 1997). Oceanic nitrogen import coincided with incredibly productive shellfish and lobster fisheries (Nixon, 1997; Jackson *et al.*, 2001)

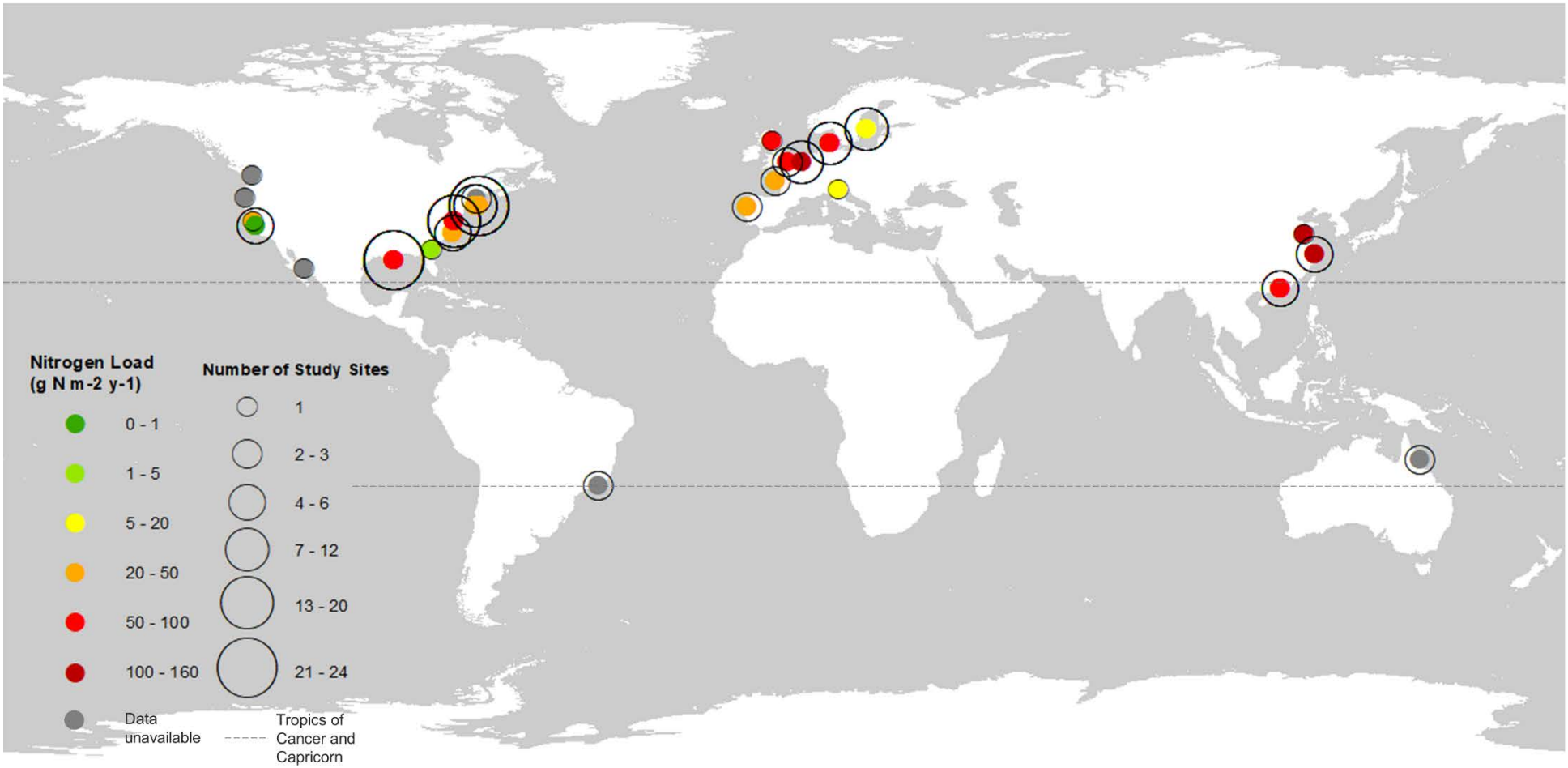

Figure 2.1. The 45 most cited, primary research studies on nitrogen cycling in estuaries from 1990-2019. Color-coded points show the annual nitrogen load to each estuarine system in g N m^{-2} y^{-1}; grey points indicate estuaries where nitrogen loading data were unavailable. Black rings represent the number of study sites located within each system. Dashed lines denote the Tropics of Cancer and Capricorn at 23.4° North and South, respectively. A clear bias can be seen toward Northern hemisphere, Atlantic systems with moderate to high N loads. See Appendix I for methodology and reference list.

This evidence suggests that estuaries that are now eutrophic were as, if not more, productive pre-eutrophication than they are today; implying fundamental shifts in ecosystem function from systems dominated by recycling and multiple ecosystem interactions to a more simple flow-through processes (Brush, 2009). This shift represents a loss of complexity and therefore a decrease in resilience with increasing nutrification (Lotze et al., 2006, Fig.2.2). In order to understand how these understudied low-nutrient systems function, our conceptual model has an oligotrophic starting point, however we acknowledge that oligotrophy is not necessarily the pristine state of all estuarine systems (Nilsson and Rosenberg, 2000; Pearson and Rosenberg, 1978).

Like pre-industrial estuaries, modern, low-nutrient estuaries function quite differently than their more eutrophic counterparts. In terms of DNF, a strong relationship has been found between increasing water column NO_3^- concentrations and increasing DNF rates in nutrified systems (Seitzinger and Nixon, 1985; Jensen, Jensen and Kristensen, 1996; Dong *et al.*, 2011). However, this relationship likely does not hold at the very low (<10 µM) nitrate concentrations found in low-nutrient and oligotrophic systems (Pina-Ochoa and Alvarez-Cobelas, 2006; Vieillard and Fulweiler, 2012). Additionally, it is well established that while nitrate fuels direct DNF when it is abundant, the coupled nitrification-denitrification pathway is dominant when nitrate concentrations are low (Valiela and Teal, 1979; Rysgaard, Risgaard-Petersen and Sloth, 1996; Eyre *et al.*, 2002; Hoffman *et al.*, 2019). The conclusion then, is that there is a different relationship between DNF and NO_3^- in low-nutrient estuaries, suggesting a fundamental shift in nitrogen retention versus removal with increasing N inputs.

Evidence also suggests that in low-nutrient systems, a far greater proportion of the bioavailable N is assimilated rather than denitrified, resulting in a net retention of N within

the system (Risgaard-Petersen *et al.*, 2003; Cook, Revill, Butler, *et al.*, 2004). This retained N is then cycled and recycled through the system ultimately supporting secondary and tertiary production (Fig. 2.2). DON may also play a larger role than DIN in oligotrophic and low-nutrient estuaries; however, DON is not commonly measured (Sundbäck, Miles and Göransson, 2000; Eyre and Ferguson, 2002a). Additionally, N fixation may be the source of a significant portion of the N demand in oligotrophic systems (Stal, 1995), but again, specific quantification of N fixation is not common in estuaries, and its quantification is plagued by methodological issues (Newell *et al.*, 2016). The data we do have paint a picture of efficient recycling of N in systems where N inputs are low; where benthic-pelagic coupling is tight, productivity is high, and fisheries are productive *(Kelly et al.*, 2007, Fig. 2.2).

Limited research in subtropical and tropical estuaries has shown that these estuaries also function fundamentally differently than their temperate counterparts. Tropical systems may be more sensitive to nutrification because the elevated temperatures ramp up ecosystem metabolism and decrease oxygen concentrations, both of which tend to amplify anthropogenic stressors that lead to eutrophication (Corredor *et al.*, 1999). Many studies have reported that DNRA can dominate over DNF when organic matter, salinity, and temperatures are higher (Burgin and Hamilton, 2007; Giblin *et al.*, 2013; Van Den Berg *et al.*, 2015). Additionally, studies in tropical/subtropical, eutrophic estuaries such as Weeks Bay, AL or Corpus Christi Bay, TX along the Gulf of Mexico (Gardner *et al.*, 2006; Bernard, Mortazavi and Kleinhuizen, 2015; Domangue and Mortazavi, 2018) and the Pearl River and Yangzte River in China (Xu *et al.*, 2005; Jiang and Li, 2014; Yin *et al.*, 2017) have reported that DNRA dominates when the sulphide transition zone is near the sediment surface.

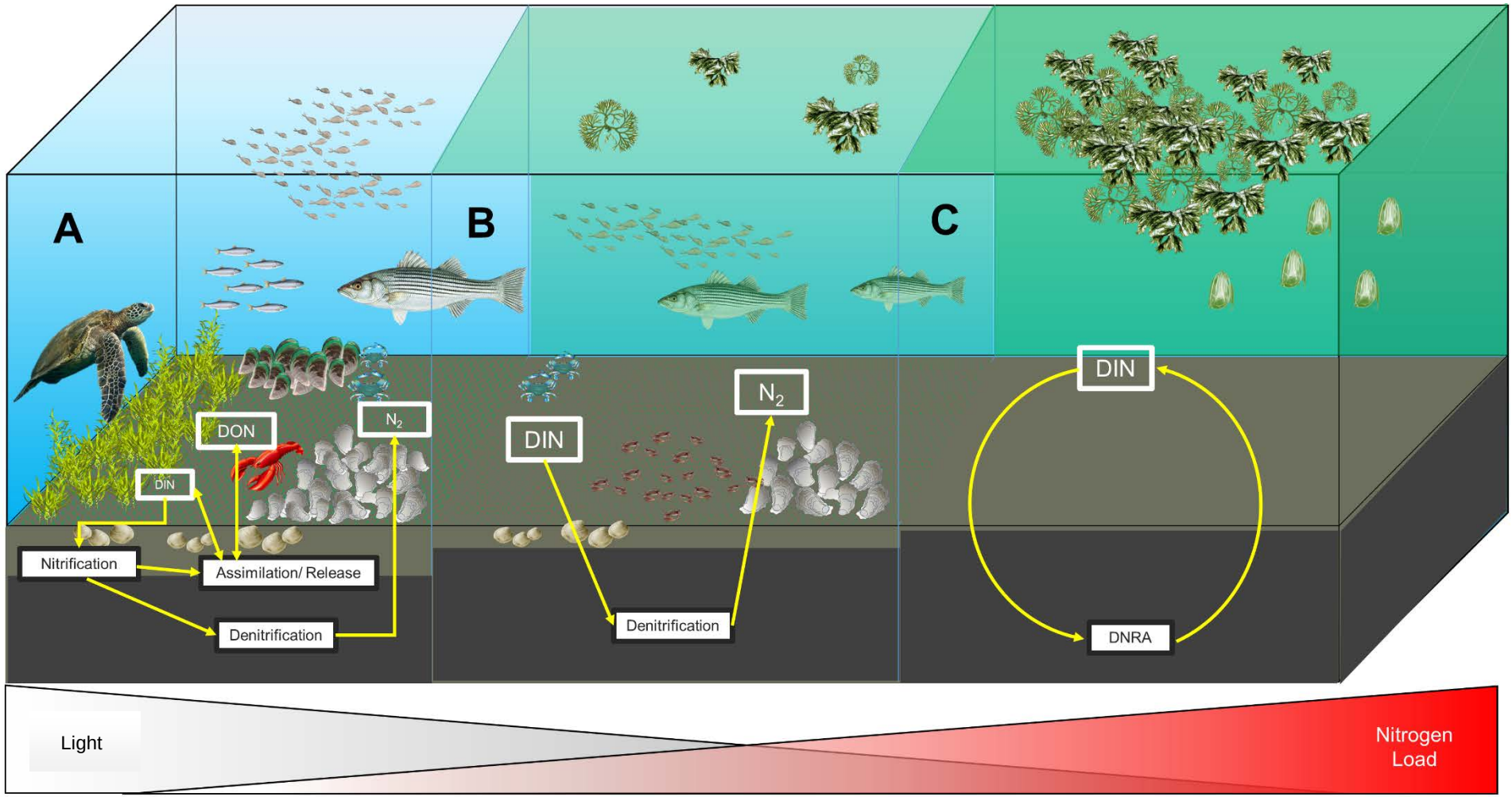

Figure 2.2. Conceptual diagram of shifts in estuarine ecosystem functionality from pristine (**A**) to eutrophic (**B**) to hyper-eutrophic (**C**). From left to right N loads increase, light attenuation decreases, and sediment oxygen penetration decreases. **A** is characterized by low N inputs, high rates of nutrient recycling, clear water, high oxygen concentrations, tight benthic-pelagic coupling, diverse benthic primary productivity, and vast secondary and tertiary production. **B** has a markedly increased nutrient load and is characterized by phytoplankton-dominated primary productivity, decreased water clarity, decreased benthic primary production, more N removal than retention, and a less diverse food web. **C** has very high nutrient inputs resulting in macroalgal blooms, extensive benthic shading, a clear decline in biodiversity, hypoxic to anoxic conditions in the sediment and overlying water, and a runaway positive feedback keeping reactive N cycling within the system.

A few studies in developing countries, such as Thailand and Indonesia (Dong *et al.*, 2011), support the more extreme findings of Oczkowski et al. (2020) of high DNRA and low-to-undetected levels of DNF, suggesting that low-latitude estuaries, like low-nutrient estuaries, likely respond differently to environmental controls than their temperate and even their subtropical counterparts.

2.5 | The Productivity Paradox

It has been established that increasing nutrient inputs increase both primary (Nixon, 1997) and secondary productivity in estuaries (Nixon and Buckley, 2002; Cole *et al.*, 2008). The question then arises: how could pre-industrial systems be so productive with so much less incoming N? First, the relationship between increasing N inputs and estuarine primary productivity only accounts for phytoplankton productivity (Nixon, 1997), so while the increase in N delivery stimulates phytoplankton growth, it often also corresponds to a loss of other forms of benthic primary production, including microalgae, kelp, and seagrass as a result of increased turbidity and benthic shading (McGlathery, 2001; Howarth, 2008). The result is a move away from diverse benthic habitats that fill multiple roles including, primary productivity as well as extensive nursery habitats, of a low-nutrient system toward more a homogeneous, phytoplankton dominated system as N inputs rise (Fig. 2.2). This represents a loss of both food and habitat diversity for a variety of secondary and tertiary consumers. Second, the relationship between fish landings and primary production, again, only accounts for phytoplankton production, and was measured only in phytoplankton-dominated, temperate systems (Cole *et al.*, 2008; Capuzzo *et al.*, 2018). Therefore, this relationship does not consider more complex ecosystems such as kelp or seagrass dominated estuaries, or tropical mangrove or reef-based systems and how increasing nutrients and phytoplankton growth might affect secondary production in those

systems. This relationship also only accounts for the fish yield by weight and does not include any changes in species composition or trophic level. These dynamics also fit into the classic benthic model of disturbance and succession, where total abundance and to a lesser extent biomass, peak as the impact of the stressor increases, but species richness continues to decline (Pearson and Rosenberg, 1978). While abundance and biomass can both increase briefly with increasing stress (such as nutrient loading), overall complexity of the benthic community will still be diminishing (Nilsson and Rosenberg, 2000).

While there are no direct comparisons between secondary and tertiary productivity in high versus low nutrient systems, there is evidence that modern, estuaries with low anthropogenic nutrient inputs can be equally, if not more productive, than high-nutrient systems (Cook, Revill, Clementson, *et al.*, 2004). Coral reefs are an example that is easy to visualize: most of these tropical systems have incredibly low nutrient inputs, yet they are some of the most diverse and productive systems on Earth, with nearly one third of the world's marine fish species found on coral reefs (Moberg and Folke, 1999). Additionally, work on temperate, artificial reefs has shown that reefs actually produce fish biomass, even in low nutrient conditions (Cresson, Ruitton and Harmelin-Vivien, 2014). Like other low-nutrient systems, these reefs rely on more diverse nutrient sources such as oceanic input and N fixation, tight nutrient coupling and recycling, and the creation of structured and varied habitat and food sources than do temperate, eutrophic systems (Cresson et al., 2014, Fig. 2.2).

In fact, these systems are not actually low in N, there is enough N from various sources to promote highly productive food webs, they are, however, low in *excess* N. These low-nutrient systems have very low water column DIN concentrations because it is recycled efficiently within the system and does not accumulate in the water column for measurement. Similarly, these systems generally have low turbidity and water column

chlorophyll levels. This, again, is a result of little *excess* phytoplankton growth. Both the nutrient cycles and food webs in these systems are efficient and tightly coupled. This efficiency is a regime shift from the heavily nutrified systems that are so well-studied, where excess and inefficiency dominate (Fig. 2.2).

When the majority of the N cycling research is conducted in temperate, eutrophic systems, N removal via DNF is the primary ecosystem service of focus. The importance then, of N retention and the wide variety of other related ecosystem services (including DNRA, which appears to play a prominent role in tropical estuaries) in other types of systems is often lost. However, it is important to understand this change in and, arguably, loss of functionality with increasing nutrification. Moreover, we need to see the value in clarifying the biogeochemical dynamics of understudied low-nutrient and tropical systems. Further understanding of these systems is necessary to balance our knowledge-base of coastal N dynamics and define benchmarks to inform the restoration and recovery of eutrophic systems.

2.6 | Benchmarks for Recovery

Estuarine restoration efforts are ongoing world-wide (Le Moal *et al.*, 2019) due to their ecological and economical value. Many countries, particularly those around the North Atlantic, have begun to try to decrease N runoff to estuaries. Strategies ranging from the reduction and management of agricultural fertilizer application, to managing urban storm water, and tertiary waste water treatment are being proposed and employed in an effort to reduce, radically in some cases, the amount of N delivered to coastal systems (Bernhardt *et al.*, 2008; Detenbeck, You and Torre, 2019). The vast majority of these programs are focused on getting DIN loads and/or concentrations to lower levels based on historic benchmarks (Kennish, 2002). While this is certainly a step in the right direction, it is difficult to know what to expect in the recovery of systems that have been

systematically polluted, and it is unclear whether turning down this one N input "dial" alone will result in the recovery of lost functionality.

In terms of recovery, if we want to "Return to Neverland" (Duarte *et al.*, 2009), we have to know what state we are trying to restore to. It is possible to look to low-nutrient systems, both temperate and tropical, as benchmarks, not just for N inputs, but for overall ecosystem functionality. Understanding, for example, the drivers of N retention versus N removal in low-nutrient systems can provide insight into how estuaries are "supposed" to function. This further understanding of ecosystem functionality in un-impacted and low-nutrient systems can help define our restoration goals more effectively than using arbitrary indices from historical data.

Modern, low-nutrient estuaries may be particularly useful in dealing with the confounding issues of regime shifts and shifting baselines that often plague restoration efforts (Duarte *et al.*, 2009). All estuaries, whether eutrophic or oligotrophic, temperate or tropical, are facing the multiple stressors of global climate change, and N dynamics are expected to change with our changing climate (Fowler *et al.*, 2015). However, unlike historical estuaries, modern low-nutrient estuaries are experiencing the same large-scale, climate induced regime and baseline shifts as the impacted estuaries we are trying to restore. Therefore, data from these low-nutrient systems across the globe can help us define better restoration goals and help characterize reasonable expectations. This approach will help establish a new normal for properly functioning, diverse, and highly productive estuarine systems. However, this requires a more thorough knowledge of the integrated biogeochemical and ecological processes governing low-nutrient estuaries. More rigorous studies of pristine, un-impacted, and low-nutrient systems are needed to establish baselines and address these questions.

Finally, we must not overlook the importance of establishing these benchmarks and management strategies for all estuarine systems, not just those in wealthy, developed nations. Developing countries have more than twice as many people living in low-lying coastal areas, making both the populations and ecosystems in these countries especially vulnerable (Neumann *et al.*, 2015). In particular, understanding the differences between tropical and temperate estuarine function will help to inform effective management of estuaries in these underserved nations.

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Chapter 3

Ecogeochemistry and denitrification in non-eutrophic estuaries*

Amanda M Vieillard & Simon F Thrush

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3.1 | Summary

Coastal nutrient pollution is an ever-present threat to estuaries world-wide. Benthic denitrification has been identified as a crucial ecosystem service to help mitigate increasing N loads to the coast. However, the controls on denitrification in low-nutrient systems are not well constrained and are likely different to those in more widely studied eutrophic systems. This study aims to identify the specific controls on denitrification in low-nutrient estuaries, including the contribution of the macrofaunal community to denitrification rates, and to understand how this important service fits into the network of ecogeochemical processes in these systems. Results show that porewater ammonium concentrations and mud content are good predictors of net N_2 flux in the dark. Additionally, models predict N_2 flux rates much more effectively in the dark than in the light, but the macrofaunal community data, specifically species richness, is a key factor in both, increasing the explanatory power of both models by nearly 20 %. Additionally, interaction networks reveal that increasing mud content results in a shift in the macrofaunal community and a reduction in the N removal capacity of these intertidal systems.

3.2 | Introduction

Coastal nutrient pollution is recognized as one of the greatest threats facing estuarine ecosystems worldwide (Steffen *et al.*, 2015), and elevated anthropogenic N and P loads to coasts have led to wide-spread coastal eutrophication. Anthropogenic nutrient sources include both acute point sources, such as wastewater outfalls, and diffuse non-point sources such as agricultural runoff and atmospheric deposition (Howarth 2008). Whether point or non-point, these nutrient loads can have disastrous consequences for coastal and estuarine ecosystems (Guignard *et al.*, 2017). In temperate, Northern hemisphere systems, N removal via DNF in estuarine sediments has been shown to help

mitigate N pollution, removing as much as 100 % of anthropogenically derived N in coastal ecosystems (Dong, Nedwell, and Stott 2006; Howarth et al. 1996). As a result, DNF is considered both an ecologically and economically valuable ecosystem service in estuaries, and its controls in heavily eutrophic systems are well studied (e.g., Seitzinger et al. 2006; Galloway et al. 2004; Burgin and Hamilton 2007). DNF is a heterotrophic, anaerobic process in which denitrifying bacteria convert bio-available N as NO_3^- into inert N_2 gas. There are two main pathways for DNF in marine sediments; the first is direct DNF where NO_3^- is supplied directly from the overlying water column via diffusion. The second is the coupled nitrification-denitrification pathway, where NO_3^- is supplied by the oxidation of NH_4^+ via nitrification. Generally, direct DNF tends to be more important in eutrophic sediments with high water column [NO_3^-], while coupled nitrification-denitrification tends to dominate in systems with lower anthropogenic N loads. Since denitrifiers are heterotrophic anaerobes, they require a carbon source and low to no oxygen conditions, as well as NO_3^-, in order for DNF to proceed but, nitrification requires oxygen (Burgin and Hamilton, 2007). As a result, there are many factors which impact the rate of DNF, including but not limited to: organic matter quantity and quality, water column N concentrations, oxygen penetration depth, grain size, temperature, redox conditions, salinity, and sulphide concentrations (Burgin and Hamilton 2007; Joye and Hollibaugh 1995; Seitzinger et al. 2006). DNF, therefore, is a product of interactions between multiple factors requiring an understanding of these drivers and how they change under different conditions in order to improve our ability to predict DNF.

Various macrofaunal species have also been shown to impact DNF rates. In particular, both polychaete worm (Pelegrí and Blackburn, 1995; Bartoli *et al.*, 2000; Bosch, Cornwell and Kemp, 2015) and bivalve dominated (Piehler and Smyth, 2011b; Higgins *et al.*, 2013a; Humphries *et al.*, 2016) communities have been shown to have high

rates of DNF. There are two primary mechanisms thought to drive this increase in DNF rates with macrofauna. First, bio-deposits from macrofauna (particularly large bivalves) are important sources of labile carbon and N to the benthos which can stimulate heterotrophic processes (Callier *et al.*, 2009), and in high volumes, lead to the low oxygen conditions ideal conditions for DNF (Kellogg *et al.*, 2013). However, due to complex interactions between organic matter loading and N cycling, bivalve bio-deposits do not always lead to increased DNF rates (Higgins *et al.*, 2013). The second mechanism is bioturbation. Burrowing organisms such as polychaete worms can create redox micro-zones alongside their burrows. By constructing a burrow from the surface, oxygen rich water is drawn down into the anoxic zone of the sediment creating greater surface area for the oxic-anoxic interface. This interface is particularly important for coupled nitrification-denitrification as the nitrification step (which converts ammonium into nitrate) requires molecular oxygen, but denitrification can only proceed in very low oxygen conditions. Therefore, the great majority of coupled nitrification-denitrification takes place along the oxic-anoxic interface. The increased area of this interface by bioturbators can greatly stimulate this coupled process. Further, it has been shown that large burrowing macrofauna can induce redox oscillations which allow for increased and sustained N removal via DNF (Volkenborn *et al.*, 2012; Gilbert *et al.*, 2016).

These are complex and interconnected processes that deserve recognition and are often poorly represented in biogeochemistry. **Ecogeochemistry** is currently defined as the application of geochemical techniques in order to answer fundamental questions about population and community ecology. Here we propose a broadening of this term to include the intersectional study of the ecological and biogeochemical dynamics of an ecosystem, whether it be using biogeochemical approaches to address ecological questions, using ecological approaches to address geochemical questions, or some combination therein.

This this highlights both the importance of macrofauna, spatial and temporal variability, and a combination of interacting processes in contributing to 'biogeochemical' fluxes.

The vast majority of the studies of macrofaunal impacts on DNF have been controlled laboratory-based studies and, while important, these cannot capture many elements of the complexity of the natural environment. However, there is evidence that the macrofaunal community may help regulate various ecosystem functions, including DNF, and reinforce resilience against future change (O'Meara *et al.*, 2020; Simon F. Thrush *et al.*, 2020). Therefore, further understanding of the connections between the ecology and biogeochemistry, or ecogeochemistry of estuarine systems *in situ* is crucial to our ability to predict important ecosystem services such as DNF.

Because of its low human population density and remote location, New Zealand has historically, largely been spared the devastating effects of excess nutrient pollution and the resulting eutrophication of its coastal systems. However, nitrogen inputs in New Zealand are now increasing at a faster rate than those in any other OECD member country (OECD, 2017). Nevertheless, many New Zealand estuaries still fall into the category of low-nutrient systems (Plew *et al.*, 2020), therefore ecosystem functions, including the controls on DNF are likely different from those in chronically eutrophic systems (Vieillard, Newell, and Thrush 2020). However these types of systems are underrepresented in the DNF literature (Vieillard, Newell and Thrush, 2020), and as a result, our understanding of the ecogeochemical factors regulating DNF rates in non-eutrophic estuaries remains limited (e.g., Cook et al., 2004). This is particularly true in New Zealand where the first directly measured rates were published in 2016 (Gongol and Savage, 2016; O'Meara *et al.*, 2020; Schenone and Thrush, 2020).

Concomitant with increasing N loads are increasing loads of terrestrial sediments to New Zealand coasts. Increasing urbanization, land use change, and sea level rise have

resulted in an increased "muddying" of New Zealand estuaries (Thrush et al., 2004). While increasing mud has had a devastating effect on coastal habitats, it has also been suggested that it may directly decrease DNF, therefore hampering a system's ability to mitigate co-occurring nutrient pollution (Gongol and Savage, 2016; O'Meara *et al.*, 2020). In order to best manage New Zealand's and other low-nutrient estuaries and prevent eutrophication from becoming inevitable, we need a reliable means to predict and optimize DNF in the face of environmental change and multiple stressors. The aim of this study was to better understand the controls on DNF *in situ*, including the interactions between the macrofaunal community and DNF, and to investigate how this crucial ecosystem service fits into a network of ecogeochemical processes across in three low-nutrient estuaries. This work will allow us to clarify the connections between the ecology and biogeochemistry of these systems and better predict DNF in the future.

3.3 | Methods

3.3.1 | Study Sites

This study was conducted in 3 tidal estuaries on the East coast of the Auckland region on New Zealand's North Island (Fig. 3.1). All three systems have extensive intertidal flats and open to the Hauraki Gulf. Okura Estuary is part of the Long Bay-Okura Marine Reserve, a 980 hectare protected area established in 1995. While part of a marine reserve, it is only 25 km North of Auckland city, and therefore has the most developed and populated catchment of the three sites. Mahurangi Harbour is the largest of the three estuaries with a catchment area of 12,100 hectares dominated by agricultural and residential land. Mahurangi has been extensively monitored by the New Zealand National Institute for Water and Atmospheric Research (NIWA) since 1994 (Halliday and Commings, 2012). Whangateau Harbour is Auckland region's northern-most, East coast estuary. It has 4,190 hectare catchment, and is the cleanest, least-developed of the three

estuaries (Cole, Lees and Wilson, 2009). Seven study sites were chosen along a grain size gradient (proportional to anthropogenic impact) with the muddiest found in Okura (4 sites, sampled April 9, 2018), and the sandier sites found in Mahurangi (2 sites, sampled April 12, 2918) and Whangateau (1 site, sampled April 6, 2018; Fig. 3.1). *In situ* benthic chamber incubations were conducted at all sites.

3.3.2 | In situ Benthic Incubations

To conduct *in situ* benthic incubations, 0.25 m^2, aluminium bases were inserted directly into the sediment and pushed to a depth of 5 cm at low tide. As the tide came in, domed acrylic lids were rid of air, placed on top of the bases, and clamped down. Foam seals on both the base and lid ensured a gas-tight seal when covered with water. When closed, approximately 41 L of seawater was enclosed within the chamber. The chamber lids had a small, 3 mm, open in-port on one side and a sample out-port which was connected to approximately 2 m of tubing attached to a stake marking the chamber (Lohrer, Thrush and Gibbs, 2004; Lohrer *et al.*, 2016, Fig. 1.3A). This set up allowed for samples to be taken from within the chamber at the surface, while the tide was in. Benthic chambers cut off natural water flow, which has the potential to alter porewater exchange rates and nutrient fluxes, therefore, small pumps were attached to the inner chamber walls to gently mix the incubated water, prevent stratification, and mimic natural conditions as closely as possible. Chambers were set up in light-dark pairs, with one chamber's clear lid left uncovered and the other covered with black plastic. These light-dark pairs were run in triplicate at each site, and temperature and light levels (as intensity, LUX) were monitored within the chambers using Pendant UA-002 data loggers (Hobo, USA).

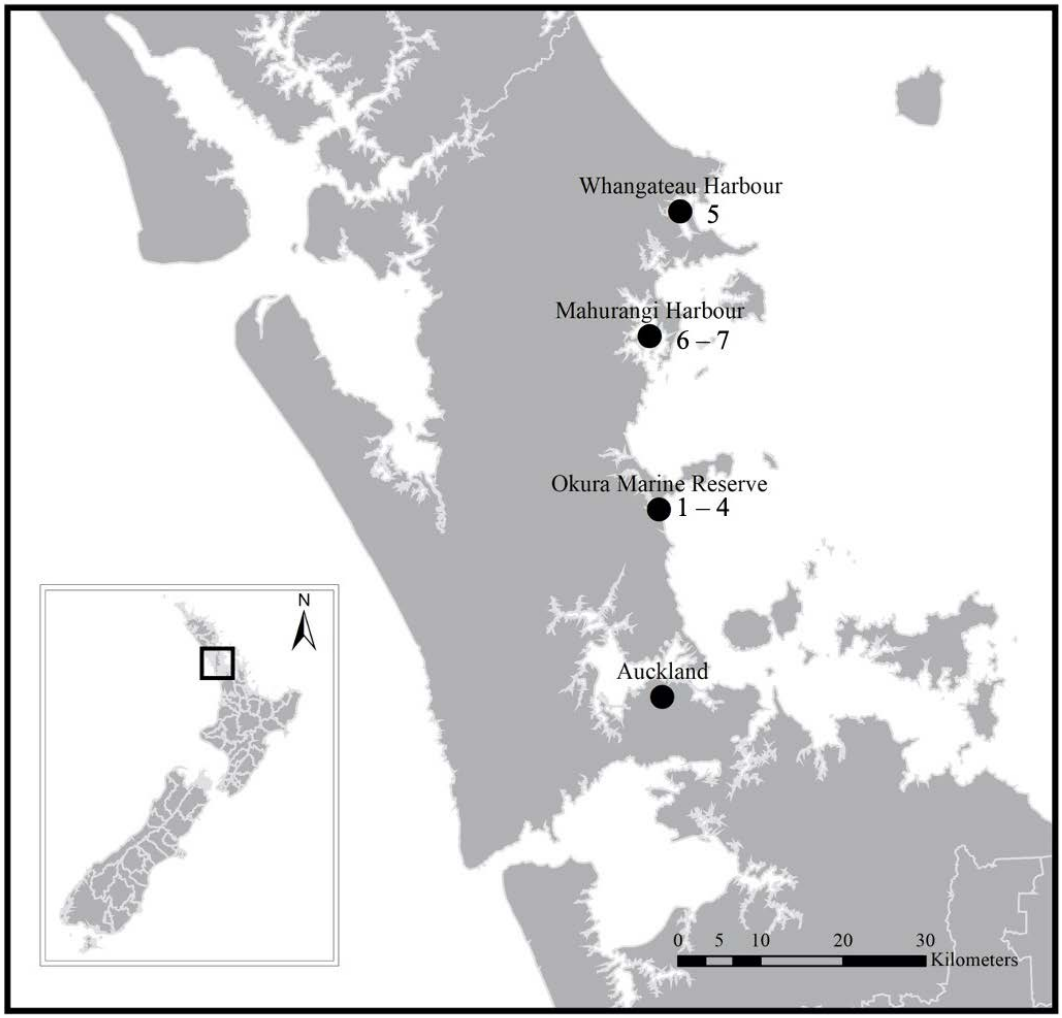

Figure 3.1 Map of estuaries in this study relative to Auckland, New Zealand. Numbers denote site numbers.

Incubations lasted 4.5 hours, on average; water temperature averaged 22.3 ºC and did not change significantly within the chambers over the course of the incubation. Light and dark bottles with no headspace were also incubated to account for water column activity.

3.3.3 | Benthic Flux Samples

Samples for the flux of dissolved NH_4^+, nitrate + nitrite (NO_x), DIP, DON, organic phosphorus (DOP), oxygen (DO), and N_2 where taken at the beginning of the incubation following the sealing of the chambers on the incoming tide, and again after approximately 4.5 hours as the tide was retreating. Samples for dissolved nutrients were collected in 60 mL centrifuge tubes, placed in the dark on ice and filtered using 0.2 µm, polycarbonate filters immediately upon returning to shore. Filtered samples were also kept on ice in the dark for the remainder of the field day and stored frozen at -20 °C upon return to the lab. Samples for dissolved gases were taken in 60 mL syringes below water level, ensuring there were no gas bubbles. Stopcocks on the ends of the syringes were closed and the syringes were placed on ice in the dark. Samples were then transferred to 12 mL, gas tight, exetainer vials (Labco, UK) and preserved with approximately 75 µL of concentrated zinc chloride solution immediately upon returning to shore. Fixed samples were then kept in cold water in the dark and stored at 4 °C upon returning to the lab.

DIN and DIP samples were analysed for NH_4^+, NO_x, and DIP on a Latchet QuickChem 8500 Flow Injection Analyser (FIA, Hach, CO, USA) using colorimetric analysis (Grasshoff, K; Ehrnhardt, M; Kremling, 1983). Detection limits for this method are 1.53, 0.85, and 0.7 µM for NH_4^+, NO_x, and DIP, respectively, with a precision of ~0.07 µM for all channels. DON and DOP were quantified by performing a persulfate digestion (Valderrama, 1981) and re-running on the FIA. This method gives total nitrogen (TP) and total phosphorus (TP), so the DIN and DIP were subtracted from TN and TP to yield DON and DOP. Samples for DO and N_2 were run on a Membrane Inlet Mass

Spectrometer (Bay Instruments, MD, USA) using the N_2/Ar method with a precision of < 0.03% (Kana et al., 1994). Benthic flux rates were then calculated using the change in analyte concentration over the course of the incubation and were normalized to water volume and sediment surface area inside the chambers, yielding fluxes in μmol m^{-2} hr^{-1}. All fluxes across the sediment-water interface are net fluxes, for DO a positive flux is net photosynthesis and a negative flux is net respiration, or SOD. For N_2, a positive flux is net DNF while a negative flux is net N fixation in the sediment.

3.3.4 | Sediment Characteristics and Macrofauna Identification

Samples for sediment grain size, porosity, chlorophyll-a concentration, organic matter content, and porewater [DIN] and [DIP] were taken by coring the undisturbed sediment just outside the chambers at low tide. Two cm diameter x 2 cm deep sub-cores were taken in replicate for grainsize, porosity & organic content, and porewater nutrients, while triplicate 1 cm x 1 cm cores were taken for chlorophyll-a. Sub-core samples for porewater nutrients were analysed individually then averaged, while other sub-core samples were pooled, homogenized, and analysed together. Grainsize samples were digested with 6 % hydrogen peroxide to remove organic matter, rinsed three times with DI water (Day, 1965), and run on a Malvern Mastersizer 3000 (Malvern Analytical, UK). Sediment mud content (% <63 μm) was then calculated. Porosity was calculated from the difference between the wet and dry mass of the sediment divided by the sediment volume. Dry sediment was then weighed again and put in the furnace at 450 °C for 4 hours after which % organic content was calculated from the loss on ignition (LOI). Porewater samples were diluted with 5 mL Mili-Q water and centrifuged at 3500 RPM for 10 minutes. The resulting supernatant was then filtered with a 0.2 μm polycarbonate filter, frozen, and analysed on for NH_4^+, NO_x, and DIP on the FIA. The sediment porosity was then used to calculate the porewater concentrations of NH_4^+, NO_x, and DIP. Chlorophyll-

a samples were stored at -80 °C, then were freeze dried in dark containers. 3 mL of 90 % acetone was added to 1 g of sediment from each sample and extracted for 24 hours at 4 °C. Extracted samples were then run on a UV-vis spectrometer in the dark and chlorophyll a concentration was calculated (Lorenzen, 1967; Wiltshire *et al.*, 1998).

Macrofaunal samples were collected using a 13 cm diameter x 15 cm deep cores from the undisturbed sediment just outside the chamber. While macrofaunal community composition can be variable at small (cm) scales, variation at larger scales tends to dominate (Thrush *et al.*, 1997). We therefore felt confident given the gradient of sites in this study that sampling for macrofauna just outside of the chamber was sufficiently representative of the local population, and that community differences between sites was greater than differences between the inside and outside of the chambers. Macrofaunal cores were sieved in the field and everything retained on the 500 µM sieves was preserved with 70 % Isopropyl alcohol and stained with Rose Bengal. Macrofauna where then separated from the remaining shell hash and detrital material and identified under a stereo microscope to the lowest possible taxonomic level.

Grazer and bioturbator classifications were acquired from an existing dataset, also from the North island of New Zealand (Thrush *et al.*, 2017). In this dataset, biological traits were identified for each of the species collected, using a species x trait matrix, that were considered relevant to ecosystem functioning. These traits included information on living position, direction of particle movement, body size, feeding behaviour, and alteration of sediment topography. From these trait data, functions were assigned for each species for further relevant processes such as grazing and bioturbation (Thrush et al. 2017; Siwicka, Thrush, and Hewitt 2020). All of these traits and functions have been either shown or hypothesized to impact various ecosystem functions including, organic matter remineralization, primary productivity, oxygenation, and sediment stability as well as

nutrient cycling and denitrification (Thrush *et al.*, 2017; Siwicka, Thrush and Hewitt, 2020). However, trait and functional classification are not necessarily mutually exclusive, for example an individual species could be both a grazer and a bioturbator, but each of these functions is likely to have a different impact on overall ecosystem functioning. This is an extensive dataset encompassing over 400 macrofauna core samples, 113 species, and 300,000 m^2 of intertidal flat, and is therefore considered to be a good representation of the macrofaunal community in the region (Thrush et al. 2017).

3.3.5 | Statistical Analysis

In order to produce a predictive model for DNF rates, we ran a series of Distance-based Linear Models (DistlM) based on distance-based redundancy analysis (Legendre and Andersson, 1999; McArdle and Anderson, 2001) using Primer v7 (PERMANOVA). DistlM statistically models the relationship between a set of multivariate data and one or more predictor variables. The multivariate data cloud is described by a resemblance matrix of distance or dissimilarities among samples, and is well suited for the comparison of multivariate environmental and ecological data to one or more key environmental variables (Anderson, Gorley and Clarke, 2008). In this study, DistlMs were run with net N_2 flux as the dependant variable, and all other non-covarying parameters as the predictor variables. Predictor variables included porewater nutrient concentrations, dissolved oxygen and nutrient fluxes, as well as various sedimentary and macrofaunal community parameters (full list in Table AII.3). Two versions of each DistlM were run, with and without macrofaunal variables, in order to elucidate the role of the macrofaunal community specifically in predicting net N_2 flux rates. All DislMs were carried out using a Euclidian distance matrix and forward selection procedure. This procedure was chosen because it presents the contribution of each individual predictor variable in the sequential tests (Primer v7, Anderson, Gorley, and Clarke 2008), allowing us to see the specific and

cumulative contribution of each variable, including the macrofaunal variables when included, to the prediction of N_2 flux rates. Step-wise selections such as the forward selection can be biased toward higher R^2 values by leaving out potentially important, though non-significant, variables and exacerbating co-linearity (Whittingham *et al.*, 2006). However, we were able to use tools within the Primer v7 DistlM package to reduce these biases; all variables were normalized, and variables that were selected as highly co-varying ($R^2 < 0.85$) were not included in the model (Anderson, Gorley and Clarke, 2008). The most notable, covarying variables were sediment mud and organic matter content. We chose to keep % mud in the model and remove % organic matter. Mud content is directly related to the stressor of interest, namely terrestrial sediment deposition. However, it is worth noting how correlated these two variables were in this study (>90 %), therefore conclusions drawn about the importance of sediment mud content may also be related to changes in sediment organic content. Sequential tests identified which predictor variables should be included in the best models to explain the greatest proportion of the variance in the net N_2 flux (Table 3.2). These sequential tests begin with the predictor variable with the greatest explanatory power and reveal the significance of, and variation in the dependent variable explained by subsequent predictor variables, while accounting for the relationship between the dependant variable and the predictor analysed immediately before it. For example, if a sequential test revealed mud content and porewater [NH_4^+] to be significant, the contribution of porewater [NH_4^+] may or may not be significant on its own, but it is significant given the relationship between mud content and the dependant variable. The sequential tests, then, best represent the inter-connected nature of the individual variables in these systems, elucidating which variables collectively work together to influence net N_2 flux rates.

In order to better understand how DNF fits into the ecogeochemical landscape, ecosystem interaction networks for the light and dark chambers were constructed using Pearson correlation coefficients. In this method, individual correlations are used to map a network of key ecosystem interactions. The links within these networks are based on the strength, rather than the significance, of relationships. In this study, relationships with $0.9 \geq r \geq 0.6$ were considered strong relationships, and those with $0.6 > r \geq 0.4$ were considered weaker, though still relevant, relationships. This allows for the consideration of the interconnectedness of ecosystem variables and processes including the presence of indirect relationships and feedback loops based on the strength and direction of the included relationships.

Additionally, interaction networks can link ecological, biogeochemical, and physical variables or processes which occur at different scales. For example, microbially mediated processes such as decomposition, and ecological processes such as bivalve feeding take place on different spatial and temporal scales, but both contribute to the flux of NH_4^+ across the sediment-water interface. An interaction network could then elucidate a feedback between, for example, oxygen consumption, bivalve density, and porewater NH_4^+, as well as how they are directly or indirectly linked to NH_4^+ flux. In this study, we use the DistlM modelling and interaction networks together to better explain DNF in an ecogeochemical context. Here the models distil our measured data down in order to understand which variables and processes interact to directly impact DNF (as measured by net N_2 flux); meanwhile, interaction networks map how DNF and its related processes fit into the wider ecogeochemical framework. This step provides much needed context, but is often lacking from traditional biogeochemical analyses of processes such as DNF (Foshtomi *et al.*, 2015).

3.4 | Results

3.4.1 | Site characteristics

Sediment mud content ranged from 0-23 % across the three estuaries with Whangateau Harbour being the lowest and Okura Marine Reserve the highest (Table 3.1). Chlorophyll-a and pheo-pigments fell in the expected range for New Zealand intertidal sediments ranging from 2.6 to 10.6 μg g^{-1} (Kromkamp *et al.*, 2006). Across all sites mud content was highly, positively correlated with chlorophyll-a, pheo-pigments, organic matter content. Additionally, mud content was negatively correlated with various macrofaunal indicators, including number of individuals, species richness, and number of bioturbators, bivalves, and grazers (Table AII.1). Porewater nutrient concentrations were also within expected ranges (Douglas *et al.*, 2017b), with porewater [NH_4^+] ranging from 50-150 μM with the highest concentrations in Okura Marine Reserve's second muddiest site, and the lowest in the sandiest site in Whangateau Harbour (Table 3.1). Porewater NO_x concentrations were generally low ranging from 2-15 μM, with slightly more elevated concentrations (up to 50 μM in Mahurangi Harbour, Table 3.1). Porewater [DIP] was generally very low ranging from the detection limit to 1.6 μM; however porewater [DIP] was significantly, positively correlated with the number of macrofauna individuals as well as the abundance of bioturbators, grazers, and bivalves (Table AII.1).

Water column nutrient concentrations were uniform across all sites, with average concentrations of 2.56, 2.63, and 25.0 μM of NO_x, DIP, and NH_4^+, respectively. NH_4^+ was by far the more abundant DIN species in all sites. In the bottle incubations for water column activity, there were small rates of net respiration in the dark bottles (-11 μmol m^{-2} h^{-1}) and net photosynthesis (37 μmol m^{-2} $h^{-}1$) in the light bottles. Chamber DO flux rates were adjusted for water column rates so that only sediment fluxes were analysed.

Table 3.1 Sediment and macrofauna community characteristics for each site. Sites 1-4 are in the Okura Marine Reserve, site 5 is in Whangateau Harbour, and sites 6&7 are in Mahurangi Harbour. Macrofauna counts are per core (1 core = 1990 cm^3). Error is reported as standard error, n = 3.

Estuary	Site	Light Intensity (Lux)	Mud content (%)	Chl-a (µg g^{-1})	PW NH_4^+ (µM)	PW NO_x (µM)	Individuals (counts)	Species (counts)
Okura	1	18515	23.1 ± 1.1	7.49 ± 0.6	79.1 ± 3.0	9.31 ± 2.3	27.3 ± 2.1	10
	2	16843	20.6 ± 1.5	6.36 ± 1.5	143.3 ± 37.1	11.2 ± 2.5	34 ± 9.8	16
	3	14318	16.7 ± 1.0	8.64 ± 1.0	53.4 ± 8.1	13.1 ± 1.8	21 ± 2.9	11
	4	11607	1.7 ± 0.3	5.01 ± 0.2	64.4 ± 4.2	4.67 ± 0.6	44 ± 4.6	16
Whangateau	5	23993	0.0 ± 0	2.89 ± 0.2	54.7 ± 3.1	5.89 ± 3.4	40 ± 7.3	12
Mahurangi	6	5454	1.2 ± 0.8	3.26 ± 0.3	112.5 ± 13.0	20.6 ± 15.1	89 ± 25.7	19
	7	4945	8.3 ± 1.4	5.34 ± 0.7	112.9 ± 18.9	15.2 ± 1.1	21.6 ± 1.9	7

There were no significant changes in dissolved nutrient, nor N_2 concentrations in the dark bottles, and only small NH_4^+ uptake rates (-23 μmol m^{-2} h^{-1}) in the light bottles. Therefore, no adjustments were made to the dark chamber fluxes, but light chamber NH_4^+ were adjusted to account for the water column uptake.

3.4.2 | Fluxes at the sediment-water interface

Net N_2 fluxes fell within the range of the two other studies with directly measured, intertidal N_2 flux rates in New Zealand (O'Meara *et al.*, 2020; Schenone and Thrush, 2020), and with a slightly larger range than other intertidal fluxes measured globally (Eyre *et al.*, 2011; Piehler and Smyth, 2011). Fluxes were generally positive (indicating net DNF), though lower in the light than in the dark, with the exceptions of sites 1&2 that had more net denitrification in the light (Fig. 3.2A). Out of 42 independent incubations there were 7 instances of net N-fixation (negative net N_2) flux: in the light at site 7, the dark at site 2, and in the light and dark at site 1 (Fig. 3.2A). Additionally, net N_2 flux was significantly, negatively correlated with mud content, and positively correlated with species richness (Table AII.1).

DO fluxes were generally positive (net photosynthesis) in the light incubations and negative (net respiration) in the dark incubations, with the exceptions of sites 6&7, which both exhibited net respiration in the light, though still at a lower rate than in the dark (Fig. 3.2B). While these fluxes were generally within the expected range and direction, the magnitude of the dark fluxes (net respiration) were generally larger than the light fluxes (net photosynthesis). The DO fluxes from the light incubations were significantly, positively correlated with NH_4^+ flux, chlorophyll-a, and mud content and negatively correlated with DON flux and abundance of bivalves (Table AII.1).

DIP and NO_x fluxes were generally low and did not correlate significantly with any other variables measured. However, NH_4^+ fluxes were large, ranging from -72 – 1153

μmol m^{-2} h^{-1} in the dark and -1134 – 354 μmol m^{-2} h^{-1} in the light (averaging 499 and -149 μmol m^{-2} h^{-1} in the dark and light, respectively). Generally, there was net NH_4^+ release in the dark and net uptake in the light, with the exception of sites 1&3 which both had an incubation with net NH_4^+ release in the light (Fig. 3.2C). NH_4^+ flux was significantly, positively correlated with DOP flux in the dark and mud content in the light (Table AII.1).

DON fluxes from this study were in the same range of light NH_4^+ fluxes and larger than dark NH_4^+ fluxes, and there was generally DON uptake in the dark and release in the light with the exception of site 2 in the Okura Marine Reserve which had the opposite. DON fluxes were significantly, positively correlated with porewater NH_4^+ concentrations, chlorophyll-a content, and mud content in the dark, but were not significantly correlated to any other variable in the light (Table AII.1). The DOP fluxes were generally smaller in the light chambers (-8 – 30 μmol m^{-2} h^{-1}) except for site 7 in Mahurangi Harbour which had an average DOP release of 128 μmol m^{-2} $h^{-1.}$ While light DOP fluxes were generally positive, most chambers had negative (net DOP uptake) in the dark with sites 4, 6&7 having the larges fluxes (-134, -122, and -96 μmol m^{-2} h^{-1}, respectively). DOP flux was significantly, positively correlated with % Mud in the dark and species richness in the light, and negatively correlated with all macrofaunal variables in the dark (Table AII.1).

3.4.3 | Contribution of macrofauna to the N_2 Flux

DistlM results show that, overall, our predictor variables do a much better job of predicting the DNF rate (i.e., the net N_2 flux) in the dark than in the light (Table 3.2). In the dark, sequential tests revealed porewater [NH_4^+] and mud content to be important predictors of net N_2 flux (explaining a total of 61 % of the variance). When the macrofaunal parameters were included, species richness was also found to be a significant predictor, given porewater [NH_4^+] and mud content, yielding a 15.5 % increase in the variation of DNF explained (Table 2).

In the light chambers there were no biogeochemical or physical parameters that were significant predictors of DNF rate (Table 3.2). However, when the macrofaunal data were

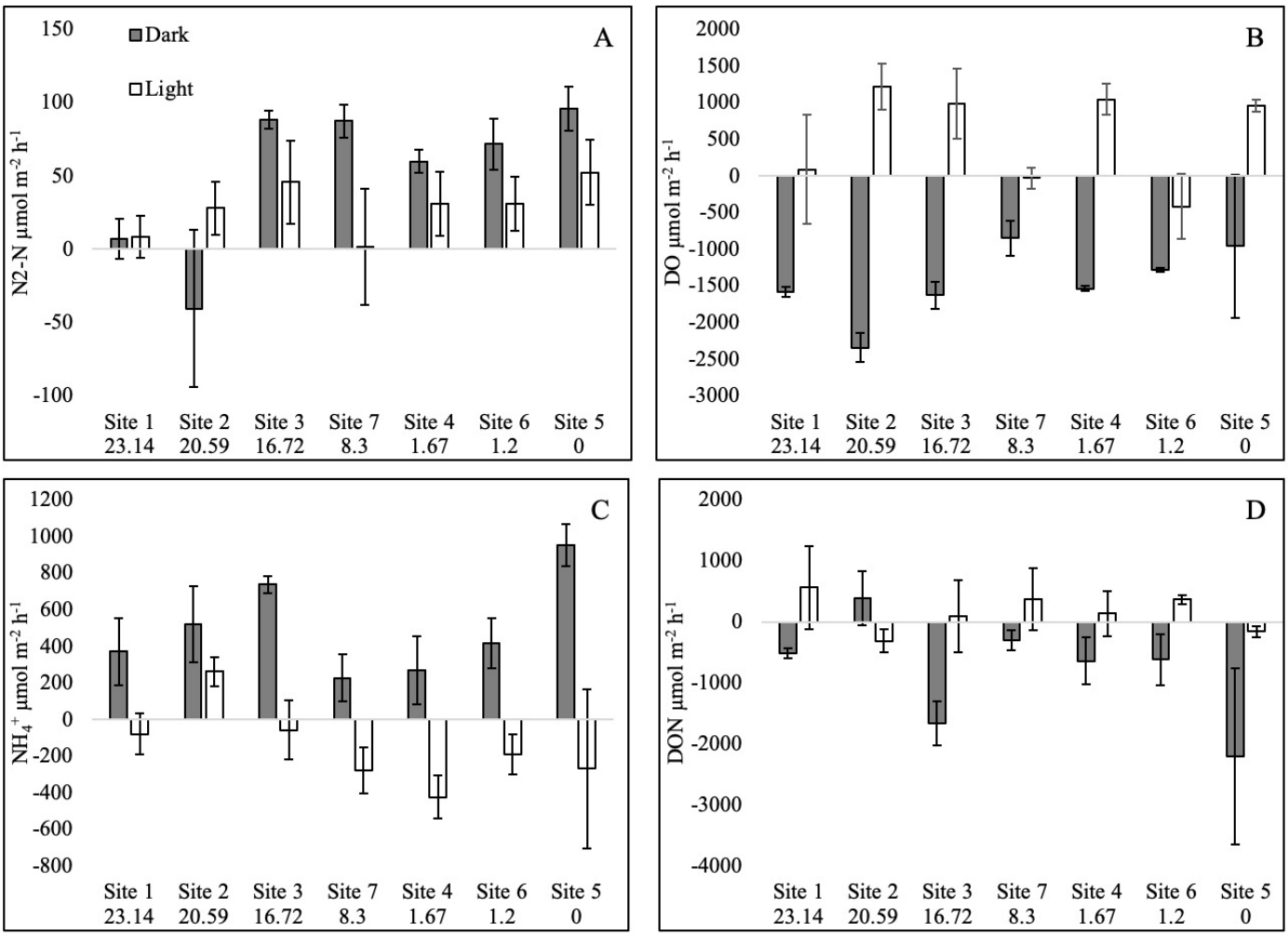

Figure 3.2: Directly measured rates of dissolved nitrogen gas (A), oxygen (B), ammonium (C) and organic nitrogen (D) fluxes across the sediment water interface within the cambers at each site. X-axis show site numbers. Dark bars indicate fluxes in the dark, while open bars indicate fluxes in the light. All fluxes are net fluxes; note the scale change in each plot, and that sites are listed in order of decreasing sediment mud content (percentage). All error is reported as standard error, n=3

included, species richness was a significant predictor, explaining 17.6 % of the variance in net N_2 flux (Table 3.2).

3.4.4 | Interaction Networks

In this study we created two interaction networks, one for the dark chambers and the other for the light (Fig. 3.3). Mechanistic differences between the light and the dark conditions immediately become apparent, and various feedback loops can be identified (Fig. 3.3). For example, in the light, there is a positive feedback loop between chlorophyll-a, oxygen production and mud content, mediated by grazer abundance. Increasing mud content is associated fewer grazers. Grazers, in turn, have a negative relationship with chlorophyll-a, which is also associated with increased oxygen production. Finally, there is a positive relationship between DO flux and mud content, completing the loop, and showing the complex interconnectedness of the macrofaunal and MPB communities (Fig. 3.3). This feedback is consistent with previous networks in these low-nutrient estuaries, but adds in the additional component of the oxygen flux (Thrush et al., 2014).

In the dark there are connections between mud content, DON flux, chlorophyll-a, species richness, and DNF rate creating a complex feedback loop whereby increasing muddiness leads to increased DON flux and increased chlorophyll-a concentration. Meanwhile, increased species richness leads to decreased chlorophyll-a and increased DNF rates. The negative relationship between mud content and DNF rate, then, completes the loop (Fig. 3.3). These interaction networks help illustrate the complex connections and feedback loops between various parameters of ecosystem function and how they change with light.

Table 3.2 DistlM sequential test results (PERMANOVA) for predicting N_2 fluxes run with and without the macrofaunal dataset. Net N_2 Flux was the dependent variable. For both light and dark chambers, the inclusion of the macrofauna data increases the predictive power of sequential models. The proportion of variance values are cumulative for the sequential tests. Bold indicates $p < 0.05$, while regular indicates $0.05 < p < 0.1$

	Excluding macrofaunal data					*Including macrofaunal data*					
Light condition	Predictor variable	p value	AICc	Pseudo-F	Proportion of variance	Predictor variable	p value	AICc	Pseudo-F	Proportion of variance	Macrofauna contribution
Dark	**PW NH_4^+**	**0.007**	**163.9**	**12.35**	**0.394**	**PW NH_4^+**	**0.008**	**163.9**	**12.35**	**0.394**	
	% Mud	**0.006**	**157.4**	**9.984**	**0.610**	**% Mud**	**0.005**	**157.4**	**9.984**	**0.610**	15.5%
						Species richness	**0.004**	**149.9**	**11.17**	**0.765**	
Light	*No significant result*					Species richness	0.060	152.1	4.065	0.176	17.6%

These interactions reveal indirect relationships, as well as the interconnectedness and complexity of these ecosystems far more than individual correlations alone.

3.5 | Discussion

Overall, results from this study show that competition dynamics between N cycle bacteria, MPB's, and macrofauna interact to drive the differences between net N_2 flux rates in the light vs. the dark. Additionally, a novel relationship between species richness and net N_2 flux is described and is found to be particularly important for predicting net DNF under light conditions.

3.5.1 | Sediment Metabolism and nutrient dynamics

The magnitude of oxygen consumption in the dark was greater than oxygen production in the light, indicating that these sediments exhibit a net heterotrophic metabolism, as is generally expected in marine sediments (Ferguson, Eyre and Gay, 2003). Sites 6&7 were strongly heterotrophic with very low rates of oxygen production or oxygen uptake in the light. This result is likely due to the fact that sites 6&7 had the highest turbidity of the sites sampled, suggesting their photosynthetic capacity, and therefore oxygen production was lowest (Table 3.1). DO flux was significantly, positively correlated with both mud content and chlorophyll-a concentration in the light. This is typical of muddier sediments, which tend to have a larger MPB community, a larger standing stock of chlorophyll-a, and greater oxygen production capacity (MacIntyre, Geider and Miller, 1996a). The relationships between NH_4^+ and DO are also consistent with sediment remineralisation consuming oxygen and releasing NH_4^+ in the dark, and MPB consuming NH_4^+ to produce oxygen in the light. However, the largest NH_4^+ fluxes were in the sandiest, lowest organic matter site (Site 5, Fig. 3.2C), indicating that NH_4^+

production by the sediment is not driven entirely by organic matter respiration. There was also a more diverse and abundant macrofaunal community in the sandier sites. In particular there were more, large bivalve and grazer species. These species produce NH_4^+ both via direct excretions and through the remineralization of the very labile organic matter in their biodeposits, making them a key source of N in these relatively low-nutrient systems.

We know that oxygen penetration is an important factor in determining the rate of coupled nitrification-denitrification, which is the dominant DNF pathway in low-nutrient systems (Gongol and Savage, 2016; Crawshaw, Schallenberg and Savage, 2019). One of the most immediate sources of oxygen to the near-surface sediment is that produced by MPB in the light, however, though coupled DNF needs oxygen to proceed, it is also in direct competition with MPB for N (Rysgaard, Christensen and Nielsen, 1995; Risgaard-Petersen, 2003). Similarly, while bioturbating macrofauna can stimulate coupled DNF, they can also be large consumers of oxygen within the sediment. Therefore a complex interaction of simultaneous benefit and competition between the N cycle bacteria, the MPB, and the macrofauna characterizes the ecogeochemistry of these systems. This interaction was further demonstrated by feedback loops identified within the interaction network between chlorophyll a, DO flux, mud content, and grazers in the light (Fig. 3.3). These loops include much more information than the simple linear relationships between various parameters.

DIP and NO_x fluxes were generally low and did not correlate significantly with any other variables measured; this is typical for these low-nutrient New Zealand estuaries (O'Meara, Hillman and Thrush, 2017). In these systems, water column NO_3^- concentrations are generally near or below detection limits, and NH_4^+ is the more abundant DIN species available (O'Meara *et al.*, 2020, Table 3.1). This is likely a result

of the fact that anthropogenic inputs to estuaries tend to come in the form of NO_3^- as oxidized runoff from septic systems, fertilizer applications, and livestock (Deegan *et al.*, 2007). Due to this low anthropogenic N load, NH_4^+ is the more abundant DIN species as it is generally a product of nutrient regeneration from within the system, rather than new N coming in.

Dissolved organic fluxes, particularly DOP sediment fluxes are rarely measured (Delaney, 1998), however the concentrations measured in this study were in the same range, if not slightly larger than those measured in the Scheldt Estuary in the Netherlands (van der Zee, Roevros and Chou, 2007). However, magnitude of the DON fluxes in this study are quite large compared to other reported fluxes from Sweden, particularly in the dark (Sundbäck *et al.*, 2004). Both DON and DOP follow the same general pattern with positive net fluxes (production) in the light and negative net fluxes (consumption) in the dark. DON and DOP are both degradation products of organic matter remineralisation, particularly of very labile sources such as MPB and biodeposits, which is reflected in the positive correlation of pheo-pigments with both DON and DOP flux (Table AII.1). DON can also be an important intermediary source of N to various N cycle bacteria, particularly in low nutrient systems (Sundbäck, Miles and Göransson, 2000), hence the observed relationships between DON and net N_2 flux in both the light and dark (Fig. 3.3). It appears then, that both DON and DOP are produced by the normal life cycle of the MPB community and then consumed by the bacterial community in the sediment in the dark (Fig. 3.2D). DON seems to be an especially important source of N to the systems in this study, as the magnitude of the DON uptake was often equal to or larger than the NH_4^+ uptake in the dark (Fig. 3.2D). Understanding the underlying mechanism of exactly how the DON pool contributes to N cycling in general, and DNF rates in particular is worthy of further study.

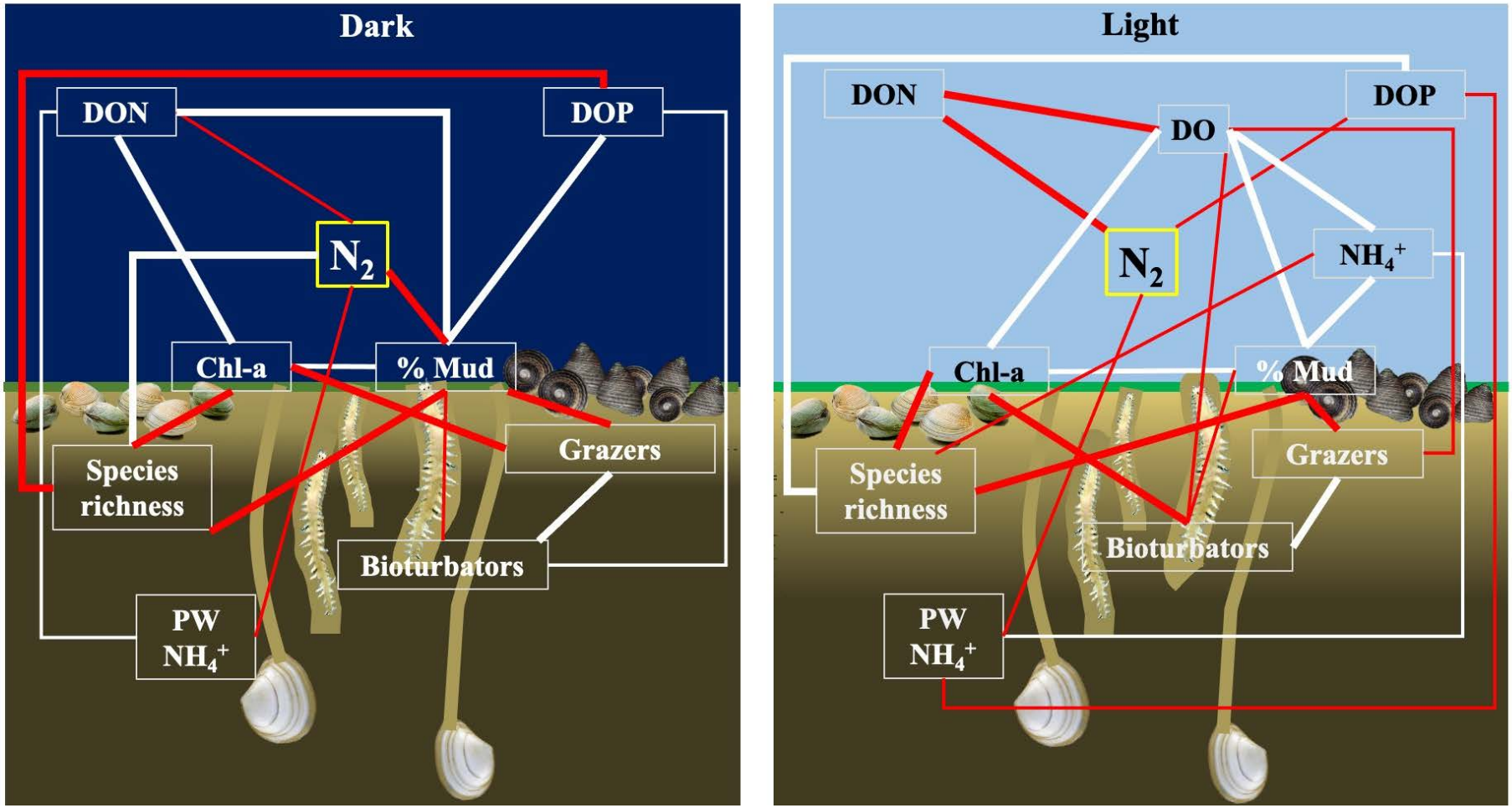

Figure 3.3 Schematic and interaction networks based on Pearson correlations between variables in the dark and light. Red lines indicate negative relationships and white lines indicate positive relationships. Strong relationships ($r \geq 0.6$) are represented by thick lines, while thin lines denote weaker relationships ($6 > r \geq 0.4$). Color gradation in the bottom half of the figure denotes sediment oxygen concentration, with lighter colors indicating higher oxic and darker colors denoting lower oxygen/anoxic conditions.

3.5.2 | Drivers of denitrification

Higher rates of net DNF in the dark are not surprising given that, under light conditions, denitrifying bacteria have to compete directly with photosynthetic sediment biofilms, or MPB for nutrients (Rysgaard, Christensen, and Nielsen 1995). As in most low-nutrient estuaries, DNF here is very likely dominated by the coupled nitrification-denitrification pathway (Burgin and Hamilton, 2007; Gongol and Savage, 2016). Therefore, the competition between MPB and denitrifiers for NO_3^-, as well as nitrifiers for NH_4^+, are both important regulators of denitrification. This is because the oxidation of NH_4^+ in to NO_3^- (nitrification) is the primary source of NO_3^- for DNF in these systems, and MPB can take up both NH_4^+ and NO_3^-.

In the dark, MPB are not photosynthesizing, therefore there is more DIN (both NH_4^+ and NO_3^-) available for nitrifiers and denitrifiers to use. However, in the light, MPB regulate the flux of nutrients across the sediment-water interface and take them up for photosynthesis, reducing the amount of N reaching nitrifiers in the oxic zone and denitrifiers in the anoxic zone of the sediment (Sundback *et al.*, 1991; An and Joye, 2001).

The lower net N_2 fluxes in the light, then, are likely a combination of this competition resulting in lower total rates of DNF as well as increased rates of N fixation. In times of competition for N with MPB, N cycle microbes may increasingly rely on N fixation as a source of N, this is particularly common in low-nutrient systems (Sundbäck, Miles and Göransson, 2000). N fixers within the sediment can break the triple bond within N_2 gas, using the resulting N for their life processes. As these organisms turn over, that fixed N becomes available in the form of NH_4^+ or DON. The microbial community has very fast turnover (on the order of hours to days) therefore, N fixers become a source of new N to other sediment microbes. Nitrifiers can use the resulting NH_4^+ from N fixation, producing NO_3^-

which can then be used by denitrifiers. This pathway links increased rates of N fixation to increased rates of DNF in low-nutrient systems (Sundbäck, Miles and Göransson, 2000). However, this relationship becomes more complicated when measuring DNF via a net N_2 flux. While increasing N fixation can stimulate DNF, N fixation and DNF are essentially opposite processes with N fixation decreasing the concentration of dissolved N_2 and DNF increasing it. N-fixation rates may also be increased in the light due to the presence of photosynthetic, N fixing, cyanobacteria in MPB assemblages (MacIntyre, Geider and Miller, 1996a). Net N-fixation linked to these mechanisms is well established in marine sediments (e.g., Fulweiler et al. 2013; Foster and Fulweiler 2014), and increasing N fixation can translate to lower or negative net N_2 fluxes, independent of changes in DNF rates. Additionally, denitrifiers can be facultative anaerobes, performing oxic respiration when oxygen is available and denitrification when it is not (Burgin and Hamilton, 2007). Therefore, increased oxygen penetration in the light as a result of photosynthesis could result in decreased denitrification rates if denitrifiers shift to aerobic respiration. Since so many processes directly or indirectly impact the production and consumption of N_2, it can be difficult to tease apart individual biogeochemical mechanisms by examining the net N_2 flux alone.

Unlike the others, sites 1&2 have the lowest overall net N_2 flux rates with equal (Site 1) and significantly higher (Site 2) net N_2 fluxes, or more net DNF, in the light ($p < 0.01$, Fig. 3.2A). These are also the muddiest sites sampled. We would generally expect, based on the literature, DNF rates to be higher in muddier sediment compared to sand (e.g., Rysgaard et al., 2001). This is due to the fact that muddier sediments generally have a larger organic matter pool, which benefits the heterotrophic denitrifiers (Burgin and Hamilton, 2007). However, the literature informing this conclusion generally comes from heavily eutrophic systems in the Northern hemisphere, where NO_3^- is so abundant that organic matter becomes

the limiting factor for DNF to proceed. Additionally, the vast majority of these studies from eutrophic systems were done using core incubations, which likely do not fully capture the effects of the macrofaunal community. In low-nutrient systems, free DIN concentration, especially NO_3^-, is very low making it the limiting factor in DNF (Table 3.1). NO_3^- limitation, therefore, makes the denitrifiers in these systems unable to utilize the abundant organic matter pool within the muddy sediments. Instead, DNF in these systems occurs primarily via the coupled nitrification-denitrification pathway (Gongol and Savage, 2016; Crawshaw, Schallenberg and Savage, 2019). The nitrification step of this process needs molecular oxygen to occur (Ward, 2008); therefore, shallower oxygen penetration in muddy sediments make them less conducive to coupled nitrification-denitrification than more porous, sandy sediments (Gongol and Savage, 2016). This requirement means that if oxygen penetration to the sediment is reduced and nitrification rates are decreased, the supply of NO_3^- is diminished and DNF cannot proceed, regardless of other available forms of N, including NH_4^+ and DON. Additionally, the lower oxygen and more reduced conditions in muddy sediments are ideal for sulphate-reducing bacteria to thrive (Oczkowski *et al.*, 2020). Many sulphate-reducing bacteria have been found to be heterotrophic N fixers (Romero *et al.*, 2015), and increasing rates of N fixation could also help account for the decrease in net N_2 flux in the mud.

DistlM results highlight the importance of mud content and porewater NH_4^+ driving net N_2 flux rates. Both of these drivers are likely mediated by nitrification rates, with increasing mud content likely decreasing nitrification rates by decreasing permeability and oxygen penetration. Overall this leads to a negative relationship between net N_2 flux and mud content, with higher rates of net DNF in sandier sediments, which is particularly significant in the dark (Table AII.1). However, interaction networks help to identify indirect drivers of net N_2 flux, including chlorophyll-a as well as grazer and bioturbator abundance

(Fig. 3.3). This pattern of decreasing DNF with increasing mud content is counter intuitive to the vast majority of literature findings, but similar results have been found in other studies in New Zealand (Gongol and Savage, 2016; O'Meara *et al.*, 2020) and Australia (Eyre, Maher and Squire, 2013). Additionally, porewater [NH_4^+], not [NO_x], is a key predictor of DNF because of its importance for nitrification. While net DNF is known to correlate with NO_3^- flux (Seitzinger and Nixon 1985), there is very little NO_3^- available in these systems to detect this relationship. Both concentrations and fluxes of NO_x were very small, and it does not accumulate in high quantities in the porewater, meaning that it is likely taken up right away when it is produced. Further, NO_x comprises both NO_3^- and nitrite (NO_2^-). NO_2^- is a very common intermediary N species in the N cycle and is produced and consumed by several different processes, including nitrification and denitrification. It is very possible, given the low anthropogenic N inputs to these systems that the NO_x is primarily made up of NO_2^- which is not known to directly correlate with DNF. This phenomena has been seen previously in a low-nutrient marsh system (Vieillard & Fulweiler, 2012). Therefore it is the porewater [NH_4^+], as the ultimate source of NO_3^- from nitrification, which predicts net N_2 flux rates in these systems.

It is also possible that the predictive control of porewater [NH_4^+] on net N_2 flux indicates N removal via the anaerobic ammonium oxidation (anammox) pathway. Anammox is an alternate N removal process in which chemoautotrophs convert $NH_4^+ + NO_2^-$ to N_2 gas. While anammox has been found in intertidal, estuarine sediments in the northern hemisphere, rates are generally low (Trimmer, Nicholls and Deflandre, 2003; Risgaard-Petersen *et al.*, 2004; Nicholls and Trimmer, 2009). Anammox tends to be more important in deeper, continental shelf sediments and has been shown to increase in importance with depth, where low organic matter inputs favour autotrophic anammox over heterotrophic DNF (Devol, 2015). In estuaries anammox generally accounts for approximately 0 – 10 % of N_2 produced

(Trimmer, Nicholls and Deflandre, 2003; Brin, Giblin and Rich, 2014), though greater contributions, up to 26 and 79 % have been reported (Risgaard-Petersen *et al.*, 2004; Teixeira *et al.*, 2012, respectively). Anammox has not been found to be corelated with water column or porewater NH_4^+, likely because it is rarely limiting in marine sediments (Dalsgaard, Thamdrup and Canfield, 2005). Overall, very few studies of anammox in estuaries, especially intertidal sediments have been done (Teixeira *et al.*, 2012; Fernandes *et al.*, 2016), and like DNF, the vast majority of data on anammox comes from temperate, chronically eutrophic systems in the North Atlantic, therefore its role in low-nutrient systems is not well constrained.

Measurements of anammox from intertidal, estuarine sediments have shown it to be primarily controlled by water column [NO_3^-] and temperature, with increasing rates with increasing [NO_3^-], maximum rates at 10 – 15 °C, and lowest rates in the summer (Teixeira *et al.*, 2012; Fernandes *et al.*, 2016). These relationships suggest that the estuaries in this study are not ideal environments for anammox to occur. Water column [NO_3^-] was very low in these systems averaging 2.56 μM, suggesting very low rates of anammox if previously described relationships hold. Additionally, this sampling was done in early autumn in New Zealand where water temperature averaged 22.3 °C, a temperature at which anammox is rarely found in temperate sediments (Teixeira *et al.*, 2012; Devol, 2015). Additionally, anammox has been found to be an unimportant N cycling process in warm, tropical sediments (Dong *et al.*, 2011). However, subtidal studies have shown anammox to be inversely related to organic matter supply to sediments, resulting in its dominance in deep, shelf sediments. Therefore, the low organic matter content, particularly, of the sites with lower mud content in this study could potentially favour anammox over DNF, as has been suggested by other intertidal work (Teixeira *et al.*, 2012; Fernandes *et al.*, 2016). Because N isotope additions were not a part of this study, rates of anammox cannot be specifically quantified, so we

cannot completely rule it out as an N removal pathway, especially with so many unknowns remaining in its study.

3.5.3 | The role of the macrofaunal community in predicting denitrification

Model results show that macrofaunal diversity also plays an important role in DNF rates (Table 3.2). Species richness is positively correlated with the net N_2 flux in these systems suggesting that greater variety of species within the macrofaunal community is conducive to higher net DNF (Table AII.1). This result supports the theoretical relationship between biodiversity and ecosystem functions (Srivastava and Vellend 2005; Thrush et al. 2017), suggesting that it is not the overall macrofaunal abundance, or the abundance of any particularly species, that directly impacts rates of DNF. Instead, this finding points to multiple species carrying out functions that collectively and potentially indirectly contribute to the net N_2 flux. As a result, overall species richness (implying high niche and resource partitioning) becomes a direct driver (Table 3.2, Fig. 3.3). Additionally, the association of species richness and not abundance is not entirely surprising given the importance of large animals in these systems (Thrush *et al.*, 2006; Hillman *et al.*, 2020). Individual abundance tends to be a more important factor in some species or groups of species more than in others. Previous work has shown that the correlation between abundance and species richness is not strong in these systems (Thrush et al., 2017), and that key large species do not have to be present in high densities to greatly impact overall ecosystem functioning (Thrush et al. 2006).

The relationship between macrofaunal species richness and directly measured DNF rates described in this study has not been found previously. However, it is supported by previous findings correlating macrofaunal community activity to increased N cycle bacterial and archaeal diversity (Foshtomi *et al.*, 2015). One mechanism underpinning this relationship between species richness and N_2 flux is bioturbation activities generating multiple oxic and anoxic interfaces at varying depths within the sediment. This is further

evidenced by the fact that both species richness and number of bioturbators are negatively correlated with mud content (Table AII.1). Therefore, there is both more bioturbation and increased permeability as the sediment becomes sandier. Bioturbation has been shown to increase DNF rates whereby bioturbating organisms bring oxygen deeper into the sediment than it can naturally diffuse, creating micro redox zones along the edges of burrows and tubes which promote greater rates of oxygen requiring nitrification and, by extension, coupled nitrification-denitrification (e.g., Crawshaw et al., 2019; Rysgaard et al., 1995).

These findings support the indirect links between the macrofaunal community and net DNF rates. However, species richness and bioturbator abundance were not directly correlated in this study, suggesting that bioturbation is not the only important mechanism causing macrofaunal community activity to impact DNF (Fig. 3.3, Table AII.1). For example, there is a feedback loop within the interaction networks between % mud, chlorophyll-a, grazers, and bioturbators (Fig. 3.3). This loop is directly linked to net N_2 flux through species richness in the light and species richness and % mud in the dark, suggesting that grazing pressure on MPBs may also play an important, if indirect role (Fig. 3.3). The mechanism underpinning this likely goes back to the competition between MPB's and N cycle bacteria for N, while increasing mud content increases MPB standing stock, increasing grazer abundance decreases it, thus leaving more N available to nitrifiers and denitrifiers. This dynamic is likely to be of particular importance under light conditions when MPB are photosynthesizing (Rysgaard, Christensen, and Nielsen 1995). The importance of different functional groups in regulating DNF is therefore reflected in the predictive power of species richness on net N_2 flux, as well as their connections within the interaction networks (Table 3.2, Fig. 3.3).

Additionally, key drivers of ecosystem function can be clarified by the number of connections within interaction networks (Thrush et al., 2012). In the dark, mud content and chlorophyll-a concentration are the key drivers, while mud, and DO are the most connected in

the light (Fig. 3.3). In both cases mud content is the most interconnected with 7 and 6 significant connections in the dark and light, respectively.

The fact that these interaction networks hinge on mud content is important given the environmental stressors facing New Zealand estuaries. While N runoff to coasts is an ever-increasing threat to New Zealand coastal systems (OECD, 2017), perhaps an even more pressing anthropogenic threat is that of increasing terrestrial sediment deposition and "muddying" of coastal systems (Thrush et al., 2004). This deposition increases both turbidity and sediment mud content and constitutes a change in both the quantity and quality of organic matter available to coastal sediments. This results in a more recalcitrant sediment organic matter pool, and can smother, particularly intertidal, sediments leading to mass shellfish die off (Thrush et al., 2004). Even without die off events, increases in terrestrial sediment deposition have been shown to decrease both macrofaunal abundance and diversity (Rodil *et al.*, 2011; Pratt *et al.*, 2014a). Additionally, this and other studies show that DNF rates decrease with this increasing mud content in low-nutrient systems (Gongol and Savage, 2016; O'Meara *et al.*, 2020). These results indicate that a reduction in the N removal capacity of these coastal systems is another side effect of increasing mud deposition. As a result, the muddying of coastal systems may be hindering their resilience against ever-increasing anthropogenic N pollution.

3.5.4 | Net N_2 flux and light

Overall, the role of macrofauna in predicting DNF appears to be especially important under light conditions (Table 3.2). While species richness was identified as a key predictor, increasing the explanatory power of the model by 15.5% in the dark (Table 3.2), it could be argued that this is a helpful but not necessary finding given the high explanatory power of the physical and biogeochemical data in the dark. However, due to the sensitivity of macrofaunal diversity to many environmental stressors, this relationship highlights potential

for changes in DNF well before major changes in environmental factors. In the light, however, species richness was the *only* significant predictor identified, taking the explained variation of the model from 0 to 17.6 % (Table 3.2). Clearly our models are much more effective at predicting net N_2 flux in the dark than in the light; however, including the macrofauna data, specifically species richness, increases the explanatory power of the models by nearly 20 % (Table 3.2).

The reason behind the discrepancy between the light and dark models is likely the origin of the benthic flux incubation method, which was originally designed to be carried out in the dark (Kana et al., 1998; Nielsen & Glud, 1996). The incubation method is primarily based on the assumption that in the dark, the production and consumption of various nutrients (including DO, N, and P) is linear. This dark linearity has been widely established in laboratory, core, and mesocosm experiments (e.g., Seitzinger & Nixon, 1985), and it is this linearity that allowed us to feel confident taking only initial and final samples in these *in situ* incubations. However, these assumptions of linearity often break down in the light (Eyre *et al.*, 2011). Generally, the introduction of light to the benthic system increases its complexity, such as the competition dynamics between N cycle bacteria and MPB. Interaction networks in this study indeed illustrate more complex networks in the light with more week and strong relationships than the dark network (Fig. 3.3). This increase in complexity can yield non-linear signals that this method is not designed to handle. This is especially true if only initial and final points are taken, whose relationship you are forced to assume is linear. As a result we end up in a situation where the fluxes predicted by our linear model likely do not reflect the true flux occurring in the environment, leading to a more limited understanding of individual processes and how they relate to each other in the light compared to the dark. Additionally, while these methods all include the macrofauna community, they are not specifically focused on their contribution and likely do not fully capture their activity, which

is generally, non-linear. Because these incubations were done *in situ* the two time points were all that was feasible with our desired level of replication; however, the results from this study show that including the macrofaunal data, helps close the gap somewhat.

The light regimes measured in this study represent, essentially, the two extremes of light conditions that these systems experience while inundated. Our results suggest that the competition and interaction dynamics between macrofauna, MPB, and N bacteria are not only influenced by the diel light/dark cycle but also potentially by other factors which influence light levels such as increased turbidity, shading, and sea level rise. All three of these factors are increasing and expected to continue to increase in New Zealand (Mangan *et al.*, 2020). Sea level rise, land clearing, and population growth are all contributing to increasing terrestrial sediment run off to coasts and therefore increasing turbidity in estuaries (Thrush et al., 2004). Additionally, as N loads increase to the coasts, shading by micro and macroalgae blooms is also expected to increase. These factors lead to overall decreasing light reaching the sediments while the tide is in. While various benthic MPB communities have been shown to photo-adapt to sub-tidal low light environments (Cahoon, 1999), in intertidal systems there is a more varied light regime, with a larger range in light intensity over time, making consistent photo-adaption less likely (Mangan *et al.*, 2020). Given the shift in ecosystem dynamics, particularly those driving DNF and N fixation rates, these more extreme changes to the light regime also have implications for important ecosystem services such as DNF. For example, in an ordinarily clear estuary, a storm event causing increased turbidity and increased N runoff, might stimulate DNF due to the darker, more N rich conditions, potentially leading to increased mitigation of the disturbance and greater resilience. Conversely, a sustained increase in turbidity can be expected to increase light attenuation and mud content in the sediment. While these darker conditions may help denitrifiers to compete with MPB, the increased mud content could reduce DNF rates and potentially stimulate N

fixation by sulphate reducers, thus diminishing the estuaries ability to mitigate increasing N loads.

3.5.5 | On the role of phosphorus

In addition to N biogeochemistry, phosphorus also clearly plays a role in the functionality of these ecosystems. New Zealand is geologically "young" and therefore has higher rates of P weathering than many other countries (Gardner, 1990). Macrofauna community variables, including the numbers of individuals, bivalves, bioturbators, and grazers where highly correlated (r=0.7 – 0.88) with porewater [DIP] (Table AII.1). This is an unusual, and to our knowledge novel, finding especially given that porewater phosphate concentrations were very low (0.05 – 1.58 μM). We tend to associate the infaunal community with elevated NH_4^+; however, this relationship holds across all three estuaries included in this study, ranging in systems from 0 – 25% mud. Other studies have found that several infaunal species excrete DIP at a rate that is approximately 50 % of the NH_4^+ excretion rate (Magni *et al.*, 2000), so their activity may be exhibiting some regulatory control on porewater [DIP]. However, the magnitude of DOP fluxes was larger than DIP fluxes, suggesting that DOP is exchanged at a higher rate than DIP. DOP flux was positively correlated with mud content and negatively correlated with species richness in the dark, and in the light DOP was positively correlated species richness and weakly, negatively correlated with net N_2 flux (Table AII.2, Fig. 3.3). These results suggest that DOP is directly related to both the macrofaunal community and N cycling in these systems. The inverse relationships with species richness in the dark vs. light indicate that this may be mediated by MPB production and decomposition, however, the role of P, particularly DOP in non-eutrophic systems such as these is worthy of further study.

3.6 | Conclusions

DNF is widely established as a critical ecosystem service, particularly in mitigating anthropogenic nutrient pollution. However, directly measuring DNF can be costly and requires very sophisticated instrumentation; it can therefore be limiting in remote or understudied systems. Here we have shown that in the low nutrient estuaries of North eastern New Zealand, porewater [NH_4^+] and mud content on their own are good predictors of net DNF rates in the dark (61 % of variation explained, Table 3.2). These parameters are both inexpensive, easy to measure, and could potentially be used as predictors of DNF in the future. However, the dark, inundated periods only represent one quarter of the experience of intertidal sediments. Future work needs to improve our capabilities for estimating DNF and N fixation in the light. Macrofauna community data are not often collected with a full suite of biogeochemical and environmental variables, this is particularly true in the case of directly measured N_2 fluxes. In this study we present a novel association between macrofaunal species richness and net N_2 fluxes, highlighting the importance of ecogeochemical connections in predicting DNF. We therefore also argue that future work on denitrification, particularly in *in situ*, light conditions, include the macrofaunal community. We also demonstrate key relationships between the DOP and the macrofauna and N cycling within these systems which is worthy of further investigation.

There is a need to connect the macrofaunal community to the nutrient cycling and biogeochemistry of these systems in order to better understand their functionality. We have addressed this need by using multi-variate modelling to distil the combined predictors of directly measured net N_2 fluxes, and by building interaction networks to clarify the role of net DNF in a broader ecogeochemical context. As both terrestrial sediment deposition and anthropogenic N loading continue to increase in New Zealand, it is now more important than ever that studies such as this one increase our understanding of how these still relatively un-

impacted systems function and what drives N removal via DNF, so that managers of these systems can be better informed in the future.

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Chapter 4

Simultaneous denitrification and nitrogen fixation in oligotrophic sediments*

Amanda M Vieillard & Simon F Thrush

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4.1 | Summary

Nitrogen fixation has been identified as the largest unknown in the global marine nitrogen budget and its quantification has been fraught with methodological issues. Nonetheless, nitrogen fixation has been shown to be ubiquitous in estuarine sediments and is a particularly important source of N to oligotrophic systems. Here we report the first simultaneously measured rates of nitrogen fixation and denitrification in an oligotrophic estuary. Isotopically labelled, dissolved $^{30}N_2$ gas was added to the overlying water of intact sediment cores from the North Island of New Zealand. Average denitrification rates were 54.9 ± 9 and 87.0 ± 6 μmol m^{-2} h^{-1} and total potential nitrogen fixation rates were -98.1 ± 4 and -74.9 ± 6 μmol m^{-2} h^{-1} in the light and dark, respectively. These rates were compared to net N_2 fluxes measured using the N_2/Ar method, and there was good agreement between both methods. These results show that, in this oligotrophic system, denitrification dominates in the dark while nitrogen fixation is more important under light conditions as a result of competition between nitrogen cycling bacteria and benthic microalgae; however both processes co-occurred in all cores. The production of $^{15}NH_4^+$ over the first 5 hours of the incubation provides further evidence of simultaneous nitrogen fixation and denitrification, with both nitrogen fixing cyanobacteria and heterotrophic diazotrophs likely responsible for the nitrogen fixation in this system.

4.2 | Introduction

Estuaries are hotspots of many ecosystem services including nutrient processing, making them some of our most valuable ecosystems. The designation of an estuary's trophic state has been a key tool for the management of coastal systems for many decades. Traditionally, the classification of trophic state for a marine system (as oligotrophic, mesotrophic, eutrophic, or hypereutrophic) is based on the supply of organic carbon to that system (Nixon, 1995). However, over time trophic classification has become inexorably

linked to N and P, as increases these nutrient concentrations are nearly always responsible for the eutrophication of coastal systems. There are many ways to classify the trophic state of an estuary, but on a nutrient basis, an oligotrophic system is defined as one which has water column DIN concentration less than 7.1 µM and a DIP concentration less than 0.3 µM (Lemley *et al.*, 2015).

Oligotrophic estuaries exist world-wide; however, because they are generally relatively un-impacted by human activities, they are often in understudied and remote regions. Despite being underrepresented, oligotrophic systems have been said to mirror the state of estuaries prior to anthropogenic eutrophication, therefore, their study is key to understanding baseline estuarine function (Cook *et al.*, 2004; Hellemann *et al.*, 2017; Vieillard, Newell and Thrush, 2020). However, the vast majority of the literature on N dynamics in estuaries comes from chronically eutrophic and hypereutrophic systems in the Northern hemisphere (Vieillard, Newell and Thrush, 2020). This paucity of data from oligotrophic estuaries limits our understanding of coastal marine N dynamics. As many of the world's chronically eutrophic systems look to various means of nutrient management in an attempt to reduce nutrient runoff and reverse eutrophication (Bernhardt *et al.*, 2008; Detenbeck, You and Torre, 2019), the study of oligotrophic estuaries as benchmark systems is now more important than ever. In this study we sought to expand our understanding of oligotrophic systems by the simultaneous quantification of two key N cycle processes: DNF and N fixation.

DNF is the primary N removal mechanism in coastal marine systems. It is a microbially mediated pathway which transforms bio-available NO_3^- into largely inert N_2, returning it to the atmosphere. While approximately 78 % of our atmosphere is comprised of N_2, very few organisms are able to utilize this massive N pool; only nitrogen fixers are able to use N_2 gas via the process of N fixation. DNF and N fixation are opposing processes which

help regulate the net N flux into (N fixation) or out of (DNF) a particular system. However, while DNF rates are widely reported, N fixation is less constrained, and has been identified as the largest unknown in global marine N budgets (Mahaffey, Michaels and Capone, 2005; Wang *et al.*, 2019).

DNF has long been identified as an important, N mitigating, process in coastal systems, and can efficiently remove anthropogenic N inputs before they reach the open ocean (e.g., Dong et al. 2009; Galloway et al. 2004). However, for many decades, N fixation was only thought to be an important source of N in open ocean systems, with the pelagic, N fixing cyanobacteria *Tricodesmium* identified as the major N fixer (Capone *et al.*, 2005). N fixation was considered an unimportant process within marine sediments (Howarth *et al.*, 1988; Howarth, Marino and Cole, 1988). This idea primarily came from the fact that N fixation is energetically expensive, requiring enough energy to break the triple bond in an N_2 molecule, and was therefore not thought to be a viable option in N-rich environments such as sediments. This was further supported by the finding that the expression of the *nitrogenase* enzyme required to fix N is repressed by the presence of NH_4^+ in cyanobacteria cultures (Lindell and Post, 2001). However, more recent work has refuted this idea, showing that N fixation can proceed with NH_4^+ concentrations has high as 200 µM in benthic environments (Knapp, 2012; Zehr and Capone, 2020).

Additionally, the quantification of N fixation has been plagued with methodological issues. The primary method for measuring N fixation has been ARA, in which acetylene gas is added to the headspace of a sediment slurry and the resulting ethylene production is measured (Dilworth, 1966; Hardy *et al.*, 1968). This method takes advantage of the fact that the *nitrogenase* enzyme which enables N fixation can also transform acetylene gas to ethylene, with one mole of N_2 reduced for every 3 moles of ethylene produced (Postgate, 1972). However, the ARA method has well-established impacts on the microbial community,

inhibiting many species responsible for key N cycle processes including: nitrification (Hynes and Knowles, 1982), DNF (Balderston, Sherr and Payne, 1976), sulphate reduction (Payne and Grant, 1982), and more recently, N fixation itself (Fulweiler *et al.*, 2015). As a result ARA is expected to vastly underestimate rates of N fixation in the environment.

Over the last decade, newer methodologies have found N fixation to be pervasive in coastal marine sediments, even in very eutrophic systems (Knapp, 2012; Fulweiler *et al.*, 2013; Newell *et al.*, 2016). Additionally, N fixation by sulphate reducing bacteria has been identified as an important N supply, particularly in tropical and eutrophic sediments (Romero *et al.*, 2015; Oczkowski *et al.*, 2020). However, the overall mechanisms driving N fixation in sediments are still poorly understood (Newell *et al.*, 2016; Zehr and Capone, 2020). While N fixation and DNF have been proven to co-occur in eutrophic sediments (Fulweiler *et al.*, 2013; Newell *et al.*, 2016), the measurement of N fixation and DNF independently is very difficult. Because both processes transform the very large background N_2 pool, N fixation and DNF are generally quantified together as a net N_2 flux via the N_2/Ar method (Kana *et al.*, 1994). As a result, the dynamics controlling the balance between these two processes are also poorly constrained.

Further, the vast majority of N fixation measurements are conducted in the dark due to potential methodological complications in the light. Oxygen production under light conditions can supersaturate the water column in a sealed incubation creating microbubbles and bubbles (Eyre *et al.*, 2002). Within these bubbles, a very strong N_2 gradient develops leading to the rapid diffusion of N_2 gas from the water column into the bubbles. This diffusion reduces the concentration of dissolved N_2 in the water column, mimicking the signal for Nfix in the N_2/Ar method (Eyre *et al.*, 2002). As a result, N fixation measured under light conditions via N_2/Ar is often written off as methodological error. However, N fixation and DNF dynamics are equally important, if not more complex, in the light as they are in the dark.

The issues with bubbles and N fixation in the light can be limited by monitoring the argon (Ar) signal within the incubation chamber. Because Ar is biologically inert, its concentration should essentially remain constant over the course of an incubation, therefore a significant decrease in the Ar concentration is indicative of diffusion into bubbles (Kana, personal communication).

The most reliable method for quantifying N fixation has proved to be the use of isotopically heavy, labelled $^{30}N_2$ gas additions. This method takes advantage of the naturally occurring difference in nitrogen stable isotopes. The vast majority of N in the environment is the lighter ^{14}N isotope with the heavier ^{15}N making up only 0.36 % of the natural N pool (Steingruber, Friedrich, Chter, *et al.*, 2001). As a result, while the background concentration of light ($^{28}N_2$) N_2 gas is very large, the concentration of the heavy ($^{30}N_2$) isotope is orders of magnitude less. By adding dissolved $^{30}N_2$ to the overlying water of a sealed system such as a core incubation, one can monitor the simultaneous production of ambient, light $^{28}N_2$ via DNF and the consumption of heavy $^{30}N_2$ via N fixation. The advantages of this method include keeping the sediment intact and not disturbing or altering the microbial community (Capone and Montoya, 2001). However, labelled $^{30}N_2$ gas is difficult to measure accurately, and is expensive, limiting its more widespread use, particularly in remote and under-studied locations.

N fixation is hypothesized to be a key N source to oligotrophic and low-nutrient systems (Sundbäck, Miles and Göransson, 2000; Vieillard, Newell and Thrush, 2020). Net N_2 fixation has been measured in these systems (e.g., Vieillard and Fulweiler 2012; Eyre, Maher, and Squire 2013; Ferguson, Eyre, and Gay 2004), but N fixation and DNF have not been quantified simultaneously in oligotrophic marine environments. In this study we simultaneously measured N fixation and DNF in oligotrophic marine sediments under both

light and dark conditions in order to quantify the relative importance of each process to the N balance in these understudied systems.

4.3 | Methods

N fixation and DNF in this study were quantified using whole core incubations. Intact sediment cores were collected at low tide from intertidal sediment in Whangateau Harbour, New Zealand. Whangateau Harbour is the northern most estuary in Auckland Region, situated on the east coast of New Zealand's North Island, and opening to the Hauraki Gulf. This is an extensively studied estuary given its close proximity to the University of Auckland's Marine Laboratory, and is considered a clean, oligotrophic system (Cole, Lees and Wilson, 2009). Twelve cores total were collected from the intertidal sediments at low tide in January 2020. Cores were 11cm (inner diameter) x 33.5 cm and sediment was collected to a depth of approximately 18cm. Upon collection the cores were immediately transported back to the laboratory and gently filled with sand-filtered sea water without disturbing the sediment surface. Cores were left with air stones in the overlying water overnight to ensure oxic conditions and incubated the following morning.

4.3.1 | Core Incubations

On the day of incubation, cores were carefully drained and refilled with seawater filtered to 1 µM without disturbing the sediment surface. Filtered seawater was used to ensure that the processes measured were occurring within the sediment, not the water column. Two sediment-free control cores were also incubated for this reason. Cores were then arranged in barrels of seawater creating a flow-through water bath to ensure a constant temperature of 19.5 °C, and topped with clear, gas-tight lids, ensuring no bubbles were present on the interior of the cores. This experiment was conducted at a time of year when the air temperature in the seawater lab was the same as the incoming seawater temperature.

Header filling water was left out overnight to allow for off-gassing and to prevent supersaturation. Six cores were placed in each tank; one tank was then covered with black plastic to ensure darkness, while the other was left under lights. Light levels in the light bath were set to match an overcast day, so as to stimulate benthic primary production, but prevent oxygen super-saturation and bubbles (~200 µmol photons m^{-2} s^{-1}). Light and temperature were monitored in each tank by Pendant UA-002 data loggers (Hobo, USA).

30 mL of isotopically enriched $^{30}N_2$ water was then added to each core and allowed to pre-incubate for 30 minutes, to ensure mixing through the water column (Carlson-Perret, Erler and Eyre, 2018). The $^{30}N_2$ solution was made by adding Mili-Q water to air-free, gas tight tedlar bags, then adding $^{30}N_2$ gas (Novachem Pty Ltd., VIC, Australia) as headspace the day prior to incubation. The bags were left shaking gently on a shaker table overnight, and added as a 30 mL slug to each core the next morning (Newell *et al.*, 2016). The concentration of dissolved $^{30}N_2$ in each core at the beginning of the incubation was 57µM, on average. Core lids were equipped with magnetic stirring bars to prevent stratification and ensure thorough mixing of the tracer. Dissolved oxygen was monitored throughout the incubation using a Firesting optical dissolved oxygen probe (PyroScience, Denmark) ensuring that oxygen dropped at least 2 mg L^{-1} from start to finish, but that hypoxic conditions (< 2 mg L^{-1}) were never reached. This ensures a long enough incubation for microbial processes to alter N concentrations, but not long enough to alter the state of the system. Total incubation time was approximately 22 hours, with dissolved gas samples taken at 5 time points throughout. Samples removed from the cores were replaced with filtered water from 5 L, gas-tight tedlar bags on the table above the tank set up. Dissolved gas samples were taken from this gravity-fed system by over-filling gas tight, 12 mL exetainer vials (Labco, UK) and ensuring there were no bubbles in the vials. Samples were immediately fixed with ~75 µL of concentrated zinc chloride. Six vials were collected from

each core at each time point, two each for N_2/Ar, $^{28,29,30}N_2$, and $^{15}NH_4^+$ analysis, all were stored under water at 4 °C until analysis. DIN and DIP samples were not taken from these cores in order to avoid excessive dilution within the cores. At the end of the incubation, three 1 cm x1 cm sub-cores were taken out of each core for chlorophyll-a and phycocyanin concentrations. These samples were collected in foil wrapped centrifuge tubes and immediately stored at -80 °C. Additionally two 2 cm x2 cm sub-cores were taken from each core for porosity and sediment composition.

4.3.2 | Sample Analysis

Porosity samples were weighed and then dried at 60°C for 3 days (until mass remained unchanged), then weighed again. Porosity was calculated from the difference between wet and dry mass, divided by the volume of sediment; the porosity for each core was then used in the diffusion flux calculations (Appendix III). Chlorophyll-a samples were freeze dried in dark jars, once dry 1 g of sediment was added to 3 ml of 90 % acetone, vortexed, and extracted for 24 h at 4 °C. After extraction samples were run on a UV-vis spectrophotometer and chlorophyll-a and pheo-pigment concentrations were calculated (Lorenzen, 1967). Phycocyanin (C-PC), a pigment associated with cyanobacteria was measured by again weighing 1 g of the freeze dried sediment and adding 3 ml of 0.05 M phosphate buffer (containing equal volumes of 0.1 M K_2HPO_4 and 0.1M KH_2PO_4). These samples were then vortexed and put through 2 freeze-thaw cycles (24h at -80°C followed by 24 h at 4 °C) in order to ensure thorough cell lysis. Samples were then measured on a UV-Vis spectrophotometer and concentrations of C-PC were calculated (Horváth *et al.*, 2013).

Dissolved gas analysis was done on a quadrapole membrane inlet mass spectrometer (MIMS). Previous oxygen tests in this instrument have shown that increasing DO does not significantly impact N_2 to Ar ratios but can slightly change the $^{30}N_2$ signal (*data not shown*). Isotopic N_2 samples ($^{28,29,30}N_2$) were measured on the MIMS the day following the incubation

using a copper reduction column and furnace set to 600 °C, so as to limit the production of NO, which also has a mass of 30 and can therefore interfere with the $^{30}N_2$ signal, and SEM detector as described by (Steingruber, Friedrich, Gächter, *et al.*, 2001). Oxygen, N_2, and Ar were then measured without the furnace or SEM according to the N_2/Ar method (Kana *et al.*, 1994). Ar concentrations were monitored on all MIMS runs and did not change more than 5% throughout the incubation, except in one of the light cores, which had a leak and was therefore not included in the analysis. The final batch of samples were oxidized with a hypobromite solution and immediately analysed for $^{15}NH_4^+$ using the OX/MIMS method (Yin *et al.*, 2014). Precision for all MIMS analysis was <0.03 % for N_2/Ar, and <0.01 %, <0.03 %, and <0.1 % for $^{28,29,30}N_2$, respectively.

4.3.3 | Flux Calculations & Statistical Analysis

The concentration of each gas was measured at each time point the used to calculate a flux. Fluxes of each gas across the sediment-water interface were calculated over the course of the incubation and normalized to core volume and surface area, yielding fluxes in μmol m^{-2} h^{-1} (Fig. AIII.1). The N_2/Ar method yields net fluxes, with a positive N_2 flux indicating net DNF and a negative flux indicating net N fixation. In the isotopic samples, the change in concentration of each N_2 isotope ($^{28,29,30}N_2$, Fig. AIII.1) was monitored independently and a flux was calculated for each (AIII, *E1*). DNF rates were calculated as the sum of $^{28+29}N_2$ production (positive fluxes; AIII, *E2*). While these are still, technically net fluxes, the only pathways for $^{30}N_2$ uptake are via diffusion and N fixation. The rate of diffusion into the sediment was calculated using Fick's Law of Diffusion (Hamersley and Howes 2005; AIII, *E3*). The measured N fixation rate was then calculated by subtracting the diffusion flux from the flux of $^{30}N_2$ into the sediment (AIII, *E5*). Because only a fraction of the total dissolved N_2 pool was labelled, the measured N fixation rate only reflects the rate of fixation of the added $^{30}N_2$. In order to calculate the potential, total N fixation rate ($Nfix_{tot}$), the proportion of

available N_2 as $^{30}N_2$ after diffusion was calculated (~12 % of the total N_2 pool, on average; AIII, *E6*). The measured $^{30}N_2$ fixation was then divided by this proportion yielding an N fixation rate for the total N_2 pool, hereafter $Nfix_{tot}$ (AIII, *E7*). A net rate was then calculated from the sum of the DNF rate and the $Nfix_{tot}$ rate (AIII, *E8*). These net rates were compared to the net rates from the N_2/Ar method as a methodological comparison. A 2-way analysis of variance with replication was used to test for significant differences in N_2 flues between the light and dark cores ($\alpha = 0.05$), t-tests were then used to test for significant difference between individual means, and the correlations and significance of relationships between variables measured were calculated as Pearson correlations. All statistical analyses were carried out in R, version 4.0.3.

4.4 | Results & Discussion

Gas fluxes in the water control cores were not significantly different from zero, and the measured [$^{15}NH_4^+$] was not significantly different from the N free Mili-Q water blank. Previous work has shown $^{30}N_2$ gas sources to be contaminated with various ^{15}N species including $^{15}NH_4^+$ (Dabundo *et al.*, 2014); however our results from the water control cores confirm that there was no significant $^{15}NH_4^+$ contamination of the $^{30}N_2$ used in this study. There were also no significant differences in overall sediment porosity and composition between the light and dark cores. C-PC content was also not significantly different. Mean chlorophyll-a content was slightly elevated in the light cores compared to the dark at the end of the incubation, although not significantly so (Table 4.1). This elevated chlorophyll-a content is potentially indicative of a stimulation of the MPB under light conditions.

4.4.1 | Dissolved Oxygen

While DO fluxes in all cores were negative (indicative of net respiration), DO consumption (or sediment oxygen demand, SOD) was higher in the dark (Table 4.1).

Table 4.1. Individual parameters measured in this study displayed by individual core and dark and light averages. Chlorophyll-a (Chl-a) and Phycocyanin (C-PC) are in µg/g sediment and all others are fluxes in µmol m^{-2} h^{-1}. $^{15}NH_4^+$ is the production of heavy ammonium over the first half of the incubation. Error is standard error (n=6 in the dark, n=5 in the light).

Core	**Chl-a**	**C-PC**	**SOD**	**N_2/Ar**	**$^{28}N_2$**	**$^{29}N_2$**	**$^{30}N_2$**	**DNF**	**$NFix_{tot}$**	**Net**	**$^{15}NH_4^+$**
1	0.43	14.0	596.0	0.0	39.36	0.07	-12.3	39.4	-65.60	-26.17	1163
2	6.94	16.7	702.2	-45.6	69.60	0.0	-12.0	69.6	-100.4	-30.75	1182
3	6.41	14.7	971.5	52.8	134.6	0.79	-7.92	135.4	-73.67	61.76	1101
4	8.06	13.6	906.8	64.8	133.2	0.0	-4.44	133.2	-58.28	74.92	989.6
5	0.94	15.4	769.4	21.0	79.56	0.0	-18.7	79.6	-84.10	-4.54	1140
6	6.22	12.7	854.9	46.8	64.74	0.25	-9.96	65.0	-67.38	-2.39	892.2
Dark Average	4.86±1.3	14.5±0.6	800 ±57	23.7±19	86.9±16	0.19±0.13	-10.9±2	87.0±16	-74.9±6	12.1±18	1078±47
7	7.04	13.9	357.2	-24.0	81.60	-0.72	-12.0	81.6	-102.8	-21.19	1187
8	7.05	15.0	262.1	-59.0	62.40	0.24	-10.2	62.6	-101.2	-38.56	1098
9	6.50	13.4	463.0	-71.0	26.41	0.10	-10.2	26.5	-88.30	-61.80	949.6
10	7.10	11.4	273.8	0.10	70.22	-0.47	-9.36	70.2	-89.57	-19.37	764.1
11	6.77	13.8	354.5	-82.2	34.02	-0.34	-10.6	34.0	-108.69	-74.67	874.8
Light Average	6.89±0.1	13.5±0.5	342±33	-39.2±19	54.9±9.7	-0.24±0.2	-10.5±0.4	54.9±9	-98.1±4	-43.1±10	974.5±69

Since the DO fluxes are net fluxes, lower SOD in the light is likely a result of increased oxygen production via photosynthesis. This is further demonstrated by the significant, positive correlation of SOD with Chl-a content in the light ($r = 0.79$, $p < 0.01$). Additionally, DO flux rates were significantly and positively correlated with DNF in the dark ($r = 0.77$, $p < 0.01$). This relationship between SOD and DNF is indicative of DNF via the coupled nitrification-denitrification pathway (Gongol and Savage 2016; Vieillard and Fulweiler 2012). Coupled DNF is often the dominant DNF pathway in low-nutrient systems, where overall DIN concentrations are low and NH_4^+ is more widely available than NO_3^- (Gongol and Savage, 2016; Crawshaw, Schallenberg and Savage, 2019). Instead of directly denitrifying NO_3^- that diffuses into the sediment, coupled nitrification-denitrification relies on nitrification to first convert NH_4^+ into NO_3^-. Nitrification is a two-step pathway which requires molecular oxygen to proceed; therefore, even though DNF itself is an anaerobic process, coupled DNF relies on oxygen-requiring nitrification, thus the correlation between SOD and DNF (Risgaard-Petersen, 2003).

4.4.2 | Denitrification and nitrogen fixation

Isotopic N_2 analysis showed net DNF of the unlabelled $^{28}N_2$ pool and net fixation of the labelled $^{30}N_2$ pool in both the light and the dark (Table 4.1). Additionally, the uptake of $^{30}N_2$ could not be explained by diffusion alone, as diffusion accounted for 7-16 % of the total measured $^{30}N_2$ flux. $^{29}N_2$ ($^{14}N + {}^{15}N$) fluxes were very low overall, and, on average exhibited net DNF in the dark and net N fixation in the light. DNF rates were higher in the dark than in the light, though this difference was not significant (Fig. 4.1). Calculated $Nfix_{tot}$ exhibited significantly more N fixation in the light ($p = 0.015$, Fig. 4.1). Additionally, while both DNF and N fixation occurred in all cores, the magnitude of the DNF rates were larger than the $Nfix_{tot}$ in the dark, and $Nfix_{tot}$ was greater in the light (Fig. 4.1). Net N_2 fluxes measured using the N_2/Ar method also showed a similar trend with net DNF in the dark and net N fixation in

the light (Fig, 4.1). While Nfix measured in the light via N_2/Ar is generally thought to be an artefact of bubble interactions (Eyre *et al.*, 2002), our analysis of the Ar signal within the light cores (namely that Ar remained constant throughout the incubation), gives us more confidence that we did not have significant bubble formation within our cores. This was aided by the lower light level which was designed to prevent oxygen super-saturation and visual inspection of the cores throughout the incubation. As a result, we consider these light N fixation rates to be legitimate and not a methodological artefact.

This pattern of net DNF in the dark and net N fixation in the light has been seen previously in low-nutrient estuaries (Chapter 3, Eyre et al. 2011), and is indicative of the competition between N cycle bacteria and MPB in sediments (Risgaard-Petersen *et al.*, 2003). In the dark, DNF is stimulated because MPB are not using DIN for photosynthesis, therefore there is more DIN available to be nitrified and denitrified. However, under light conditions, MPB are active and regulate the flux of nutrient across the sediment-water interface, directly competing with denitrifiers (Sundbäck, Miles and Göransson, 2000; Risgaard-Petersen *et al.*, 2003). It is under these conditions also that N fixation becomes an important source of N, particularly in oligotrophic sediments such as these (Eyre *et al.*, 2011). Our results follow this same pattern and provide conclusive evidence of DNF and N fixation co-occurring across in these oligotrophic sediments.

There was good agreement between the net N_2 fluxes calculated from the individual N_2 isotopes and those from the N_2/Ar method (Fig. 4.2). Averaged net fluxes from these two methods were not significantly different from each other in either the light or the dark (Fig. 4.1).

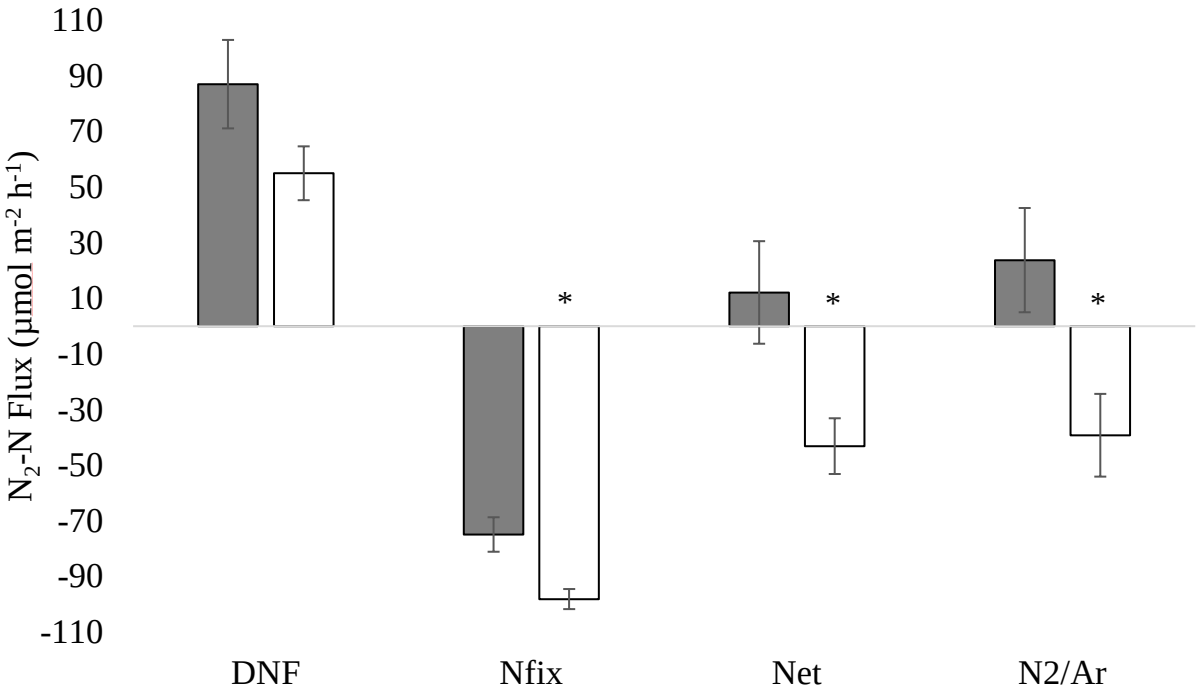

Figure 4.1. Total and net N_2 flux rates (as N_2-N) measured as denitrification (DNF, $^{28}N_2$), nitrogen fixation ($Nfix_{tot}$), net (DNF + $Nfix_{tot}$), and the N_2/Ar method for comparison. Positive fluxes signify denitrification and negative fluxes signify N fixation. Dark bars are rates from cores kept in the dark and open bars are from cores exposed to light. * denote significant differences ($p < 0.05$) between light and dark, and error is standard error (n=6)

This agreement is promising and helps to expand our understanding of the relative importance of each of these two processes, while also corroborating the legitimacy of net methods were not significantly different from each other in either the light or the dark (Figure 1). in the light. Figure 4.2 shows that compared to the N_2/Ar method, the calculation of net N_2 fluxes using isotopes slightly underestimated DNF rates and slightly overestimated methods were not significantly different from each other in either the light or the dark (Figure 1). rates (Fig. 4.2). The reason for this discrepancy likely lies in the fact that the DNF rate, calculated primarily from $^{28}N_2$ fluxes, is still a net rate. Therefore, the DNF rate includes any fixation of $^{28}N_2$. While the calculation of $Nfix_{tot}$ accounts for the fixation of $^{28}N_2$, it is not possible (without the addition of a labelled, ^{15}N- NO_3^- source) to expressly partition DNF from N fixation in the $^{28}N_2$ flux. As a result there is likely some overlap, or double counting of $^{28}N_2$ fixation (~ 18 µmol m^{-2} h^{-2}, on average) resulting in a net underestimation of total DNF by 0 – 30 %. Similarly, the $^{30}N_2$ flux is also, technically a net flux, and does not account for any $^{30}N_2$ that may be produced later in the incubation. However, given the high level of correlation between the calculated Net N_2 flux and the N_2/Ar N_2 flux, we can assume that this underestimation of DNF and overestimation of N fixation is relatively small in most cases. While we see good agreement between these two methods in this study, future work is needed to investigate whether this relationship holds in other environments.

$^{15}NH_4^+$ was measured as further evidence of the occurrence of N fixation. Because the only heavy isotopic label that was added to these cores was added as $^{30}N_2$, any heavy, ^{15}N measured in the ammonium pool, must first have been fixed. $^{15}NH_4^+$ exhibited a parabolic curve over the course of the incubation with $^{15}NH_4^+$ produced in the first 5.5 hours, and an average maximum concentration of 23.8 µM $^{15}NH_4^+$ (Fig. 4.3A). Slightly more $^{15}NH_4^+$ was produced in the dark cores compared to the light, with average production rates over the first 5.5 hours of the incubation of 1078 ± 47 and 974.5 ± 69 µmol m^{-2} h^{-1} in the dark and light,

respectively (Table 4.1). This result shows that not only did fixation of the added $^{30}N_2$ begin immediately, but that heavy N fixed into organic matter was turned over and respired into the ammonium pool in a matter of hours. While $^{15}NH_4^+$ was likely produced throughout the incubation, the shape of the curve suggests that more was consumed than produced over the remaining incubation time yielding net negative fluxes (Fig. 4.3A). There was significantly more net consumption of $^{15}NH_4^+$ in the dark with average fluxes of -105.1 ± 14 and -24.3 ± 10 μmol m^{-2} h^{-1} in the dark and light, respectively ($p = 0.040$). This pattern of $^{15}NH_4^+$ production followed by consumption has been seen previously (Newell *et al.*, 2016).

$^{15}NH_4^+$ production was also significantly, positively correlated with sediment phycocyanin (C-PC) content across all cores (Fig. 4.3B). C-PC is one of the primary, accessory pigments for various cyanobacteria (Horváth *et al.*, 2013), including N fixing species, which are often important components of the MPB biofilm (MacIntyre, Geider and Miller, 1996). Therefore the relationship between $^{15}NH_4^+$, originally fixed from the added heavy $^{30}N_2$, and C-PC suggests that ~65 % of the variation in $^{15}NH_4^+$ produced is associated with C-PC in cyanobacteria (Fig. 4.3B). Interestingly, this relationship holds in both the light and dark cores. However, for many species of cyanobacteria, the N fixing mechanism is heterotrophic and decoupled from photosynthetic pathway, allowing for N fixation as needed in the light or dark (Summers *et al.*, 1995). As N fixation and DNF co-occurred in all cores, this result suggests that cyanobacteria in this system play a key role in N fixation regardless of light condition. While $^{15}NH_4^+$ production is highly correlated with C-PC, both measured N fixation and calculated $Nfix_{tot}$ are not. These results suggest that multiple N fixation pathways are likely co-occurring. While the N fixing cyanobacteria seem to be working and turning over rapidly, producing $^{15}NH_4^+$ in the MPB biofilm on the sediment surface, other microorganisms may be responsible for the remainder of N fixation occurring in these sediments.

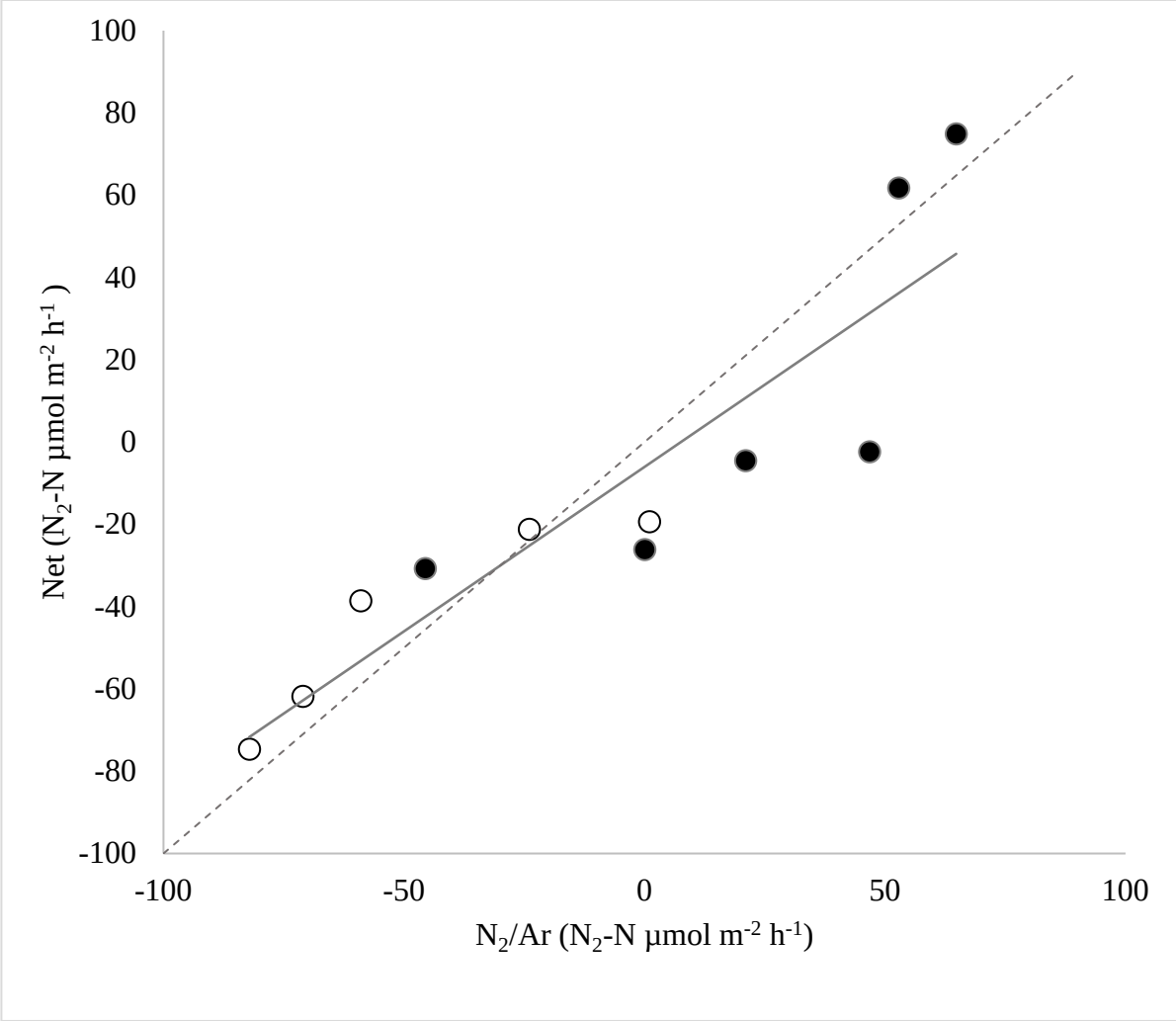

Figure 4.2. Comparison of Net N_2 flux calculated from individual N_2 isotopes (y-axis) and from the N_2/Ar method (x-axis) across all cores. Dark points denote fluxes from the dark cores and open points denote fluxes from light cores. The darker line is the data trend line ($y=0.80x - 6.0$, $R^2= 0.81$, $p<0.001$), while the dashed line is the 1:1 line for reference.

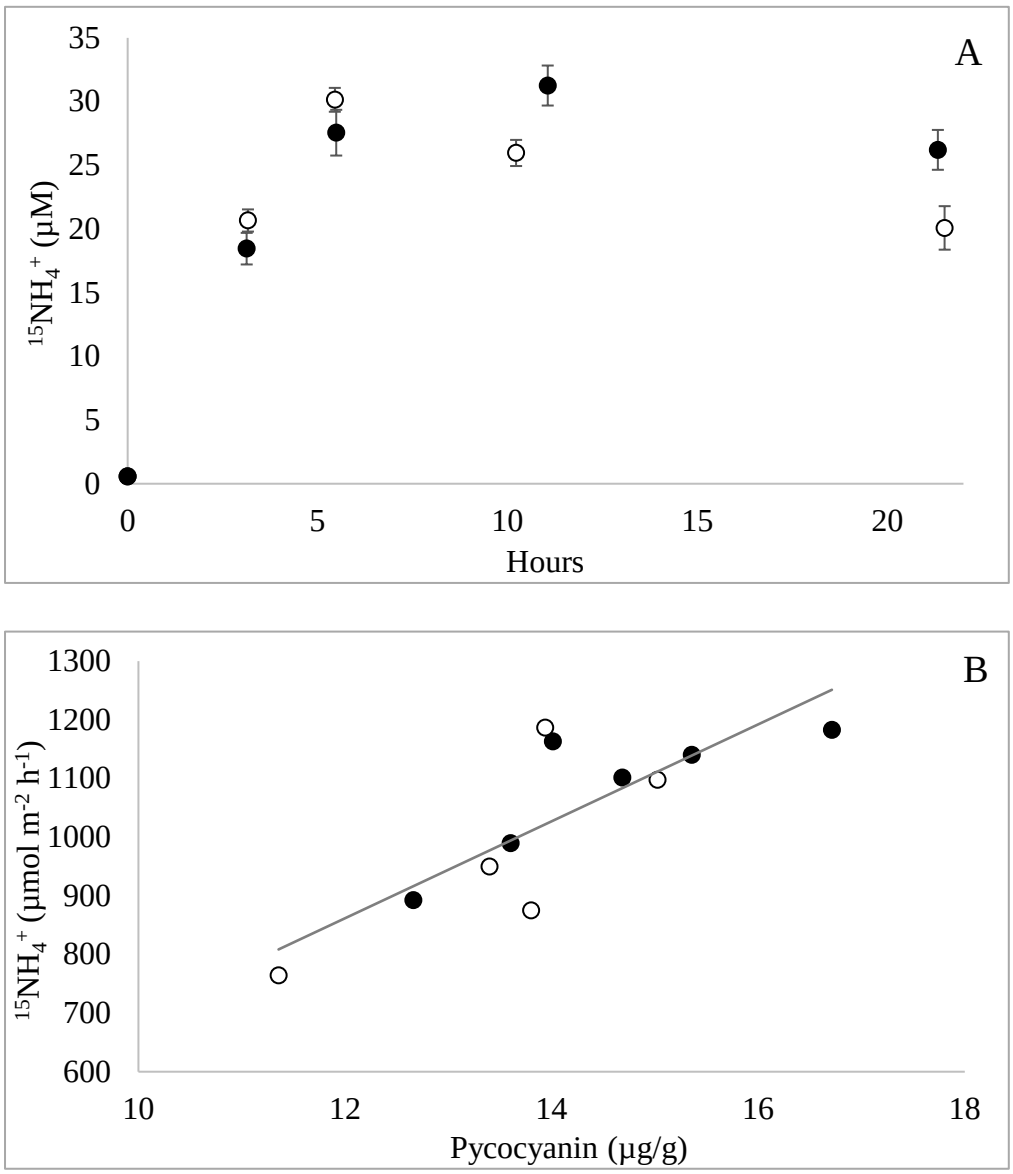

Figure 4.3. A.) Isotopically enriched ammonium ($^{15}NH_4^+$)concentration over the course of the incubation. Error = standard error, n= 6 B.) $^{15}NH_4^+$ production in the first 5 hours vs sediment phycocyanin content. y= 82.6x – 130, R^2= 0.654, p< 0.001. Open circles are light cores and closed circles are dark cores for both plots.

N fixing bacteria (diazotrophs) have been shown to be important sources of N in a variety of marine ecosystems including salt marshes (Brown, Friez and Lovell, 2003; Lovell *et al.*, 2008), mangroves (Romero *et al.*, 2015; Oczkowski *et al.*, 2020) and estuarine sediments (Zehr *et al.*, 2003; Bhavya *et al.*, 2016), and in many of these systems heterotrophic N fixation has been strongly linked to organic matter and carbon loading, rather than N availability (Fulweiler *et al.*, 2013; Bhavya *et al.*, 2016; Shiau *et al.*, 2017). Many of these diazotrophs have specifically been linked to sulphate reduction in sediments, even in heavily eutrophic systems (Romero *et al.*, 2015; Oczkowski *et al.*, 2020). Though sulphate was not quantified in this study, sulphate reduction is a common and important process in estuarine sediments, it is reasonable to suspect that N fixation via sulphate reduction is also playing an important role in this system, particularly in the deeper, anoxic zones of the sediment.

4.5 | Conclusions

Overall this study has teased apart the relative importance of DNF and N fixation in oligotrophic marine sediments, while maintaining good agreement between the addition of $^{30}N_2$ and the more widely used N_2/Ar method. We have demonstrated that N fixation is just as important in these systems as DNF, with fluxes in the same order of magnitude and at higher rates in under light conditions than DNF. Additionally, these N fixation rates in the light are very likely true and not an artefact of interaction with bubbles. $^{15}NH_4^+$ production in the first 5 hours of the incubation is further evidence of the co-occurrence of N fixation and DNF. As in many low-nutrient sediments, the coupled nitrification-denitrification pathway seems to dominate, and the relative differences between DNF and N fixation in the light and dark are likely driven by competitive interactions with MPB. Further, cyanobacteria within the MPB were found to be key, though not the only important, N fixers. These findings show

that like DNF, N fixation is a key ecosystem service in this low-nutrient estuary, providing an important source of N to the system.

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Chapter 5

Reaching the tipping point: Terrestrial sediment deposition in oligotrophic sediments

Amanda M Vieillard, Judi E Hewitt, Simon F Thrush

5.1 | Summary

Increasing terrestrial sediment deposition is a recognised threat to estuaries world-wide, particularly throughout the Pacific Rim. Climate change, increasing coastal populations, and land use change have led to an increased delivery of terrestrial sediment to coastal systems, which can smother benthic infauna and alter biogeochemical cycling. A series of *in situ* benthic incubations were carried out along a grainsize gradient in a marine reserve affected by terrestrial sediment deposition in Northern New Zealand. This space for time substitution allowed for the quantification of how this protected ecosystem is responding to this increasing anthropogenic stressor. Results show that a tipping point is reached in this system at only 3 % sediment mud content with decreased total macrofaunal abundance and bioturbator abundance. There were also decreased net denitrification rates in the higher mud sites. In the low mud sites, a novel negative relationship was found between net N_2 flux and dissolved organic phosphorus uptake. In the high mud sites, net N_2 flux was positively correlated to the release of nitrate + nitrite. Additionally, multivariate linear models to predict net denitrification identified different drivers of denitrification in the low vs. higher mud sites. This work highlights how increasing terrestrial sediment deposition is decreasing the N removal capacity within this system and emphasizes the need to embrace variability and non-linear responses within ecosystems in order to better understand how they respond to stress.

5.2 | Introduction

Though they may seem simple at first glance, intertidal sand flats are extremely complex and important ecosystems, and the organisms within them are adapted to a dynamic environment. The daily tidal cycles cause intertidal systems to experience conditions ranging from fully submerged and possibly dark to completely exposed and baking in the midday sun. Despite these challenges, organisms that can tolerate these changing conditions benefit from a

high level of nutrient cycling (Joye, De Beer and Cook, 2009; Longphuirt *et al.*, 2009; Schutte *et al.*, 2019) and primary productivity (MacIntyre, Geider and Miller, 1996a; De Brouwer, De Deckere and Stal, 2003; Piehler, Currin and Hall, 2010). This allows estuarine species to thrive and drives some of our most ecologically and economically valuable food webs (Brown and Herbert Wilson, 1997; Smaal, Van Stralen and Schuiling, 2001). Intertidal flats are vital foraging grounds for shorebirds (Galbraith *et al.*, 2002; Danufsky and Colwell, 2003) and are key to coastal stabilization and storm surge protection (Murray, Ma and Fuller, 2015). However, their coastal location and exposed nature also make intertidal systems vulnerable to human impacts; over fishing, dredging, coastal engineering, and recreation can all physically degrade the intertidal environment, particularly in soft-sediments (Brown and McLachlan, 2002; Murray, Ma and Fuller, 2015). Additionally, there are many forms of anthropogenic pollution that heavily impact intertidal systems, including nutrient pollution and terrestrial sediment deposition.

Estuarine ecosystems receive higher nutrient inputs per unit area than any other ecosystem (Howarth, 1993). These N inputs very often lead to coastal eutrophication, resulting in shading and die-off of benthic primary producers, shifts in phytoplankton community composition, increased harmful algal blooms, and extensive hypoxia, all of which impede estuarine function (Howarth, 2008; Howarth *et al.*, 2011) and the delivery of ecosystem services (Barbier *et al.*, 2011). Eutrophication remains one of the greatest threats facing coastal marine ecosystems, therefore managing it is key to maintaining and restoring vital estuarine ecosystem functions and services (Steffen *et al.*, 2015; Le Moal *et al.*, 2019). DNF is a service that has received particular attention; it is a microbial process which can convert bio-available NO_3^- into inert, N_2 gas, thus removing it from the system and helping to mitigate anthropogenic nutrient loads. Maximizing DNF in estuarine systems, is therefore one important mechanism in preventing or lessening coastal eutrophication.

Another threat facing coastal systems worldwide, particularly on the Pacific rim, is increasing coastal erosion and terrestrial sediment deposition. Land clearing, land use change, coastal development, and climate change have all contributed to increased rates of terrestrial sediment loads to coastal systems (Lohrer, Hewitt and Thrush, 2006). Sediment loading is recognized as a threat to estuarine systems (GESAMP *et al.*, 1994), and has been shown to reduce their biodiversity and ecological value (Thrush *et al.*, 2004). There are two primary types of terrestrial sediment loading: catastrophic events and gradual increases. Much of the terrestrial sediment entering coastal ecosystems is a result of events such as landslides and major rain storms which can elevate coastal suspended sediment loads orders of magnitude above average levels (Hicks, Gomez and Trustrum, 2004). A large proportion of this incident sediment load is highly charged silt and clay particles which quickly flocculate and settle in seawater, smothering the benthos particularly in shallow and intertidal environments (Thrush *et al.*, 2004). Field-based experiments have shown that once terrestrially derived sediment settles and forms a layer 2 cm thick, the sediment beneath rapidly becomes anoxic and causes die-off in much of the seagrass and infaunal population (e.g., Norkko *et al.*, 2002; Cummings *et al.*, 2003; Hewitt *et al.*, 2003; Thrush, Hewitt and Norkko, 2003). Even if less sediment settles to the benthos, as little as 2 mm of deposited silt has been shown to alter macrofaunal (Lohrer *et al.*, 2004; Hohaia, Vopel and Pilditch, 2014) and MPB (Wulf *et al.*, 1997) community composition. These decreases in sediment microphytobenthos, as well as in macrofaunal diversity and biomass have detrimental consequences for higher trophic levels and ecosystem functions.

Gradual increases in sediment loading are typically the result of increased coastal erosion from land clearing, construction, or other land use change. These more subtle increases generally result in higher sustained turbidity, as a result of the elevated suspended sediment content, and gradual decreases in sediment grain size and permeability. Increased

turbidity decreases light reaching the benthos, reducing primary productivity and oxygen production on the sediment surface by seagrass and MPB's. When prolonged, this shading can cause reduction or die-off of benthic primary producers and a switch to a phytoplankton dominated system (Duarte, 1991; McGlathery, 2001). Phytoplankton blooms then further shade the benthos, and their decomposition reduces bottom water oxygen concentrations. Increased suspended sediment can also clog the filter feeding mechanisms of suspension feeding organisms and reduce their feeding efficiency (Ellis *et al.*, 2002; Ward and Shumway, 2004). Many suspension feeding organisms, especially bivalves, are key to maintaining benthic-pelagic coupling by transferring water column nutrients to the benthos via their feeding process. Therefore a reduction in their feeding efficiency also reduces organic carbon and nutrient delivery into the sediment, further affecting nutrient cycling within the system (Hammen, Miller and Geer, 1966; Dame, Zingmark and Haskin, 1984).

Decreases in grain size and sediment permeability limits oxygen penetration into the sediment changing biogeochemical, redox, and hydrological conditions. This muddying of coastal sediments has been shown to alter coastal nutrient cycling (Pratt *et al.*, 2014a) and decrease rates of DNF, particularly in low-nutrient systems (O'Meara *et al.*, 2020; Schenone and Thrush, 2020, Chapter 3). The mechanism for this decrease in DNF is thought to be three-fold. First, the decrease in oxygen penetration could decrease nitrification rates and therefore coupled nitrification-denitrification which dominates in low-nutrient environments. Because NH_4^+ is generally the more dominant DIN species in these systems, nitrification which converts NH_4^+ to NO_3^-, is the primary source of NO_3^- for DNF. However, nitrification requires molecular oxygen to occur, therefore the reduction in oxygen penetration would reduce nitrification. Second, higher mud content promotes greater MPB biomass. As MPB's have been shown to regulate the flux of nutrients across the sediment water interface (Joye, De Beer and Cook, 2009) and compete directly with denitrifying bacteria (Risgaard-Petersen,

2003), an increase in their biomass could result in decreased DNF rates. Finally, muddier sediments have less oxygen penetration and higher rates of sulphate reduction and hydrogen sulphide. Many sulphate reducing bacteria have been shown to be N fixers, even in high N environments such as sediments (Shiau *et al.*, 2017; Zehr and Capone, 2020), and an increase in N fixation results in a decrease in net DNF or even a flip to net N fixation (Fulweiler *et al.*, 2007). Additionally, there is some evidence that very high levels of hydrogen sulphide can directly impede denitrification (Joye and Hollibaugh, 1995).

All of these degradations of coastal functioning by increasing terrestrial sediment deposition are exacerbated by global climate change. Sea level rise, as well as the increased rainfall and incidents of major storm events predicted in many coastal regions will only accelerate coastal erosion. It is therefore crucial that we understand how coastal ecosystems will respond to this increasing stress, particularly in the context of long-term, cumulative change. However, the complex, interactions within these systems and their often non-linear response to stress mean that shifts in ecosystem structure and function under increasing anthropogenic impact are still not well understood. It is these interactions that require further study.

Both nutrient and sediment pollution are examples of stressors that can induce regime shifts or tipping points in an estuarine system leading to catastrophic change in and loss of ecosystem functions and services. A tipping point is the point at which abrupt ecological shifts, induced either by forces external to a system or by internal non-linear responses, occur, impacting how an ecosystem functions (Carpenter *et al.*, 2001; Scheffer *et al.*, 2001). Crossing the threshold of a tipping point rapidly transitions an ecosystem between two functionally different states. This process often triggers a series of runaway, positive feedbacks which make the transition very difficult to reverse (van Nes *et al.*, 2016; Thrush *et al.*, 2020). The shift from an oligo or mesotrophic estuary (or lake) to a eutrophic one is an

example of a tipping point. Unimpacted, low-nutrient systems have a certain degree of resilience, or buffering capacity, against increasing nutrient loads which allow them to function normally. However, when nutrient loads become high enough, that buffering capacity is overloaded, and the system switches quite quickly to a new, eutrophic state. This eutrophic state has built in hysteresis and positive feedbacks which, for example, favour reactive nutrients cycling within the system, rather than being removed via DNF, exacerbating the problem and making the return to an oligotrophic state, even with reduced N inputs, very difficult (Boesch, 2019; Vieillard, Newell and Thrush, 2020). Because of this complex nature, our understanding of, and ability to predict tipping points in the environment remains extremely limited (Ratajczak *et al.*, 2018; Hewitt and Thrush, 2019).

There is, therefore, a recognized need to understand the conditions which lead to tipping points so that we are better equipped to anticipate them or better able to assess the risk of crossing them (Nicholson *et al.*, 2009; Scheffer *et al.*, 2009). However, in order to do this, our research needs to include the complexity within ecosystems, including understanding the gradual changes that affect resilience (Scheffer *et al.*, 2001). Complex ecosystem dynamics such as feedback loops, and breaks in feedbacks have been shown to be key in generating tipping points (van Nes *et al.*, 2016), but because these dynamics are difficult to quantify we are often left without any early warning signs of an impending tipping point. Therefore, taking a more wholistic approach to the study of ecosystem functioning, including the complex interactions of reactions, processes, organisms, and functions, is the best way forward in understanding non-linear dynamics such as tipping points.

The high degree of variability with in estuarine, particularly intertidal estuarine, ecosystems has generally been thought of as a challenge to their study and is often averaged out over multiple replicates. However, changes in variability have been suggested to be early warning signs of tipping points (Wang *et al.*, 2012); therefore, variability can also be an

advantage when trying to parse out these more complex, non-linear dynamics within a system. In this study we sought to use the information held within the variability of estuarine ecosystem function to our advantage to help quantify non-linear functional shifts induced by increasing mud content. By working along a grain size gradient in an estuary heavily impacted by increasing sediment loads, we aimed to locate the sediment mud concentration that would induce a tipping point in key ecosystem functions, particularly DNF, and the macrofaunal community.

5.3 | Methods

5.3.1 | Study Site

This work was conducted in Okura Estuary, part of the Long Bay- Okura Marine Reserve. The Marine Reserve is a 980 hectare protected area established in 1995. Despite its protection, the location of the Okura Estuary, just 25 km North of New Zealand's largest city, Auckland, makes it vulnerable to anthropogenic stress. In particular, expanding urbanization, land clearing, and construction have massively increased the amount of sedimentation to Okura over the last twenty years (Ford, Anderson and Davison, 2004; Hewitt and Carter, 2020). In recent years, sedimentation has intensified, with increasing fine particle deposition, decreases in the density of the dominant bivalve, *Austrovenus stutchburyi*, from 2015-2018, and a die off event in April 2018 (Hewitt and Carter, 2020). Additionally, the recreational kayak tour company that has been operating in Okura for decades has stopped its tours in the reserve, anecdotally citing the degraded state of the system and diminished shore bird and ray populations (Pete Townend, *personal communication*).

5.3.2 | Field Sampling

In order to assess the impacts of increasing terrestrial sediment deposition in Okura, thirty-six benthic chamber incubations were conducted *in situ* along a grain size gradient in December 2018. This gradient serves as a space for time substitution which can help

demonstrate how the system will respond to increasing stress. The chamber methods used in this study are described elsewhere (e.g., Lohrer *et al.*, 2010; Gladstone-Gallagher *et al.*, 2016; Hillman *et al.*, 2020, Fig. 1.3B); briefly, half-sphere acrylic chambers were deployed on an incoming, midday tide incubating approximately 1L of water over the sediment surface for ~ 4 hours. The chambers used in this study were dark preventing photosynthesis. Water samples were withdrawn from the sealed chambers at the beginning and end of the incubation for dissolved gas and nutrient analysis. Dark bottles filled with site water were also incubated to account for any water column activity. Nutrient samples were filtered immediately upon collection with 0.2 µm, polycarbonate filters and stored in the dark until delivery to the laboratory where they were frozen to -20 °C until analysis. Dissolved gas samples were collected, air-free in syringes with stopcocks which were closed underwater immediately after collection. These syringes were kept in cool, dark coolers until returning to shore where they were immediately transferred to 12 ml gas tight, exetainer vials (Labco, UK) and fixed with 75 µL of a concentrated zinc chloride solution. Upon returning to the lab, exetainers were stored at 4 °C until analysis. Core samples for sediment grain size, organic matter, chlorophyll-a content, porewater nutrients, and macrofauna community composition were taken adjacent to each chamber. Two 2 cm diameter cores were taken to a depth of 2 cm for grain size, organic content, and porewater, three 1 cm diameter cores were taken to 1 cm depth for chlorophyll-a, and one 13 cm diameter core was taken for macrofauna to a depth of 15 cm at each incubation site. Sediment cores were stored in 50 mL centrifuge tubes (darkened with aluminium foil for chlorophyll-a samples), stored on ice, and frozen upon returning to the lab. Porewater samples were centrifuged and the resulting water was filtered to 0.2 µm and frozen until nutrient analysis. Macrofauna cores were sieved in the field to retain everything >500 µm. Content retained on the sieve was stored in 70 % isopropyl alcohol and stained with ~ 1 mL of a concentrated Rose Bengal solution. Macrofauna were

sorted and identified to the lowest practical level of taxonomic resolution. Macrofaunal traits and functions were then assigned based on an existing dataset that included a macrofaunal survey of over 400 macrofauna cores and 113 species from an nearby estuary in Northern New Zealand (Thrush *et al.*, 2017). Briefly, a species x trait matrix was used to identify the biological traits that were considered pertinent to ecosystem functions for each species (Thrush *et al.*, 2017). From these trait data, functions were assigned for relevant processes such as grazing or bioturbation (Siwicka, Thrush and Hewitt, 2020). We then applied these classifications to the species found in Okura during this study. An additional classification of rare species was defined which included species that occurred two or less times in the macrofaunal dataset derived from Okura.

5.3.3 | Laboratory Analysis

Dissolved gas samples were analysed for dissolved N_2, O_2, and Ar on a quadrupole membrane inlet mass spectrometer (MIMS) using the N_2/Ar method, precision for this analysis is <0.03 % (Kana *et al.*, 1994). Ar was also monitored independently for each sample to check for air bubbles or leaks. Ar concentrations did not change more than 5 % throughout the course of the incubation, indicating that there were no major bubbles or leaks in all but one chamber; data from the leaky chamber was not included in the analysis (Kana *personal communication*). Dissolved nutrient samples were analysed on a Latchet QuickChem 8500 Flow Injection Analyser (FIA, Hach, CO, USA) for NH_4^+, NO_x and DIP using colorimetric analysis (Grasshoff, Ehrnhardt and Kremling, 1983). Precision for this method is ~0.7 µM for all channels and detection limits are 1.53, 0.85, and 0.7 µM for NH_4^+, NO_x, and DIP, respectively. DON and DOP were also measured by digesting samples with persulfate and running again on the FIA (Valderrama, 1981). This method gives total N and phosphorus; DON was calculated by subtracting TN – DIN, and the same for P. Benthic flux rates were calculated for O_2, N_2, DIN, DIP, DON, and DOP using the change in concentration

(in µM) over time in the incubation, then normalized to chamber volume and surface area yielding fluxes in µmol m^{-2} h^{-1}. All of these are net fluxes across the sediment water interface, for N_2 a positive flux is net DNF while a negative flux is net N fixation.

Grain size samples were digested with 6 % hydrogen peroxide and run on a Malvern Mastersizer 3000 (Malvern Analytical, UK). Chlorophyll-a samples were freeze dried in the dark and homogenised. 1 g of dry sediment was then extracted in 3 mL of 90 % Acetone for 24 h, then run on a UV-vis spectrometer and Chlorophyll-a and pheo-pigment concentrations were calculated (Lorenzen, 1967; Wiltshire *et al.*, 1998). Organic matter was measured via loss on ignition in a furnace set to 450 °C for four hours. Macrofauna were sorted from remaining shell hash and organic debris and were then identified under a stereo microscope to the lowest possible taxonomic level. Shannon diversity index was calculated for each dataset using the "vegan" R package (Oksanen, 2019; Table 5.1).

5.3.4 | Statistical Analysis

Differences between mean values in each dataset were assessed using *t*-tests. Pearson correlations between all variables were calculated in R, version 4.0.3. Due to the number of correlations run, we do not give p-values for individual relationships, instead, we report the strength of relationships. Correlations with $0.6 \geq r$ considered strong relationships and those with $0.6 > r \geq 0.4$ considered weaker, though still important relationships. However, scatter plots also suggested non-linear relationships between mud and many of the various biogeochemical and ecological variables. We therefore used regression tree analysis to identify possible break-points or threshold responses within the system using the "rpart" package in R (Therneau *et al.*, 2015). Regression tree analysis explains variation in a single dependent variable by dividing the data into homogeneous groups, using the statistically best predictor variable for each split. These splits indicate significant changes in the relationship between the dependant and predictor variables (Thrush *et al.*, 2020). Many environmental

and macrofaunal community variables had splits between 100-150 µmol m^{-2} h^{-1} N_2, and when predicting N_2, the data split at 3.1 % mud. This split explained 30 % of the variance in the N_2 flux, more than a simple linear regression of mud content and N_2 flux which explained less than 9 % of the total variance. We therefore identified 3 % mud as a potential tipping point and divided the dataset into low mud (<3 % mud, n=18) and higher mud (>3 % mud, n=15) subsets.

Following regression tree analysis, quantile regressions were performed on each data subset, using mud content as the independent variable with the "quantreg" R package (Koenker, 2009). These quantile regressions at the 95th percentile indicate the upper edge of an envelope in which 95 % of the data can be expected to fall as the predictor variable changes (Cade and Noon, 2003). These quantile regressions can thus be used in factor ceiling analysis where the total variation and heterogeneity in the data is considered (Blackburn, Lawton and Perry, 1992). In this analysis, the quantile regression represents the upper bound or "ceiling" where changes in the predictor variable limit the possible range of the dependent variable. Changes in factor ceilings can then be used to indicate threshold responses within an ecosystem (Thrush, Hewitt and Lohrer, 2012). Here, 95th quantile regressions were computed for each dataset, then were plotted together on the same plot to show changes in the regressions (ceilings) between the low and higher mud data subsets (Fig. 5.1). These analyses were found to be the most appropriate and robust way to identify a tipping point in this system given the size of the dataset (Hewitt and Thrush, 2019).

The factor ceiling analyses convey important information about the ecological potential of a system, and may therefore be more sensitive to change in highly variable ecosystems such as estuaries (Cade and Noon, 2003). Sampling along a gradient allowed for space-for-time substitution in order for us to better asses how intertidal systems like those in Okura are responding to increasing sediment deposition. The combination of these two

techniques is particularly useful because ecosystem functioning is dependent on many factors and increased stressors along an environmental gradient are expected to induce constrained variation and reduced ecological potential (Thrush *et al.*, 2008). However, mean response and simple linear correlation are not designed to capture these kinds of responses.

Separate multiple linear regression (MLR) models were then computed to predict N_2 in each subset of data in order to better understand the drivers of DNF in the low vs higher mud datasets. The strongest predictors for each dataset were selected using a best subset analysis (ols_step_best_subset, R), and Mallows Cp as the primary selection criterion. These variables were then used to create the MLR models for predicting net N_2 flux in each dataset.

5.4 | Results

5.4.1 | Sediment composition

A grain size gradient was captured in this sampling, with mud content (proportion of particles <63 μm) ranging from 0 – 28.32 % (Table 5.1). Organic matter and chlorophyll-a content both increased with increasing mud; this increase was linear until ~10 % mud after which it began to level off, forming a non-linear relationship overall (Table 5.1, Fig. 5.1). Pheo-pigments were more variable, ranging from 0.5 – 9.3 μg g^{-1}, with no significant relationship with mud content detected. When split into the low and higher mud datasets, mud content and chlorophyll-a were both significantly greater in the higher mud dataset with averages of 11.7 %, and 1.5 % mud ($p = 1.62 \times 10^{-5}$) and 6.7 and 3.1 μg of chlorophyll-a per gram of sediment ($p = 2.20 \times 10^{-5}$), higher and low mud datasets respectively (Table 5.1).

5.4.2. | Dissolved oxygen and nutrients

Dark bottles incubated for water column activity did not reveal N_2, dissolved inorganic, or organic nutrient fluxes that were significantly different from zero.

Table 5.1 Key variables divided into the low and high mud subsets. The last two rows show average values for each variable in each dataset (listed as average ± standard error; n=18 for low mud and n=15 for high mud), bold text in this row denotes a significant difference between the average of the high and low mud datasets ($p<0.05$). Mud content, (% mud, proportion <63 um), chlorophyll-a (Chl-a), porewater NO_x (NO_x), porewater phosphorus (DIP), porewater ammonium (NH_4^+), net N_2 flux (N_2-N flux), sediment oxygen demand (SOD), NO_x Flux, and macrofaunal community variables are listed.

	% Mud	Chl-a (µg/g)	NO_x (µM)	DIP (µM)	NH_4^+ (µM)	N_2-N flux (µmol m^{-2} h^{-1})	SOD (µmol m^{-2} h^{-1})	NO_x flux (µmol m^{-2} h^{-1})	Individuals (per core)	Species (per core)	Bivalves (per core)	Polychaetes (per core)	Shannon Diversity
Low Mud	0.00	2.68	17.40	48.70	521.9	51.34	223.92	5.31	27	13	14	6	2.31
	0.00	2.01	20.88	107.97	1102.7	123.36	174.94	-4.59	35	12	18	8	2.27
	0.00	2.53	20.34	83.79	1220.2	91.77	251.24	0.56	20	8	17	3	1.63
	0.00	0.25	18.44	47.06	458.0	123.39	227.28	15.25	74	15	28	28	2.17
	0.01	3.29	13.31	59.42	408.9	189.59	18.05	-22.05	32	8	27	1	1.78
	0.02	3.36	19.47	57.37	377.4	221.33	381.11	-0.21	32	7	25	5	1.69
	1.47	3.11	11.29	78.21	737.7	129.50	156.23	3.45	33	8	26	4	1.65
	1.62	1.00	21.05	92.34	881.1	151.66	228.69	-3.90	32	7	25	5	1.37
	2.00	3.37	45.07	48.59	541.2	111.59	342.44	-11.24	53	8	19	0	1.31
	2.13	3.97	11.86	48.75	497.7	141.21	446.29	3.18	13	8	2	5	1.99
	2.15	2.98	19.61	46.47	425.2	313.24	564.19	-0.60	44	6	42	2	1.36
	2.26	2.88	6.06	54.93	734.2	150.33	383.48	1.54	52	10	25	25	1.87
	2.30	4.65	6.56	51.14	922.7	95.51	138.37	3.42	25	10	16	2	1.75
	2.36	3.51	7.40	17.46	228.4	256.66	558.43	-5.41	26	8	18	6	1.68
	2.46	2.20	2.54	21.44	327.5	93.88	287.14	-2.05	35	8	8	23	1.65
	2.64	4.80	5.85	117.64	727.6	132.95	280.36	-1.99	83	17	43	32	2.23
	2.64	2.74	36.44	42.95	466.5	95.06	210.63	8.01	39	9	20	1	1.71
	2.84	5.88	9.76	471.45	2853.3	146.09	394.80	-0.38	62	11	40	15	1.38

Table 5.1 Continued

	% Mud	Chl-a (µg/g)	NO_x (µM)	DIP (µM)	NH_4^+ (µM)	N_2-N flux (µmol m^{-2} h^{-1})	SOD (µmol m^{-2} h^{-1})	NO_x flux (µmol m^{-2} h^{-1})	Individuals (per core)	Species (per core)	Bivalves (per core)	Polychaetes (per core)	Shannon Diversity
High Mud	3.36	4.98	11.38	65.41	565.0	-51.74	126.95	-3.78	49	15	25	16	2.19
	3.89	4.75	11.19	44.06	552.6	164.30	397.27	4.21	0	0	0	0	1.71
	4.00	6.04	20.97	393.09	3025.2	142.04	195.29	31.89	49	11	35	7	2.16
	4.36	8.91	11.77	51.79	666.1	68.62	101.42	-2.26	55	16	29	16	2.22
	4.74	2.38	12.77	59.80	795.6	80.88	176.72	-0.40	63	15	43	9	1.34
	5.06	6.32	7.87	63.80	736.8	26.65	185.38	0.04	19	6	15	3	2.13
	7.70	7.32	2.64	21.88	424.0	44.70	5.70	-0.80	32	11	16	9	1.93
	9.11	8.14	4.79	51.18	647.0	69.99	206.32	11.07	48	12	33	6	1.95
	10.15	6.01	9.96	29.12	586.0	1.13	58.98	-0.16	21	9	9	10	1.83
	13.77	8.08	5.77	17.77	423.8	97.05	188.02	-2.28	41	12	24	10	2.18
	13.99	10.73	3.19	71.01	739.4	132.24	161.89	7.86	30	11	11	6	1.95
	20.57	0.48	22.54	45.25	1353.8	62.05	864.17	3.93	0	0	0	0	2.34
	23.30	9.06	13.09	24.14	783.7	101.71	672.42	-2.06	36	10	20	12	2.13
	23.43	7.88	8.37	18.80	426.5	50.51	303.38	-0.12	26	13	9	10	2.21
	28.32	9.14	7.35	20.99	630.7	38.65	980.13	-2.46	27	9	9	8	2.07
Average Low Mud	**1.5 ± 0.3**	**3.1 ± 0.3**	**16.3 ± 1.8**	83.0 ± 43	746 ± 139	**145.5 ± 15**	267.8 ±44	**-0.65 ±1.8**	**40 ± 4.4**	9.6 ± 0.7	23 ± 2.6	9.5 ± 2.4	1.77 ± 0.1
Average High Mud	**11.7 ± 2.2**	**6.7 ± 0.7**	**10.2 ± 1.5**	65.2 ± 24	823 ± 168	**68.6 ± 14**	266.6 ± 86	**2.97 ± 2.3**	**33.3 ± 3.2**	11.1 ± 3.2	17.7 ± 2.4	8.7 ± 1.0	2.02 ±0.1

However, there was some respiration averaging ~22 μmol O_2 m^{-2} h^{-1}, and the benthic chamber oxygen fluxes were adjusted accordingly. SOD varied from 5.7 – 980 μmol m^{-2} h^{-1} and was not significantly different between the low and higher mud datasets, averaging 268 and 267 μmol m^{-2} h^{-1} in the low and higher mud, respectively (Table 5.1). However, SOD did exhibit a strong, positive correlation with mud content higher mud dataset (r = 0.78) and a weaker, positive correlation with mud content in the low mud dataset (r = 0.59), as well as overall (r = 0.56).

Porewater nutrient concentrations were not significantly different between datasets, with the exception of porewater [NO_x] which was significantly greater in the low mud (16.3 μM) compared to the higher mud (10.2 μM) dataset (p = 0.007, Table 5.1). NH_4^+ was by far the dominant DIN species in the porewater with concentrations ranging from 228 – 3025 μM, compared to NO_x which ranged from 2.5 – 45 μM (Table 5.1). Porewater NO_x was strongly, negatively correlated with chlorophyll-a in the higher mud dataset (r = -0.66). Porewater[DIP] was more variable than NO_x, ranging from 17.5 – 471 μM, and was not significantly correlated with mud content, but was strongly, positively correlated with porewater [NH_4^+] (r = 0.92).

NO_x flux showed both uptake and release, ranging from -22.0 – 31.8 μmol m^{-2} h^{-1}, and was significantly greater in the higher mud dataset which had an average NO_x release of 2.97 μmol m^{-2} h^{-1}, on average, compared to the low mud dataset which had average NO_x uptake of -0.65 μmol m^{-2} h^{-1} (Table 5.1). NO_x flux was weakly, positively correlated with net N_2 flux (r = 0.52), and strongly, positively correlated with porewater [NH_4^+] (r = 0.87) and [DIP] (r = 0.90) in the higher mud dataset only. The 95th percentile regression for NO_x flux was also different between low and high mud datasets, with a steeper slope and greater range in low mud (Fig. 5.1). NH_4^+ flux was not significantly different between datasets and mostly exhibited release from the sediment, however, there were

some cases of NH_4^+ uptake. NH_4^+ flux ranged from -81.9 – 567 μmol m^{-2} h^{-1} and was positively correlated with pheo-pigments across both datasets (r = 0.56). DIP flux was not significantly different between the higher and low mud datasets and ranged from -140 – 136 μmol m^{-2} h^{-1}. DIP flux was strongly, positively correlated with mud content (r = 0.63) and with pheo-pigments (r = 0.71) in the higher mud sites only.

DON flux was highly variable, ranging from -412 – 584 μmol m^{-2} h^{-1}, and was not significantly related to mud content. DOP flux was also highly variable ranging from -799 – 94.2 μmol m^{-2} h^{-1}, but there was also significantly more DOP uptake in the higher mud sites with averages of -42.6 and -210 μmol m^{-2} h^{-1} in the low and higher mud datasets, respectively. DOP flux was weakly, positively correlated with SOD (r = 0.52) and strongly, negatively correlated with mud content (r = -0.69) and net N_2 flux (r = -0.76) in the low mud dataset.

5.4.3. | Macrofauna

The most abundant species were the surface-dwelling New Zealand cockle (*Austrovenus stutchburyi*), deposit feeding polychaete (*Scoloplos cylindrifer*), deep-dwelling bivalve (*Macomona liliana*), juvenile surface dwelling bivalve (*Nucula hartvigiana*), and another deposit feeding polychaete (*Prionospio aucklandica*; Table AIV.1). In the low mud sites there were greater total abundances of individuals, bivalves, rare species, polychaetes, surface to depth feeders, depth to surface feeders, deep dwelling organisms, tube worms, motile organisms, bioturbators, predators, and surface dwelling organisms (Table AIV.1 & AIV.2). The higher mud sites had greater total abundances of crabs, grazers, and organisms which create permanent borrows (Table AIV.1 & AIV.2). On average, there were significantly more individuals per core in the low mud sites (average of 40 per core compared to 33.3 in the higher mud sites, p = 0.043), but Shannon diversity was not significantly different (Table 5.1).

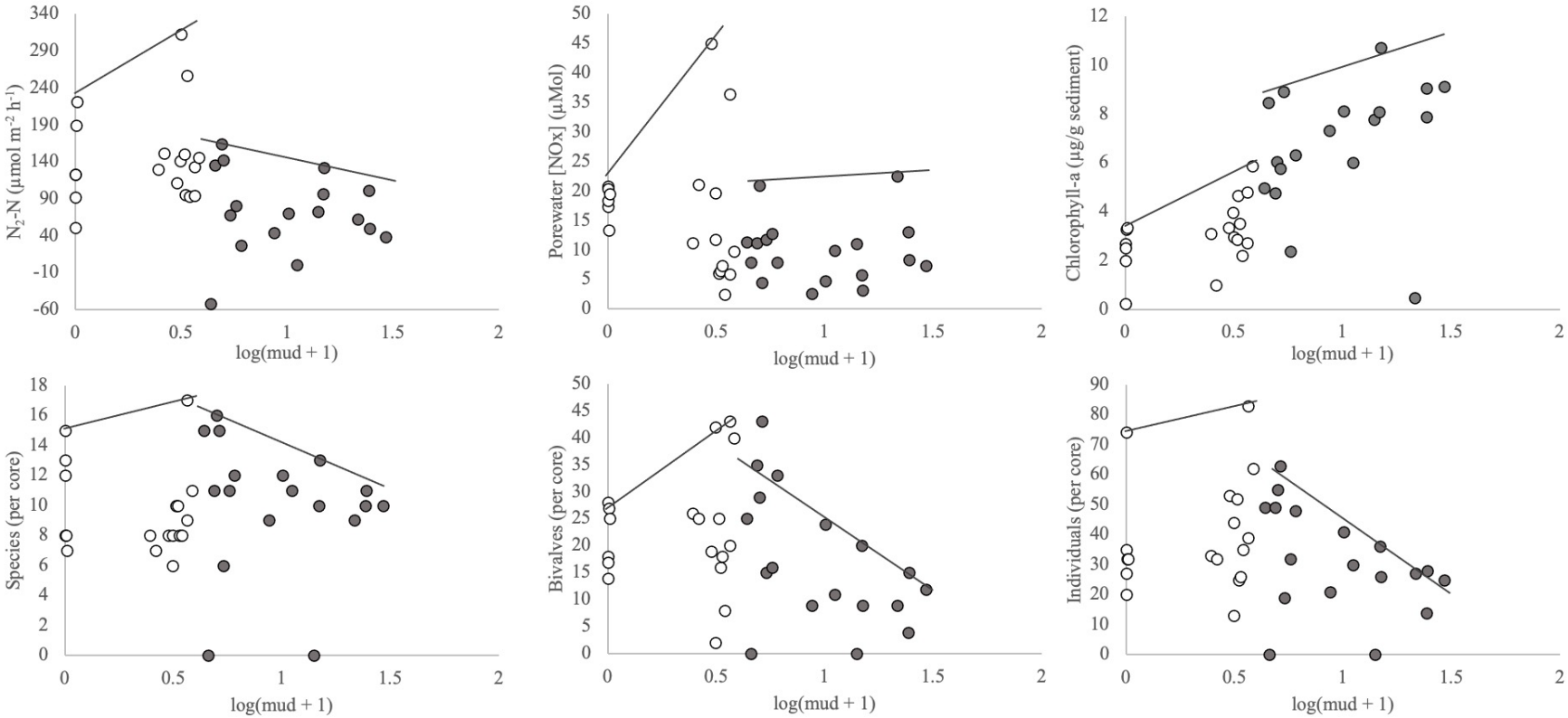

Figure 5.1 Quantile regressions of net N_2 flux, porewater [NO_x], chlorophyll-a, as well as the number of species, bivalves, and individuals per core with mud content (log transformed) as the predictor variable. X-axis is log(mud + 1), therefore the cut off of 3 % mud between the low and higher mud datasets is at 0.6. Open points are from the low mud (<3 %) sites and darkened points are from the higher mud (>3 %) sites. 95th quantile regressions are shown, and macrofauna cores are 1,990 cm ^{3}.

Additionally, bioturbating organisms made up a larger proportion of total abundance in the low mud sites and were dominated by *M. liliana;* in the high mud sites the bioturbator population was more diverse, but comprised a smaller proportion of individuals (Fig. 5.2). Across both datasets, the abundance of mud crabs was weakly, positively corelated with sediment mud content (r = 0.55). In the low mud sites, porewater [NO_x] was strongly, positively correlated to surface dwellers and epifauna (r = 0.79) and negatively correlated with deep dwelling animals (r = 0.61).

In the higher mud sites, mud content was weakly, negatively correlated with deep dwellers (r = -0.51), and NO_x flux was weakly, positively correlated with the number of individuals (r = 0.51), species (r = 0.59), and depth to surface feeders (r = 0.57), with stronger positive correlations to motile organisms (r = 0.62), and bioturbators (r = 0.60) per core. Conversely, NH_4^+ flux was strongly, negatively correlated with motile organisms (r = -0.64), and weakly, negatively correlated with bioturbators (r = -0.55). DON flux was weakly, negatively correlated with grazer abundance (r = -0.59), and porewater [NO_x] was strongly, positively correlated with the abundance of rare individuals (r = 0.62). Additionally, porewater [DIP] was strongly, positively correlated with the number of species (r = 0.62) and bioturbators (r = 0.60) per core, and weakly, positively correlated with polychaete (r = 0.56), deep dweller (r = 0.58), and motile organism (r = 0.57) abundance. Many macrofauna community variables, such as the number of individuals, species, and bivalves also exhibited different factor ceiling relationships, with 95^{th} quantile regressions that exhibit positive slopes with increasing mud and higher ceilings in the low mud sites, compared to the negative slopes and lower ceilings in the high mud sites (Fig. 5.1). These variables appear to have maxima at or near 3 % mud (Fig. 5.1).

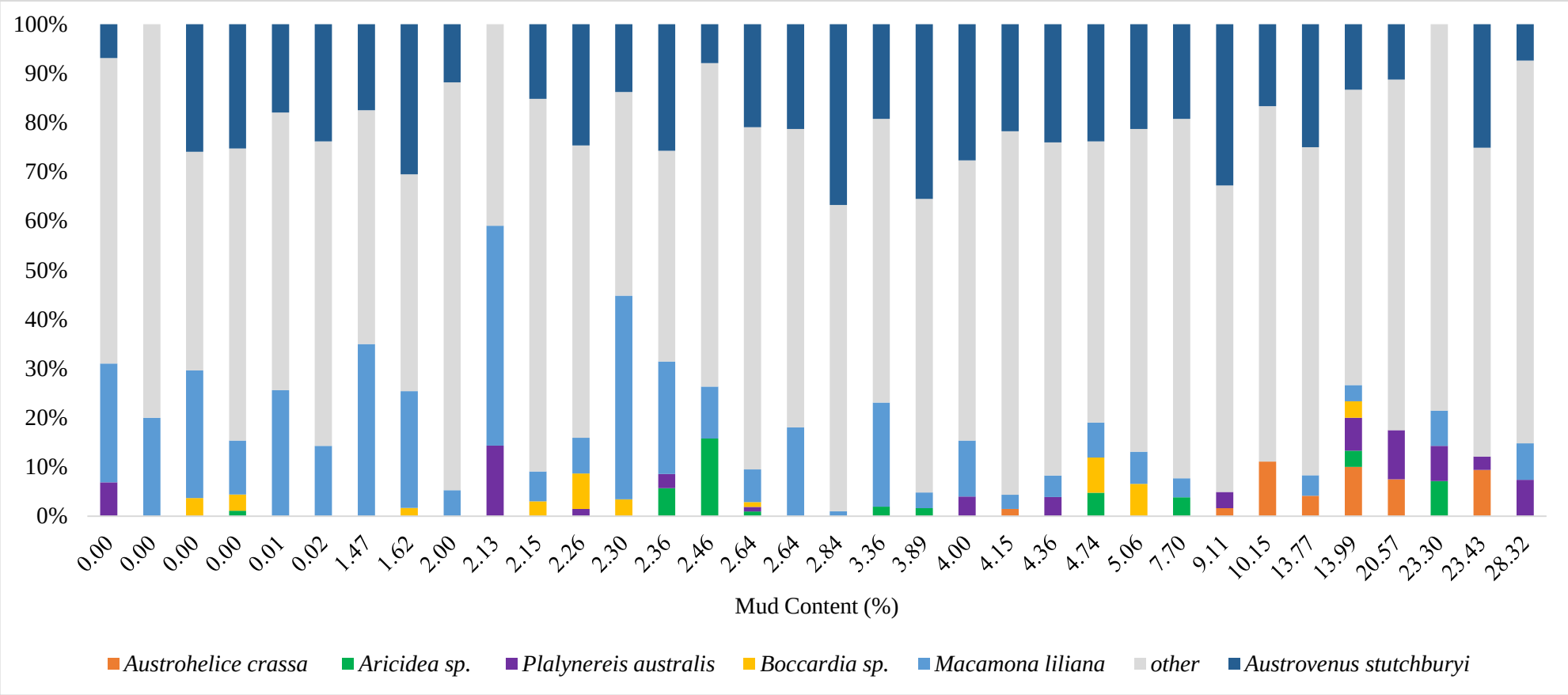

Figure 5.2 Proportion of dominant species per core at each site. Cores are 1,990 cm ^{3}, and x-axis is mud content (%). Colourful bars at the bottom show proportion of the dominant bioturbating species (*M. liliana, Boccardia sp., Plalynereis australis, Aricidea sp., and A. crasa*), the dark blue bars at the top show the proportion of the dominant suspension feeding bivalve *A. stutchburyi*, and grey in the middle show the proportion of all other species. Bioturbators generally comprise a larger proportion of the total than *A. stutchburyi* in the low mud sites (3 % mud), and *A. stutchburyi* generally makes up a larger proportion than bioturbators in the higher mud sites (<3 % mud). *M. liliana* dominate bivalve populations in the low mud sites, while the burrowing mud crab *A. crassa* and polychaetes (*P. australis, Aricidea sp., and Boccardia, sp*) contribute more to the bioturbator population in the higher mud sites.

5.4.5. | Net denitrification

All sites exhibited positive net N_2 fluxes (net DNF) except one of the higher mud sites (3.36 % mud) which had a negative flux, exhibiting net N fixation. Overall, net N_2 fluxes ranged from -51.7 – 313 μmol m^{-2} h^{-1} and were significantly greater in the low mud sites with averages of 146 and 68.6 μmol m^{-2} h^{-1} in the low and higher datasets, respectively ($p = 0.028$, Table 5.1). Net N_2 flux was strongly, positively corelated with SOD ($r = 0.67$) and strongly, negatively correlated with DOP ($r = 0.76$) in the low mud sites. In the higher mud sites, net N_2 flux was strongly, positively correlated with NOx flux ($r = 0.62$).

MLR models to predict net N_2 flux on the split dataset explain 77 % and 40.5 % of the low and higher mud data, respectively (Table 5.2). In the low mud sites, the best fit model included mud content, SOD, porewater [NO_x], and the number of species per core as predictor variables. NO_x flux, porewater [DIP] chlorophyll-a, and the number of individuals per core were used in the higher mud model. Additionally, porewater [NH_4^+] and bivalve abundance were key variables in both models (5.2).

5.5 | Discussion

Overall, variations in nutrient cycling and the macrofaunal community demonstrate how the intertidal environments of Okura Marine Reserve are responding to increasing terrestrial sediment deposition. Differences in Pearson correlations indicate differing ecosystem interactions and functioning between the low and higher mud datasets, and regression tree and factor ceiling analysis point to a tipping point in this system at 3 % mud.

Table 5.2 Multiple Linear Regression results using the best fit variables to predict N_2 flux in the low and higher mud datasets. Significance levels are as follows: ***<0.001, highly significant; ** < 0.01, highly significant; * <0.05, significant; • <0.1, moderately significant.; -- not significant. Adjusted R^2 and p-value for each model as a whole is listed below each table.

Low Mud			**Higher Mud**		
Variable	**p-value**	**Significance Level**	**Variable**	**p-value**	**Significance Level**
Mud Content	0.1161	--	Porewater $[NH_4^+]$	0.0627	•
SOD	0.0048	**	NO_x Flux	0.1578	--
Porewater $[NH_4^+]$	0.0339	*	Porewater $[PO_4^+]$	0.0948	•
Porewater $[NO_x]$	0.0826	•	Chlorophyll-a	0.0491	*
# Species/ core	0.0029	**	# Bivalves/ core	0.0138	*
# Bivalves/ core	0.0004	***	# Individuals/ core	0.0157	*
Adjusted R^2 = 0.773	p-value = **0.0005**		Adjusted R^2 = 0.4047	p-value = 0.0716	

Additionally, MLR models to predict net DNF suggest that the drivers of this important processes are different on either side of the 3 % mud tipping point. Results from this study also show that sites with more than 3 % mud have an altered macrofaunal community, and decreased capacity to remove nitrogen via DNF.

5.5.1 | Sediment Metabolism

The basic sediment and microalgal characteristics in this study behaved as expected, with Chlorophyll-a, pheo-pigment, and organic matter content all increasing non-linearly with increasing mud content (Table 5.1, Fig.5.1). This non-linear relationship is characteristic of intertidal MPB communities (Jesus *et al.*, 2009). It is also well established that while chlorophyll concentrations and MPB standing stocks tend to be greater in muddier sediments, this does not necessarily translate to increased rates of primary productivity and photosynthesis (MacIntyre, Geider and Miller, 1996). The observed correlation between porewater NO_x and chlorophyll-a does suggest increased MPB activity, as chlorophyll-a and mud content increase, especially since porewater samples were taken outside the incubation chambers in light conditions. However, the *in-situ* incubations in this study were done in dark conditions where this increasing MPB biomass did not appear to impact mean aerobic respiration rates in the chambers. While average SOD rates were not significantly different between the low and higher mud sites, the significant, positive relationship between SOD and mud as well as organic content in each dataset separately does suggest that the increased terrestrial sediment deposition did have an effect on the metabolism of the system, with more aerobic respiration with increasing mud content (Table 5.1). SOD is a measure of net respiration by the entire sediment community, including both microbes and infauna. Increased respiration seen here is likely a result of the increased organic matter in the surface sediments being aerobically decomposed. This finding is supported by the positive correlation between

NH_4^+ flux with pheo-pigments (degraded chlorophyll-a by-products) suggesting increased decomposition and remineralization to NH_4^+ with increased mud content and MPB biomass. Further, DIP flux was also positively corelated with mud and pheo-pigment content in the high mud dataset supporting the conclusion of enhanced decomposition and remineralization as mud content increased.

5.5.2 | Nutrient cycling along the mud gradient

NH_4^+ was by far the dominant DIN species as seen in both the porewater and DIN fluxes. This is typical for systems like Okura in Northeast New Zealand, where anthropogenic N inputs are quite low and the vast majority of available N is recycled into NH_4^+ (O'Meara *et al.*, 2020, Chapter 3). The only significant difference in mean porewater concentration between the low and higher mud sites was in the NO_x pool (Table 1). This difference in porewater [NO_x] is also revealed by factor ceiling analysis, as 95th quantile is greater in the low mud sites compared to the high mud sites (Fig. 5.1). The significantly higher concentration and ceiling of porewater [NO_x] in the low mud sites may be attributed to the increased oxygen penetration in permeable sediment, which could allow for increased rates of nitrification. Nitrification is a two-step N recycling processes which converts NH_4^+ to NO_3^- and is the first step in coupled nitrification-denitrification. As nitrification requires molecular oxygen to proceed, it is reasonable that there would be higher rates in more permeable sediments (Burgin and Hamilton, 2007).

NO_x dynamics in the estuary highlight the potential coupling of sediment deposition impacts and N processing as sediments become muddier. On average, there was net NO_x release (positive flux) from the high mud sites and net uptake (negative flux) by the low mud sediment (Table 5.1), which suggests more uptake and use of water column NO_x by the low mud sites and increased NO_x production in the high mud sites. However, the fluxes are quite variable and water column [NO_x] in Okura is very low

(average of 0.2 μM), therefore the water column is unlikely to be a large source. There is another potential source of NO_x to the high mud sites in particular. The vast majority of the sediment deposited in Okura consists of clay particles (Thrush *et al.*, 2004; Hewitt and Carter, 2020), and NO_3^- in terrestrial runoff can adsorb to clay particles (Mohsenipour, Shahid and Ebrahimi, 2015). This adsorption of NO_3^- is actually used as a technique to mitigate N runoff to heavily nutrified systems, since the NO_3^- is then bound and sequestered in terrestrial soils rather than running off into streams or the marine environment (Meghdadi, 2018). However, if that NO_3^- containing soil is eroded and ends up in a marine system, the salinity and redox conditions of the marine environment can release the bound NO_3^- (Abdulgawad, 2010). Sediment processing by macrofauna could accelerate this NO_3^- release. This could then be an added source of NO_3^-, potentially fuelling NO_x release in the higher mud sites. Additionally, there was a positive relationship between NO_x flux and porewater [NH_4^+] as well as porewater [DIP] in the high mud sites. Given that the macrofaunal community is known to influence the concentrations of porewater nutrients, this interaction suggests that the NO_x flux may also be impacted by the activities of the macrofaunal community.

DIP flux was also influenced by increasing mud content. As with NO_3^-, we suggest that the deposited mud itself could be a source of this increased DIP. The ultimate source of P to marine ecosystems is the physical and chemical weathering of terrestrial crust and soils (Kolowith and Berner, 2002), terrestrial soils therefore, tend to have higher concentrations of DIP both adsorbed to soil particles and within the soil porewaters. As a geologically young landmass, New Zealand soils, in particular, contain high concentrations of P (Gardner, 1990). If that soil is then deposited in the marine environment, its ability to retain DIP diminishes due to lower oxygen and higher salinity and sulphate concentrations (Caraco, Cole and Likens, 1989; Delaney, 1998), thus

increasing release of DIP from the sediment, particularly as it is processed by benthic infauna (Foster and Fulweiler, 2016). These phenomenon could explain the significant increases in DIP efflux from the sediment with increasing mud content in the higher mud sites.

In the low mud sites, DOP uptake by the sediment increased with increasing mud content. This result is indicative of the oligotrophic nature of the low mud sediments where DON and DOP are much more important components of the elemental cycles of N and P than in their more eutrophic counterparts (Fig 2.2, Chapter 3). DOP is very seldom measured in marine sediments, and its role in marine biogeochemistry remains unclear. Some terrestrial studies have shown P concentrations to impact rates of both nitrification and denitrification (Minami and Fukushi, 1983; Hall and Matson, 1999), and more recent work in estuaries in the North-eastern United States have found evidence of P limitation in denitrification (Amanda M. Vieillard and Fulweiler, 2012) and other N cycle processes (Foster and Fulweiler, 2014, 2016). However, it is more likely that these organic rich, eutrophic sediments are P limited, than the sandy, oligotrophic sediments in this study, which are generally N limited. Also, the fact that DIP was, on average, released from the low mud sites, while DOP was taken up suggests that this dynamic may be more tied to organic matter than to P. Unlike inorganic compounds, DON and DOP molecules include organic carbon, therefore we may be able to treat DOP flux as a proxy for organic carbon flux, which was not measured in this study. DOP has been suggested to be a useful indicator of organic matter turnover from both primary production and remineralization (van Beusekom and de Jonge, 2012). Heterotrophic processes in these low mud, low OM sediments may be limited by the supply of organic carbon, as well as N, which is reflected in the uptake of DOP. This carbon limitation is likely even stronger in the high mud sites, where increased nutrient availability through the release of bound N and P likely also

increase the demand for organic carbon. While DOP in the high mud sites was not correlated with mud content, there was significantly more DOP uptake in the high mud sites, signalling and increase in demand of DOP, and its associated organic carbon, by the sediment microbial community.

5.5.3 | Macrofaunal community

Many macrofaunal species have been found to be important mediators in estuarine N cycling (e.g., Lohrer, Thrush and Gibbs, 2004). In New Zealand intertidal sand flats, two functional groups have been found to be key to community architecture and biogeochemical cycling: Large, deep-dwelling, deposit feeding bivalves dominated by *M. liliana,* and large, motile, suspension-feeding bivalves at or near the surface, dominated by *A. stutchburyi* (Thrush, Hewitt and Lohrer, 2012; Thrush *et al.*, 2014; Pratt *et al.*, 2015). Large, deep-dwelling and bioturbating organisms such as *M. liliana* are surface to depth feeders which pump oxygen-rich water and organic matter down from the surface into the anoxic zone of the sediment, creating an oxic micro-zone around themselves. This is a physical bioturbating and bioadvective processes that has been shown to impact N cycling by stimulating processes such as coupled nitrification-denitrification, and by pressurizing porewaters, physically altering nutrient fluxes. Bioadvection by large bivalves like *M. liliana* has also been shown to be an important nutrient source to MPB (Woodin *et al.*, 2016). Bioturbating organisms, dominated by *M. liliana,* comprised a larger proportion of total macrofaunal abundance in the low mud sites, suggesting higher rates of bioturbation than in the higher mud sites (Fig. 5.2).

Highly motile, surface suspension feeders graze on suspended microalgae, including re-suspended MPB and are key to maintaining benthic-pelagic coupling. These organisms effectively transfer nutrients and organic matter from the water column to the sediment, which has been shown to impact various N cycle processes (e.g., Higgins *et al.*,

2013; Kellogg *et al.*, 2013). While high densities of these bivalves can reduce MPB biomass, they are largely thought to stimulate MPB growth via the excretion of dissolved nutrients (Sandwell, Pilditch and Lohrer, 2009). This stimulation of MPB can indirectly influence sediment DNF via completion with MPB (Rysgaard, Christensen and Nielsen, 1995). The dominant surface-dwelling, suspension-feeding bivalve in these systems is the New Zealand cockle, *A. stutchburyi*. While *A. stutchburyi* abundances were fairly uniform across all sites, they generally made up a larger proportion of the total abundance in the higher mud sites, suggesting that their activity plays a greater role than bioturbation as mud content increases (Fig. 5.2).

The low mud sites in this study had greater total abundances of nearly every macrofaunal category and functional trait group (Table AIV.1 & AIV.2), with only crabs and grazers increasing in abundance in the high mud sites (Table AIV.1 & AIV.2). This shift in the macrofaunal community structure and trait composition, shifts the functions provided by these organisms in low vs. higher mud sites. This shift is confirmed by factor ceiling analysis which shows that there is a peak in the ceiling of many macrofauna community variables, including the number of individuals, species, and bivalves per core, at ~ 3 % mud (Fig. 5.1). These findings indicate that the macrofauna community likely benefits from small increases in mud content which increase food stocks (as MPB), but do not change sediment permeability and suspended sediment concentrations enough to inhibit the dominant species. However, above 3 %, increasing mud becomes more detrimental than beneficial to the community. Similar community responses have been seen previously (Pratt *et al.*, 2014a), however, this inflection point has never been specifically identified, nor has it been related to directly measured denitrification rates.

Across both datasets, the increase in crabs, particularly the burrowing mud crab, *Austrohelice crassa*, and grazer species with increasing mud content was expected (Table

AIV.1 & AIV.2). *A. crassa* is an important bioturbating species, which builds large, permanent borrows in New Zealand estuaries. It prefers muddy sediments, with both abundance and burrow density shown to significantly increase with decreasing grain size (Needham *et al.*, 2010). Additionally, the increase in chlorophyll-a and organic matter in the muddier sites would explain the increase in grazer abundance in those sites as well. The pattern of more species and individuals in sandier compared to muddier sites is also typical of these systems (Thrush *et al.*, 2003; Pratt *et al.*, 2014).

In the low mud sites, there was a positive relationship between porewater [NO_x] and surface-dwelling organisms, and a negative relationship with deep dwelling organisms. These relationships are likely related to the high abundance of *A. stutchburyi.* These surface-dwelling bivalves were the most abundant species (Table AIV.1 & AIV.2). While bivalves in general are associated with the direct excretion of NH_4^+, some cockle species have been shown to excrete substantial amounts of NO_x as well, locally increasing porewater [NO_x] (Magni *et al.*, 2000). If *A. stutchburyi* share this characteristic, they could be responsible for the positive correlation between porewater [NO_x] and surface dwelling organisms, while also explaining the inverse relationship with deep dwelling organisms that may not directly produce NO_x. High densities of *A. stutchburyi* in surface sediments, then, may also contribute to the elevated mean porewater [NO_x] in the low mud sites.

In the high mud sites, increasing mud was related to a decrease in bioturbators and deep dwelling organisms. While there is overlap between these two categories, these relationships indicate a reduction in deep bioturbation and the porewater pumping that is often performed by deep, bioturbating organisms such as the bivalve *M. liliana* (Fig. 5.2). *M. liliana* is a tellinid bivalve common to New Zealand intertidal sediments which lives at depths 10 - 15 cm below the sediment surface (Thrush, Pridmore and Hewitt, 1994).

These deposit feeders create bioadvective flows though their feeding process which pull oxygen and particle-rich water down from the surface, oxygenating the sediment around the organism, pressurising the porewater, and increasing the surface area of the oxic-anoxic interface within the sediment (Nils Volkenborn *et al.*, 2012). These flows in turn impact redox conditions and nutrient cycling within the sediments. However, *M. liliana* is not mud tolerant, even millimetre thick terrestrial sediment deposits have been shown to significantly decrease settlement and burial of juvenile *M. liliana* (Cummings *et al.*, 2003; Hohaia, Vopel and Pilditch, 2014), and adult densities have been found to be strongly related to sediment grain size (Hewitt *et al.*, 1996), therefore their abundance was substantially decreased in the high mud sites (Fig. 5.2).

The macrofaunal community in the high mud sites also directly influenced nutrient cycling via the N and P fluxes. DON flux was negatively correlated with grazer abundance; as DON is primarily a decomposition product of MPB on the sediment surface, increased grazing activity would likely decrease the flux of DON from the sediment surface, especially in the dark. NO_x flux was positively correlated with bioturbators, and bioturbating functions such as motility and depth to surface feeding suggesting that bioturbation and bioadvection by the macrofauna community contributed the observed NO_x flux. The NO_x flux in the high mud sites was quite variable, with the largest release of NO_x associated with the most bioturbators and lower mud content (4 %), while the smallest bioturbator community was associated with NO_x uptake and higher mud content (23.3 %) suggesting that the bioadvection by large, deep dwelling bioturbators may be pressurising the porewater, helping to release NO_x to the water column.

Conversely to NO_x, NH_4^+ flux was negatively corelated with motile and bioturbating organisms found in Okura estuary. While estuarine sediments tend to be

sources of NH_4^+, especially in the dark, net NH_4^+ fluxes in the high mud sites were quite low, despite high porewater NH_4^+ concentrations. These low net fluxes suggest that either, the vast majority of porewater NH_4^+ is consumed and transformed within the sediment before it can be released, that there is as much or more uptake of water column NH_4^+ as release, or some combination of the two. The negative correlation with bioturbators suggests that the same macrofaunal processes that are releasing NO_x from the sediment are drawing NH_4^+ into it, perhaps fuelling nitrification and creating a surplus of NO_x. However, porewater NO_x concentrations were, on average, lower in the high mud sites, so if there is a surplus of NO_x created within the sediments, it is very quickly either taken up in other processes or fluxed out of the sediment. This negative relationship between NH_4^+ flux and bioturbating macrofauna is contrary to what has been previously seen in nearby systems (Lohrer, Thrush and Gibbs, 2004).

The NO_x and NH_4^+ dynamics in this system are indicative of relatively high rates of nitrification, which takes up NH_4^+ and produces NO_3^- in oxic sediments. Typically, this NO_3^- does not accumulate in the porewater or flux out of the system because it is rapidly denitrified in the coupled nitrification-denitrification pathway. While decreased oxygen penetration in muddier sediments has the potential to limit nitrification rates, the increased rate of NO_x production in the muddier sediments in this system points toward a limitation of DNF rather than nitrification. Because nitrification requires oxygen and denitrification is an anoxic process, the vast majority of coupled nitrification-denitrification is thought to occur along the oxic-anoxic interface. Bioturbating organisms have been shown to increase the area of this interface by drawing oxygen-rich water into anoxic zones of the sediment, creating redox oscillations, and therefore stimulating DNF (Pelegrí and Blackburn, 1995; Volkenborn *et al.*, 2012; Gilbert *et al.*, 2016). It is possible that the shift from more deep-dwelling bioturbators toward grazers and epifauna with increasing mud

content resulted in a decrease in the total area of the oxic-anoxic interface, therefore limiting the amount of NO_3^- supplied in the oxic zone that could be denitrified in the anoxic zone. This would then help account for the drawdown of NH_4^+ and efflux of NO_x in the higher mud sites.

Additionally, the production of NO_x combined with the release of NH_4^+, combined with the predictive power of porewater [NH_4^+] on net N_2, could also be explained by anammox contributing to N removal in these sites. As anammox uses NH_4^+ and NO_2^-, producing N_2 and some NO_3^- this is possible, however the combined nature of NO_x makes this difficult to determine. Anammox is generally not considered an important process in intertidal and shallow estuarine systems (Devol, 2015); however, it has been found to contribute to N removal in two sandy intertidal systems in the winter (Teixeira *et al.*, 2012; Fernandes *et al.*, 2016).

Porewater [DIP] was positively correlated with many macrofaunal community variables including the species richness as well as polychaete, deep dweller, motile, and bioturbator abundances. While there is certainly co-variance among these variables, these correlations indicate a clear relationship between the deep, bioturbator community and porewater [DIP]. Previous work in these systems has also found porewater DIP to be a strongly related to the macrofaunal community (Chapter 3); and as various macrofauna species have been shown to directly excrete DIP, this relationship suggests that the macrofaunal community exhibits a strong control on P dynamics within sediments (Welsh, 2003). Relatively few studies have investigated the impact of estuarine infauna on P cycling, especially in low-nutrient systems, however, bioturbating infauna are thought to physically mix particulate organic P away from surface sediments where remineralization rates are highest into deeper layers of the (Dale *et al.*, 2016). P dynamics are highly dependent on the redox conditions within the sediments, where oxidized metal compounds

can chemically bind P, which can be released to the porewater if conditions become reduced (Sundby *et al.*, 1992; Föllmi, 1996), therefore its location within the sediment is key to its reactivity. However, macrofaunal communities, particularly bioturbating macrofauna, have been shown to create redox oscillations within the sediment, which can expand microbial P uptake (Dale *et al.*, 2016) and may further contribute to their regulatory control on P (Volkenborn *et al.*, 2012a; Volkenborn *et al.*, 2012b).

5.5.4. | Increasing mud decreases net denitrification

While all but one of the chambers in this study exhibited net DNF, both DNF and N fixation have been found to be important processes which occur simultaneously within oligotrophic systems like the one in this study (Chapter 4). Therefore, while we are working with the net N_2 flux, we must acknowledge that both DNF and N fixation are contributing to these values. For example, decreases in net DNF may be a result of decreased DNF, increased N fixation, or some combination of the two.

In the low mud sites, the positive correlation between SOD and net DNF is indicative of coupled nitrification-denitrification (Risgaard-Petersen, 2003; Vieillard and Fulweiler, 2012). Though DNF itself is an anaerobic process, nitrification requires oxygen, and since nitrification is the primary source of NO_3^- to low-nutrient systems such as these (Rysgaard, Christensen and Nielsen, 1995; Crawshaw, Schallenberg and Savage, 2019), oxygen drawdown becomes correlated to DNF. Coupled nitrification-denitrification is typically the dominant DNF pathway in low-nutrient systems and has been found to dominate in other New Zealand estuaries (Gongol and Savage, 2016; Crawshaw, Schallenberg and Savage, 2019). The elevated porewater [NO_x] in the low mud sites relative to the higher mud sites, may also be an indication of increased nitrification, and the relationships between porewater [NO_x] and surface vs deep dwelling macrofauna suggest that infauna may have a mediating role in the supply of NO_3^- for

DNF. Net DNF was also negatively correlated with DOP flux, with greater DOP uptake corresponding to more net DNF (Fig. 5.3). While DNF has found to be P limited in some environments (Vieillard and Fulweiler, 2012; Foster and Fulweiler, 2014), as with the relationship between DOP and mud content, we hypothesize that this relationship is more likely about the associated organic carbon than the P itself. These low mud sediments also have a low organic matter content, which likely limits the rates of DNF, a heterotrophic process. Therefore, organic compounds (like DOP) taken up from the water column may be a key source of organic matter for heterotrophic microbial processes including DNF (van Beusekom and de Jonge, 2012). The organic carbon limitation of DNF is also supported by the factor ceiling analysis, which demonstrates that the DNF ceiling increases with increasing mud up to 3 %, suggesting that the corresponding increase in organic matter helps to stimulate DNF rates, without diminishing permeability and oxygen penetration, therefore limiting nitrification rates. Above 3 % mud, however, the detrimental impacts of decreased permeability likely outweigh any incremental increases in organic matter (Fig.1).

In the high mud sites, net N_2 flux was positively correlated with NO_x flux. This is an unexpected result; it is common, especially in more eutrophic systems, to see a negative relationship between N_2 and NO_3^- flux (Seitzinger and Nixon, 1985). This type of relationship is indicative of direct denitrification, where the NO_3^- for DNF comes directly from NO_3^- the water column, but this pathway requires much higher water column [NO_3^-] than we see in this study. The positive relationship between the net N_2 and NO_x flux is likely linked to the positive relationship between NO_x flux and bioturbating macrofauna, and could serve as evidence that rates of nitrification become somewhat uncoupled from DNF, leading to lower net DNF rates in the higher mud sites (Fig. 5.3).

MLR models to predict N_2 flux also demonstrated the difference in functionality between the low and higher mud sites. While porewater [NH_4^+] and bivalve abundance were common predictors in both models, the other selected predictor variables differed between the two datasets. SOD, as well as porewater [NO_x] and [NH_4^+] were selected as key predictors of net DNF in the low mud sites, highlighting the dominance of the coupled nitrification-denitrification pathway. Species richness and bivalve abundance were also selected as key components of the model, highlighting the importance of the bioturbation and bioadvection in stimulating net DNF. In the higher mud sites, the model included NO_x flux, porewater [DIP], and chlorophyll-a as predictors of N_2 flux, all of which can be linked to the shift from deep-dwelling bioturbators in the low mud sites to surface-dwelling grazers with increasing mud.

5.5.5. | The Tipping Point

The many lines of evidence used in this study all point to a threshold at 3 % mud which acts as a tipping point within this system. This tipping point is induced at a much lower mud content than previously observed (Lohrer *et al.*, 2010; Pratt *et al.*, 2014, 2015), demonstrating how even small changes in terrestrial sediment deposition can have important implications for the macrofaunal community composition, biogeochemical cycling, resilience to increasing nitrogen loads, and overall functionality of these important ecosystems. Below 3 % mud, small increases in sediment mud content do not appear to be detrimental and may even benefit overall ecosystem function. However, increasing the mud content above this threshold has clear impacts on this system. Macrofaunal abundances in nearly every category show that increasing mud limits abundances above the 3% threshold, including in the deep-dwelling and bioturbating species whose bioadvective processes are so key in stimulating DNF.

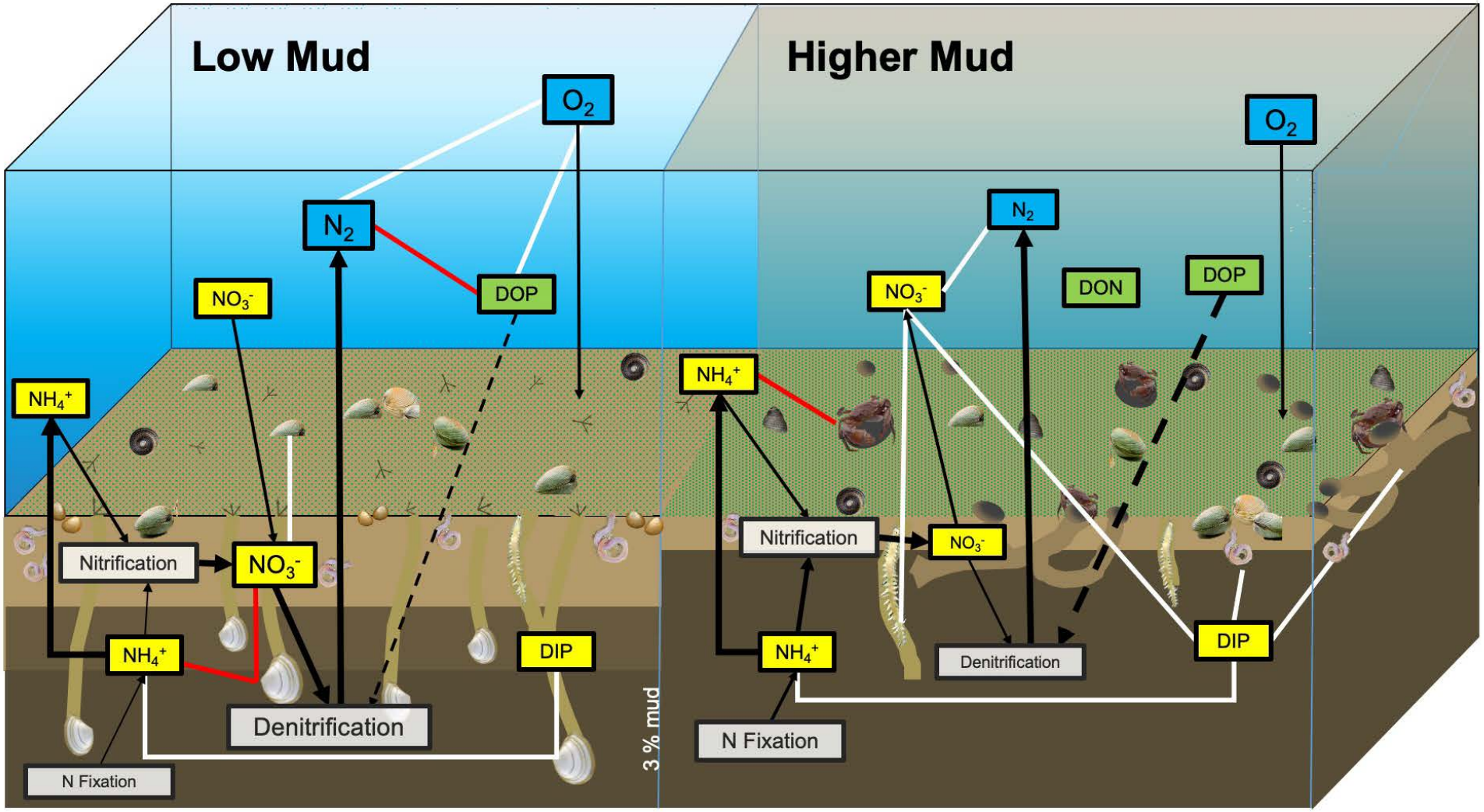

Figure 5.3 Conceptual diagram of the ecogeochemistry of Okura Marine Reserve on either side of the 3 % mud tipping point in the dark. Yellow boxes denote dissolved inorganic species, green are dissolved organic species, and blue are dissolved gasses. White boxes denote N cycle processes, and size of boxes is proportional to the size of the pool or rate of the process. Black arrows represent fluxes or nutrient flows, and white lines show strong correlations between various ecosystem components. Thickness of the line or arrow is proportional to the rate of the flux. Dominant macrofauna are represented. "Bird foot" patterns on the surface in the low mud sites indicate *M. liliana* below, and dark ovals on the surface in the higher mud sites represent *A. crassa* borrows. Colour gradation in the sediment boxes indicate oxygen concentrations with lighter colours indicative of higher oxygen.

The community shifts to one with fewer deep bioturbators and more surface-dwelling and grazer species. This shift, combined with the decreased permeability of muddy sediments, limits bioadvection and nutrient exchange to the deeper, anoxic sediments, reducing the rate of coupled nitrification-denitrification, and likely increasing rates of heterotrophic N fixation. This shift, therefore, also has consequences for the balance of N removal vs. N retention within the system, with less N removed and more reactive N recycling in the higher mud sites. These dynamics indicate that in addition to its well-established impacts on the benthic primary producer and infaunal community, increasing sediment deposition is limiting this system's ability to remove N. This effect has been increasingly documented in the non-eutrophic estuaries of New Zealand (O'Meara *et al.*, 2020; Schenone and Thrush, 2020, Chapter 3). As both sediment deposition and anthropogenic N pollution are expected to increase in this region of New Zealand, this limitation could have catastrophic consequences, leading to further and larger regime shifts in the future.

When considering tipping points, we tend to think of the more catastrophic examples that lead to extensive changes in ecosystem function and massive loss of ecosystem service delivery. The thought of tipping points brings to mind sudden fisheries collapse, complete kelp or seagrass die off, or massive macroalgal or jellyfish blooms (e.g., Möllmann and Diekmann, 2012; Selkoe *et al.*, 2015). These are the tipping points that make headlines, and while these shifts appear to be sudden, we have evidence that there are early warning signs (Scheffer *et al.*, 2009; Wang *et al.*, 2012; Dakos *et al.*, 2019). However, a tipping point refers to a change of state, and has no inherent measure of magnitude. Therefore, theoretically, when a system reaches such a massive tipping point, a series of smaller, less catastrophic tipping points have very likely been crossed, creating a cascade of tipping points, like knocking over larger and larger dominos (Fig. 6.1). While these smaller tipping points may not drastically change the functioning of a system

or its provisioning of ecosystem services, they are symptoms of slow, non-linear, yet relentlessly increasing stress.

The common trope in the ecology of tipping points is that in order to identify a threshold, you have to cross it. However, we may be able to better predict and prevent the big tipping points, if we can identify and explain the small ones. The situation in the Okura Marine Reserve is a prime example of this. Anecdotally and quantitatively we know that conditions in this system are deteriorating (Hewitt and Carter, 2020). However, it can still be classified as an oligotrophic system with water column [DIN] of 5.3 μM and [DIP] of 0.25 μM (Lemley *et al.*, 2015). Nonetheless, results from this study show dynamics such as decreased macrofaunal and bioturbator abundance, and increased NO_x flux which suggest Okura is already beginning to shift toward behaving like a more heavily impacted, eutrophic system. Additionally, the steadily increasing terrestrial sediment inputs are decreasing net DNF rates, reducing this system's capacity to mitigate New Zealand's ever-increasing N pollution (OECD, 2017). Identifying seemingly more minor tipping points in a system, such as the one identified in this study, can be an early warning sign and hopefully, help inform more effective management of these vulnerable systems.

Threshold responses like tipping points present particularly difficult challenges to managers because "they change the rules of the game" (Levin and Möllmann, 2015). However, evidence suggests that management practices that specifically address tipping points lead to improved outcomes (Kelly *et al.*, 2015). Due to hysteresis and runaway feedbacks associated with tipping points, it has also been suggested that proactive measures to preserve ecosystem resilience are likely more practical, effective, and affordable than attempts to stop or reverse a tipping point (Selkoe *et al.*, 2015). While advances in ecosystem-based management (EBM) theoretically equip managers with the

ability to deal with tipping points, particularly if integrative ecosystem assessments (IEA) are used (Levin and Möllmann, 2015), these constructs need to be underpinned by empirical data. Therefore, studies like this one are critical to establishing the underlying mechanisms and stressors that lead to tipping points (Levin and Möllmann, 2015; Selkoe *et al.*, 2015), so as to better inform future estuarine management and protection.

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Chapter 6

General Discussion & Conclusions

Amanda M Vieillard

6.1 | Synthesis

In this dissertation research, I investigated how the ecology and biogeochemistry of intertidal, soft sediment ecosystems are interlinked, and how they jointly contribute to the key ecosystem service of denitrification. I used a combination of *in situ* field studies, laboratory experimentation, and multi-variate modelling techniques to expand our understanding of ecogeochemistry in oligotrophic estuarine systems, which are understudied and underrepresented in the literature. Overall the results of this dissertation research highlight the key interactions between the macrofaunal ecology and biogeochemical processes within oligotrophic intertidal sediments, showing that they function differently than their more eutrophic counterparts. However, the pressure of increasing terrestrial sediment deposition is ultimately limiting the nitrogen removal capacity via denitrification in these sediments, and therefore impeding their ability to help mitigate ever-increasing nitrogen loads to New Zealand's coasts.

In Chapter 2, I quantified the underrepresentation of oligotrophic systems in marine biogeochemical research, showing that 83 % of sites in the most highly cited studies on nitrogen in estuaries were in North Atlantic systems, and a staggering 95 % were in eutrophic or hyper-eutrophic estuaries. I demonstrated that the limited work that has been done in oligotrophic systems suggests that they function differently from chronically eutrophic systems, supporting more diverse and productive food webs, and reflecting the condition of systems pre-eutrophication. I also emphasized the importance of increasing our understanding of low-nutrient systems, and their utility in creating benchmarks for the recovery of chronically eutrophic estuaries. The motivation for this chapter was born out of my own personal biases toward the eutrophic estuaries of the North Atlantic, and lead me to further investigate low-nutrient systems myself.

When I began this research in 2017, the rates and controls of denitrification in New Zealand's oligotrophic estuaries were not well constrained, with the first directly measured rates reported in 2016 (Gongol and Savage, 2016). I therefore conducted a field survey of *in situ* incubations to measure denitrification along with other biogeochemical and ecological parameters across an anthropogenic impact and grain size gradient in Chapter 3. The results of this chapter, including the ecosystem interaction network I created, highlighted the importance of the macrofaunal ecology in influencing net denitrification rates, revealing a novel relationship between species richness and net N_2 flux. I also found that net denitrification rates were lower in muddier sediments, sometimes flipping to net nitrogen fixation with increasing mud content, which is contrary to most literature findings (Rysgaard, Fossing and Jensen, 2001). My proposed explanation of this finding was a framework where increasing mud decreased sediment permeability and oxygen penetration, limiting rates of nitrification and therefore decreasing the coupled nitrification-denitrification pathway. I also hypothesized that nitrogen fixation could be an important source of nitrogen to these low-nutrient estuaries, as has been seen previously (Sundbäck, Miles and Göransson, 2000; Eyre and Ferguson, 2002b). However, while the method I was using to measure N_2 fluxes (N_2/Ar method) allows for the direct quantification of N_2 flux across the sediment-water interface without disturbing the sediment microbial or infaunal communities, it only yields net fluxes which make it impossible to directly discern the specific contribution of denitrification and nitrogen fixation independently.

Since I had hypothesized that nitrogen fixation was also an important process in these systems, I set up a laboratory experiment to simultaneously measure denitrification and nitrogen fixation in Chapter 4. In this experiment I modified an isotopic tracer method, using the consumption of labelled $^{30}N_2$ gas to quantify nitrogen fixation while

using the production of $^{28}N_2$ do quantify denitrification at the same time. Results from Chapter 4 show that nitrogen fixation, as well as denitrification, is an important process in these systems, with denitrification dominating in the dark and nitrogen fixation dominating in the light. Additionally, I conducted a methodological comparison with N_2/Ar and showed that the net of the denitrification and nitrogen fixation rates agreed well with the net N_2 flux measured by N_2/Ar. Through the development of this method, I was also able to address common concerns with measuring net N_2 fluxes and nitrogen fixation rates under light conditions.

Building on the findings of Chapters 2-4, I wanted to understand more about the impact of increasing terrestrial sediment deposition on estuarine function, and about the role of the macrofaunal community on regulating biogeochemical processes. It was clear that there was a network of interacting mechanisms and processes that were governing ecosystem function within these oligotrophic systems that went beyond simple cause and effect relationships (Fig. 3.3, 5.3), and that the structure of these networks was likely to break and change with increasing stress (Thrush, Hewitt and Lohrer, 2012; Thrush *et al.*, 2020). Therefore in Chapter 5, I conducted a series of *in situ* incubations along a grain size gradient in a marine reserve that is heavily impacted by increasing terrestrial sediment deposition. This space for time substitution allowed me to assess how the macrofaunal community and ecosystem functions were responding to this important anthropogenic stressor. As in Chapter 3, I saw decreased rates of net denitrification with increasing mud, as well as decreased macrofaunal abundance, both of which had been documented in other systems (e.g., Pratt *et al.*, 2014; O'Meara *et al.*, 2020). I also observed shifts in the macrofaunal community which corresponded to shifts in the biogeochemical processing of both nitrogen and phosphorus. The results from this study pointed toward more

"desirable" functionality in the low mud sites and more eutrophic-like characteristics in the higher mud sites, despite their continued oligotrophic status.

In Chapter 5, I also demonstrated how variability within a system can be a valuable tool to contribute to our understanding of ecosystem functionality, rather than pesky error bars on averaged data. Embracing variability and non-linear relationships, I identified a tipping point in this ecosystem at 3 % mud. Tipping points within the infaunal community in response to increasing mud have been proposed previously between 10-15 % mud (Thrush, Hewitt and Lohrer, 2012). However, this finding highlights the ability of even small increases in anthropogenic stress to degrade these ecosystems to a different functional state.

These small, incremental changes of state could be the result of a cascade of tipping points with smaller, less functionally drastic steps preceding larger, more catastrophic shifts (Fig. 6.1). The theoretical tipping points cascade (or domino effect) is widely used on a more global scale in climate change science (Kinzig *et al.*, 2006; Lenton and Williams, 2013; Klose *et al.*, 2020), and suggests that tipping points in one ecosystem or "tipping element" increase the likelihood of reaching a tipping point in a different but interconnected system (Klose *et al.*, 2020). The basis of this theory is that real-world tipping points and elements are not independent, and that there are complex interactions between them, therefore tipping point cascades can occur within ecosystem networks and between groups of networks (Watts, 2002; Parshani, Buldyrev and Havlin, 2010). The classic example of this is in the eutrophication of lake chains connected by small streams. Increasing nutrient inputs and subsequent eutrophication of one lake (once a tipping point is reached) leads to eutrophic conditions in connected lakes, even if increasing nutrient loads are not detected (Scheffer *et al.*, 1993; Carpenter and Lathrop, 2014; van Gerven *et al.*, 2017). Based on the results of my dissertation research as well as other work on

tipping points in the marine environment (e.g., Thrush and Dayton, 2010), I believe that this tipping points cascade theory can also be applied within one ecosystem. In this way, increasing stress leads to a series, or cascade, of tipping points within a system, each representing a measurable change in state and functionality, and increasing in scale and impact (Fig. 6.1). The larger and more catastrophic the tipping point, the greater the likelihood that it will impact a larger number of more interconnected components of that system, and ultimately influence tipping points in connected ecosystems (Lenton and Williams, 2013). This is particularly likely in highly connected ecosystems such as intertidal flats, which are linked to both terrestrial and marine systems. These smaller tipping points could then be important, real-world early warning signs of larger, more impactful tipping points to come, which would be incredibly valuable knowledge for ecosystem management and protection.

Understating these non-linear and interconnected processes and how they impact ecosystem function is key to the more wholistic study and management of important ecosystems such as estuaries. A more interdisciplinary approach, such as ecogeochemistry, is therefore needed to capture these complex ecosystem dynamics. Biogeochemical studies of nutrient cycling in marine systems too often disregard the role of the animal community, likewise, ecological studies often do not account for the microbial and chemical mechanisms underpinning ecosystem function. As a result, there is a gap in our collective understanding of how macrofaunal activities and biogeochemical processes interact to produce functions and ecosystem services, and how these processes and interactions respond to stress.

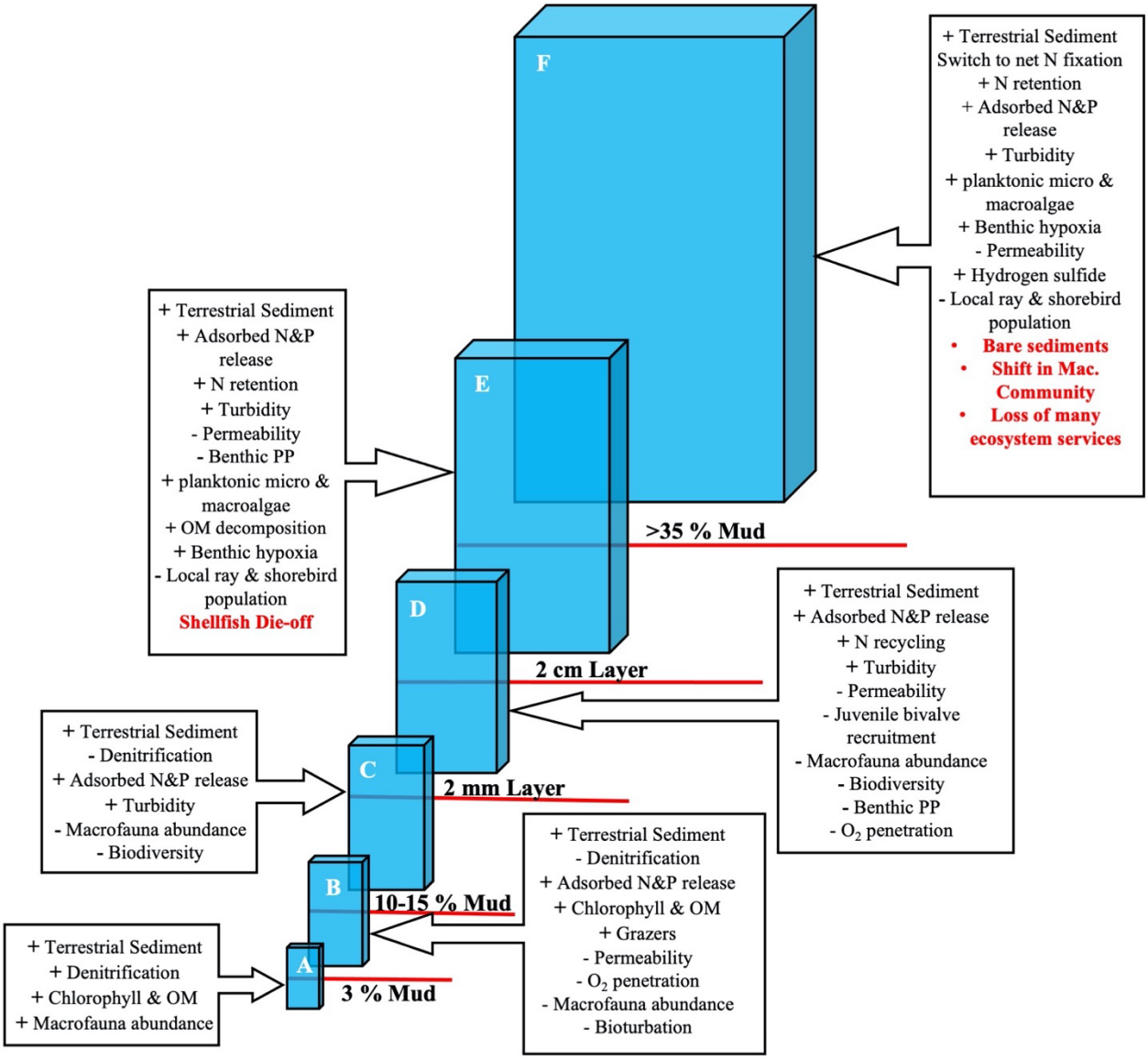

Figure 6.1 Conceptual diagram of the tipping point cascade. This example is the cascade of tipping points as a result of increasing terrestrial sediment deposition in the oligotrophic intertidal flats of Northern New Zealand, converting them from sand to mud flats. Each blue box represents a separate stable state within the ecosystem, the larger the box the further the system is from its unimpacted state and the more components of the ecosystem are affected. Terrestrial sediment deposition (measured as % <63 μm) is increasing from box A to F. Inside text boxes symbols indicate the increase in (+) or decrease in (-) a key ecosystem component or process and red text indicate more catastrophic conditions of that state. These changes in ecosystem function are synthesized from this dissertation work as well as key studies in these systems (full reference list in Appendix V). Red lines between the boxes denote the hypothesized tipping points. The hypothesized tipping points and resulting state changes are also informed by in situ studies of these environments including this work and others. References as follows: A-B (Chapter 5), B-C (Thrush, Hewitt and Lohrer, 2012), C-D (Cummings & Thrush, 2004; Hohaia, Vopel and Pilditch, 2014), D-E (Norkko *et al.*, 2002; Thrush, Hewitt and Norkko, 2003), E-F (Chapter 3 & Vieillard *anecdotal observation*).

My goal in this dissertation research was to begin to bridge this gap, and elucidate the interconnectivity of the biogeochemical and ecological processes within estuarine sediments, showing that key interactions among and between these ecosystem components together drive the delivery of vital ecosystem services like denitrification.

6.2 | Generalities and methodological constraints

While this research made significant in-roads in constraining the controls on denitrification in oligotrophic sediments, and in understanding the linkages between ecology and biogeochemical nitrogen cycling, it does of course, have its limitations. This research was limited to three estuaries, with Chapters 4 and 5 only encompassing one estuary each. These estuaries do appear to be representative of the region, however, I cannot assume that the findings from these systems will translate everywhere. This is particularly true for other oligotrophic systems outside of New Zealand, since the combination of geology, topography, and climate found in Northern New Zealand is not as common world-wide. This therefore highlights the importance of further study in these underrepresented systems, around the globe. This issue also touches on the spatial heterogeneity of ecosystems at various scales, and whether *in situ* measurements, that are technical and time consuming, are scalable. While I was not expressly interested in scaling up these processes, I did endeavour to capture as much of the spatial heterogeneity of intertidal soft sediments as possible. I used as much replication as was tractable, working along gradients, and was therefore able to describe the natural variability in and interconnected networks of interactions within these systems.

This is particularly true under light conditions where I could only predict 17 % of the variation in net N_2 flux at best. Contributing to this result are methodological limitations in the light that mean the light fluxes measured likely do not match the true flux happening in the environment (Chapter 3). There is therefore further refinement of

the application of some of these methods (e.g., calculation of benthic fluxes) which were designed to be used in the dark, but are now often employed under light conditions as well. The light sensitivity of photosynthetic processes can lead to non-linear fluxes; therefore the linear assumption of benthic flux calculations may often not be a good fit for light conditions. However, I did help to constrain one of these issues (i.e. nitrogen fixation in the light) in Chapter 4. I also think that the patchiness of intertidal sediments contributes to this challenge (Gladstone-Gallagher *et al.*, 2019). For example, taking macrofauna cores outside of the benthic incubation chambers gives a good estimate of the local community, but will not yield the exact community composition inside the chamber. This is likely exacerbated by the importance of relatively large bioturbators like *M. liliana* and the fact that these animals are not generally found at high densities at my study sites. The direct impact of each macrofauna individual (including its burrows, tubes, or bioadvection) will not be exactly reflected in the fluxes measured over a different patch. However, studies have shown that spatial heterogeneity and macrofaunal distribution patterns exist over centimetre to hundreds of metre scales (Thrush, 1991), and that small scale spatial variance is likely not the only important factor in the functioning of these systems (Thrush, 1991; Azhar *et al.*, 2020; Schenone and Thrush, 2020). The advantage of benthic chamber methods is that they can be used to explain functions across a wide range of environments and environmental conditions, which was what was needed for this work.

Finally, these studies are also limited in temporal scale, serving as snapshots of ecosystem function. Long-term monitoring data have been proposed to be crucial in managing for non-linear ecosystem responses like tipping points (Selkoe *et al.*, 2015; Hewitt and Thrush, 2019). Promising early warning signs of tipping points have been shown to include changes in variation or autocorrelation of biomass, densities, and rate of

recovery (e.g., Scheffer *et al.*, 2009; Wang *et al.*, 2012; Dakos *et al.*, 2019), however, these early warnings remain largely theoretical. In real-world systems, time series, or space for time substitutions, are often required to detect these shifts. It is therefore up to individual countries or regions to "invest in good data" by supporting long-term monitoring in order to help manage for impending tipping points (Selkoe *et al.*, 2015). Space for time substitution and snapshot studies like those in this dissertation can, however, help identify systems that may be particularly vulnerable and worthy of further study.

6.3 | Future Directions and Applications

This work offers many opportunities for future research, and I have outlined a few that I find most compelling here.

Much of my dissertation relied on the net fluxes of solutes across the sediment-water interface, which offer a clear and much needed "big picture" understanding of what is ultimately being taken up and released by physical, chemical, microbial, and macrofaunal processes within the sediment. However, this approach can make specific mechanistic determination difficult. I therefore think incorporating more stable isotope tracers could clarify some key processes and interactions in these systems. A criticism of isotope biogeochemistry is that it can make the focus too narrow and on too small a scale, however, I believe that when used appropriately it can bring great clarity. For example, isotopic tracers would allow us to say more definitively how individual nitrogen cycle processes like nitrification, denitrification, nitrogen fixation, and anammox contribute to net fluxes, and therefore more accurately assess their relationship to the macrofaunal community. In Chapters 3 and 5, I identified some characteristics of the N biogeochemistry, such as the relationship between porewater ammonium concentration

and net N_2 flux, that suggest that anammox could, potentially, be a component of N removal in these systems. Even though anammox is generally not considered important in shallow, estuarine sediments (Devol, 2015), it has recently been shown to play a significant role in N removal in, particularly in sandy, intertidal flats with low organic matter supply (Fernandes *et al.*, 2016). It would therefore be interesting to investigate whether anammox is an important process in the oligotrophic sediments of New Zealand, especially given its capacity, like denitrification, to mitigate anthropogenic nutrient pollution. In my opinion this would strengthen our understanding of ecogeochemistry in these systems and therefore contribute further knowledge of how they are changing and responding to anthropogenic stress.

My dissertation work also revealed some very interesting relationships between the macrofaunal community and phosphorus, as well as novel links between the phosphorus and nitrogen cycles. I found many macrofaunal variables to be very highly correlated with porewater inorganic phosphate concentrations in both chapters 3 and 5, suggesting that macrofauna exhibit regulatory control over the release of dissolved inorganic phosphorus in these environments. Many species are known to directly excrete phosphate (Magni *et al.*, 2000; Welsh, 2003), and it has been proposed that the sediment processing carried out by infaunal organisms could change redox conditions, and therefore the adsorption or release of P (Dale *et al.*, 2016). However, very little work has been done on the specific relationships between macrofaunal communities and estuarine phosphorus cycling (Tuominen *et al.*, 1999; Karlson *et al.*, 2005). Even less work has been done on dissolved organic phosphorus, particularly in coastal ecosystems (van der Zee, Roevros and Chou, 2007). However, in Chapter 5 I found a strong negative correlation between DOP flux and net denitrification which is, to my knowledge, a novel finding. I therefore suggest

that future work could focus on the role of phosphorus on coastal N cycling and ecogeochemistry, and elucidate the specific role of DOP in the oligotrophic N cycle.

Some of the interactions among benthic N cycle bacteria, MPB, and macrofauna could be further elucidated by measuring and incorporating meiofauna into these networks as well. Ranging from 63-500 μm, meiofauna are intermediate in size and scale to the bacterial/microalgal communities and the macrofaunal communities. Previous work has found meiofauna to link these populations and to modify interactions between various macrofaunal species (Piot *et al.,* 2013). Additionally, meiofauna have been shown to directly impact denitrification rates in Baltic sediments (Bonaglia *et al*., 2014). However the specific mechanisms underlying meiofaunal impacts remain understudied and poorly understood.

Overall this work supports the idea of relationships between biodiversity and ecosystem function (Srivastava and Vellend, 2005), and by extension the connection between biodiversity and ecosystem services (Barbier *et al.*, 2008, 2011; Daily *et al.*, 2009; Siwicka and Thrush, 2020). As a result, I think there is an opportunity to expand this work to other ecosystem services and into the management sector. EBM is becoming the standard for the management of marine systems, but many EBM strategies still ignore some of the more complex non-linear ecosystem dynamics such a tipping points (Levin and Möllmann, 2015; Selkoe *et al.*, 2015). Those that do account for tipping points have been shown to be more successful overall (Kelly *et al.*, 2015). However, these kinds of strategies need to be underpinned by empirical data to be effective. I therefore believe that while the kind of integrated, intersectional research conducted in this dissertation can be challenging to both conduct and interpret, it is the way forward for better management and protection of our vital costal ecosystems and the services they provide.

I therefore think the idea of a tipping points cascade is worthy of further study. I have shown in this work that small, subtle tipping points can occur within a system as a result of even very small increases in an anthropogenic stressor. I propose that there are a series of tipping points of increasing intensity that likely occur before a seemingly sudden, catastrophic regime shift (Fig. 6.1). This tipping points cascade has wide reaching implications for EBM. While there are many promising ideas for early warning signs of tipping points, they remain largely theoretical (e.g., Scheffer *et al.*, 2009). However, smaller, earlier tipping points as a response to increasing stress (like the one identified in chapter 5), can be empirically measured, and could therefore potentially be used as the canaries in the coal mine, indicating that further damaging regime shifts are on the way. If some of these tipping points could be identified as a result of common stressors, they could inform tiered management strategies such as IEA and EBM (Kelly *et al.*, 2015; Selkoe *et al.*, 2015; Dakos *et al.*, 2019; Hewitt and Thrush, 2019). Ecogeochemistry is therefore also important for EBM, as it hinges on ecosystem functions. In coastal sediments, these functions are directly linked to estuarine dynamics, risk assessment (i.e. tipping points), and ecosystem services, all of which are, ideally, optimized by EBM.

I also think that there is an additional need to use ecogeochemistry to further understand the interconnected nature of systems that have crossed tipping points and that we are now trying to restore. Eutrophication mitigation efforts in chronically eutrophic systems have shown very mixed results (Boesch, 2019; Le Moal *et al.*, 2019). Focusing on, particularly the non-linear relationships between the faunal and biogeochemical processes will help elucidate why turning back the nutrient input “dial” on its own is generally not sufficient to mitigate decades of eutrophication. These efforts could be further supported by looking to modern oligotrophic and low-nutrient systems as benchmarks for recovery (Chapter 2). With such high rates of background change due to

various human activities and global climate change, historical restoration benchmarks may no longer be relevant or attainable. While we may not be able to return to "Neverland" (Duarte *et al.*, 2009), we may be able to identify a new normal condition which accounts for all aspects of ecosystem function and allows for the revival of chronically polluted ecosystems, including their associated ecosystem services.

Finally, I think this work could be expanded into other understudied and underrepresented ecosystems. For example, polar systems are disproportionately affected by global climate change, are changing at an unprecedented rate (IPCC 2014), and are therefore in need of further interdisciplinary research. Similarly, tropical systems are also remain understudied. While I touched on the importance of tropical estuaries in Chapter 2, the bulk of this dissertation work was done in sub-tropical ecosystems, bordering on temperate (36 °S). Only 7.8 % of study sites in the most highly cited estuarine N papers were in the tropics, and none were located in polar regions (Chapter 2), but like low-nutrient estuaries, both tropical and polar systems function differently than their more widely studied, temperate counterparts (e.g., Falardeau and Bennett 2019; Oczkowski *et al.*, 2020). Additionally, about one third of the human population resides in low-elevation coastal zones, 75 % of which are in tropical, developing nations where they are particularly dependent on marine fisheries and local ecosystem services (Neumann *et al.*, 2015). Further, polar Indigenous communities are especially reliant on marine resources for survival and have deep, cultural ties to the sea (Larsen and Fondahl 2015). These are human communities that will be the most heavily impacted by climate change. Therefore, the further understanding and better management of underrepresented systems is not only a scientific issue, but also a social justice one. Expanding our knowledge of the ecogeochemistry of these systems could help preserve the vital ecosystem services they

provide, and inform more effective management of all estuarine ecosystems, not just those in wealthy, developed communities.

6.4 | Concluding Remarks

My dissertation research has shown that the oligotrophic, soft sediment ecosystems of northern New Zealand function differently than their more eutrophic counterparts, and therefore exhibit dynamics and relationships that are unexpected based on the biased literature base. I have identified a variety of controls on the key ecosystem service of denitrification that are driven by macrofaunal community composition and how they are impacted by anthropogenic stress. I have also confirmed nitrogen fixation to be an important N source to these low-nutrient systems, particularly in the light, and have shown that as little as 3 % mud can cause a shift in the functional state of these ecosystems. Additionally, I have demonstrated that increasing terrestrial sediment deposition is decreasing net denitrification rates in these systems via a suite of ecogeochemical mechanisms. This anthropogenic stressor is therefore decreasing the nitrogen removal capacity of these important systems, reducing their resilience against nutrient loading. With the rate of anthropogenic N runoff to New Zealand coastal systems steadily increasing, it is my hope that this work might help contribute to further research in and better, more wholistic management of these crucial ecosystems. I hope that we can learn from past experiences and keep eutrophication from becoming an inevitability in New Zealand.

Appendix I

Supplemental Materials for Chapter 2

Figure 2.1 Methods

Figure 2.1 was produced by first conducting a Web of Science search on March 13, 2019 with the search terms "estuary + nitrogen." Results were sorted by "times cited" and the top 45 most cited, primary research articles published from 1990 to 2019 were selected. Reviews and meta-analyses were excluded, as were laboratory-based manipulations and studies where nitrogen cycling within an estuary was not one of the primary focuses of the study. Each of the 45 studies was then mined for the GPS coordinates and nitrogen load of each sample site. GPS coordinates were approximated using given figures and Google Earth if they were not explicitly listed. If the nitrogen load to a given estuary was not listed, every effort was made to find it either from other studies or published reports. All loads were then converted into g N m^{-2} y^{-1}, with the square meters referring to the surface area of the estuary.

The final list included 140 individual study sites from 45 studies. These sites were then binned by estuarine system; for example, sites in the Potomac River estuary and the York River estuary were binned into the Chesapeake Bay system. An average of available N load data for all the sites within a system was calculated to yield an average N load for that system.

Each bin was then plotted on a world map using ArcMap 10.5.1 (ArcGIS Desktop: Release 10, ESRI, Redlands, CA). Points were color-coded for N load with lower loads in green tones and higher loads in reds; grey points were used for bins for which an N load had not been reported. Rings around each point denote the number of study sites included

in each bin. The finished map shows a clear bias toward the Northern hemisphere, particularly the North Atlantic, and for sites with high N loads.

Table AI.2 Study sites used in Figure 2.1 including GPS location, annual nitrogen load in g N m^{-2} y^{-1}, and bin to which each site was assigned.

Location	Lat	Long	N Load	Bin
Cairns, QLD, Australia	-16.9011	145.7817	ND	Australia
Townsville, QLD, Australia	-19.2686	146.8376	ND	Australia
Bornholm basin	54.85887	17.56962	45.90	Baltic
Gulf of Riga	57.79027	23.82594	6.29	Baltic
Gulf of Finland	60.30069	26.3651	4.20	Baltic
Gulf of Finland	60.34563	27.97186	4.20	Baltic
Gulf of Finland	59.50001	27.11551	4.20	Baltic
Gulf of Finland	60.00174	24.72914	4.20	Baltic
Baltic Sea	59.19092	18.57181	2.97	Baltic
Baltic Sea	58.75402	19.24597	2.97	Baltic
Itaipu Lagoon, Brazil	-22.9607	-43.0421	ND	Brazil
Piratininga Lagoon, Brazil	-22.9476	-43.0748	ND	Brazil
Elkhorn Slough, CA	36.80562	-121.79	0.33	CA
Elkhorn Slough, CA, USA	36.80562	-121.79	0.33	CA
Tijuana Estuary, CA, USA	32.55364	-117.128	ND	CA
San Dieguito Lagoon, CA, USA	32.97028	-117.262	ND	CA
Tomales Bay, CA, USA	38.5	-122.5	ND	CA
Choptank River Estuary, MD, USA	38.65859	-76.2693	90.00	Chesapeake
Nanticoke River Estuary, MD, USA	38.21007	-76.0082	90.00	Chesapeake
Susquehanna River Estuary, MD, USA	39.51734	-76.5117	85.00	Chesapeake
Patapsco River Estuary, MD, USA	39.34199	-76.3268	50.00	Chesapeake
Chester River Estuary, MD, USA	39.08408	-76.1635	30.00	Chesapeake
Delaware Bay, Lews, DE, USA	39.11576	75.25197	29.40	Chesapeake
Potomac River Estuary, MD, USA	38.05139	76.44632	29.32	Chesapeake
Chesapeake Bay, VA, USA	36.9962	-76.176	29.30	Chesapeake
Potomac River Estuary, MD, USA	38.01509	-76.3637	28.00	Chesapeake
Delaware Bay, Lews, DE, USA	38.78466	-75.1376	26.00	Chesapeake
Chesapeake Bay, VA, USA	37.24642	-76.1469	21.00	Chesapeake
Chesapeake Bay, MD, USA	38.10993	-76.2279	20.54	Chesapeake
Chesapeake Bay, MD, USA	37.99303	-76.2658	20.54	Chesapeake
Chesapeake Bay, MD, USA	38.46667	-76.3993	20.54	Chesapeake
Chesapeake Bay, MD, USA	37.63979	-76.1112	20.00	Chesapeake
James River Estuary, VA, USA	36.97669	-76.3405	20.00	Chesapeake
York River Estuary, VA, USA	37.23689	-76.406	20.00	Chesapeake
Rappahannock River Estuary, VA, USA	37.59255	-76.3406	20.00	Chesapeake
Patuxent River Estuary, MD, USA	38.31577	-76.4176	20.00	Chesapeake
Chesapeake Bay, MD, USA	37.91637	76.15571	21.00	Chesapeake
Pensacola Bay, FL	30.41306	-87.1318	14.00	FL
Nick's Hole, Apalachicola Bay, FL, USA	29.84728	-84.6677	10.00	FL
Yent's Bayou, Apalachicola Bay, FL, USA	29.79303	-84.8729	10.00	FL

Table AI.3 Continued

Location	Lat	Long	N Load	Bin
Rookery Bay, FL	26.02815	-81.7447	0.00	FL
Loire Estuary, France	47.27352	2.178369	68.00	France
Gironde Estuary, France	45.44758	0.417714	65.00	France
Sapelo Island, GA	31.50016	-81.2654	1.60	GA
Mississippi River Delta, LA, USA	29.07503	-90.0032	100.00	Gulf of Mexico
Breton Sound, LA, USA	29.60648	-89.5298	100.00	Gulf of Mexico
Mississippi River Delta, LA, USA	29.15454	-89.4762	100.00	Gulf of Mexico
Ochlockonee Bay, FL, USA	29.9687	-84.4022	83.93	Gulf of Mexico
Tampa Bay, FL, USA	27.75444	-82.5479	62.40	Gulf of Mexico
Apalachicola Bay, FL	29.66892	-85.0031	50.00	Gulf of Mexico
Corpus Christi Bay, TX, USA	27.78872	-97.2935	40.00	Gulf of Mexico
Upper Laguna Madre, TX, USA	26.71147	-97.4332	26.00	Gulf of Mexico
Guadalupe Estuary, LA, USA	28.45598	96.78996	25.96	Gulf of Mexico
Nueces River Mouth, TX, USA	27.83766	-97.4747	18.00	Gulf of Mexico
Baffin Bay, TX, USA	27.27279	-97.468	17.75	Gulf of Mexico
Sabine Lake, TX, USA	29.88003	-93.8383	17.75	Gulf of Mexico
East Matagorda Bay, TX, USA	28.69766	-95.8473	17.75	Gulf of Mexico
Baffin Bay, TX, USA	27.26614	-97.5174	17.75	Gulf of Mexico
Weeks Bay, AL	30.39879	-87.8307	17.00	Gulf of Mexico
Guadalupe Estuary, LA, USA	28.37865	96.75957	7.28	Gulf of Mexico
North Adriatic Sea, Venice, Italy	45.33922	12.29348	12.81	Italy
Norsminde Fjord, Denmark	56.01485	10.23736	45.00	Kattegat
Randers Fjord Estuary, Denmark	56.58118	10.27185	115.21	Kattegat
Randers Fjord Estuary, Denmark	56.58118	10.27185	115.21	Kattegat
Arkona basin	54.57749	12.25203	45.90	Kattegat
Norsminde Fjord, Denmark	56.01452	10.23763	45.00	Kattegat
Kerteminde Fjord, Denmark	55.44094	10.56429	20.00	Kattegat
Horsens Fjord, Denmark	55.85413	10.02648	11.97	Kattegat
Jehu Pond, Cape Cod, MA, USA	41.56722	-70.4971	29.80	MA
Childs River, Waquoit Bay, MA, USA	41.57403	-70.5275	26.83	MA
Childs River, Waquoit Bay, MA, USA	41.57403	-70.5275	26.83	MA
Waquoit Bay, MA, USA	41.56243	-70.5218	12.90	MA
Plum Island Sound, MA, USA	42.72732	-70.8094	12.90	MA
Waquoit Bay, MA, USA	41.56243	-70.5218	12.90	MA
Waquoit Bay, MA, USA	41.6	-70.5	12.90	MA
Waquoit Bay, MA, USA	41.56243	-70.5218	12.90	MA
Great Pond, MA, USA	41.83384	-69.9892	12.90	MA
Green Pond, MA, USA	41.55398	-70.5703	12.90	MA
Childs River, Cape Cod, MA, USA	41.57144	-70.5346	12.90	MA
West River, Plum Island, MA, USA	42.73708	-70.8503	10.00	MA
Hamblin Pond, Cape Cod, MA, USA	41.57213	-70.5075	6.27	MA

Table AI.4 Continued

Location	Lat	Long	N Load	Bin
Quashnet River, Cape Cod, MA, USA	41.5771	-70.5151	3.01	MA
Oyster Pond, Chatham, MA, USA	41.67784	-69.9797	2.00	MA
Miacomet Pond, Nantucket, MA, USA	41.24834	-70.1155	1.38	MA
Eel Pond, Cape Cod, MA, USA	41.55541	-70.5464	0.76	MA
Timms Pond, Cape Cod, MA, USA	41.55307	-70.5406	0.53	MA
Sagelot Pond, Waquoit Bay, MA, USA	41.55351	-70.5095	0.06	MA
Sagelot Pond, Waquoit Bay, MA, USA	41.55351	-70.5095	0.06	MA
Sweeney River, Plum Island, MA, USA	42.72194	-70.8473	150.00	MA
Boston Harbor, MA, USA	42.3346	-70.9761	110.47	MA
Quashnet River, MA, USA	41.57619	-70.5157	101.80	MA
Sagelot Pond, Cape Cod, MA, USA	41.55388	-70.5091	40.70	MA
Mashpee River, MA, USA	41.59214	-70.4601	29.80	MA
Baha del To´bari bay, Mexico	27.13333	-110.6	ND	Mexico
Apponaug Cove, RI, USA	41.69358	-71.447	28.00	Narragansett
Bissel Cove, RI, USA	41.55056	-71.432	28.00	Narragansett
Brush Neck Cove, RI, USA	41.69162	-71.4048	28.00	Narragansett
Donavan, Narragansett Bay, RI, USA	41.45971	-71.2259	28.00	Narragansett
Fogland, Narragansett Bay, RI, USA	41.5568	-71.2166	28.00	Narragansett
Foxhill Pond, Narragansett Bay, RI, USA	41.49038	-71.396	28.00	Narragansett
Jenny Pond, Narragansett Bay, RI, USA	41.63202	-71.3372	28.00	Narragansett
Old Mill Creek, Narragansett Bay, RI, USA	41.71323	-71.3662	28.00	Narragansett
Passeonquis Cove, Narragansett Bay, RI, USA	41.74648	-71.3851	28.00	Narragansett
Wathemoket Cove, Narragansett Bay, RI, USA	41.7998	-71.3786	28.00	Narragansett
Narragansett Bay, RI, USA	41.62202	-71.3528	27.83	Narragansett
Pamlico Sound, NC, USA	35.12033	-76.4777	100.40	NC
Pamlico Sound, NC, USA	35.34334	-75.8755	100.40	NC
Neuse River Estuary, NC, USA	35.23417	-75.9646	34.00	NC
Lower Neuse River Estuary, NC, USA	35.16772	-76.5097	34.00	NC
Neuse River Estuary, NC, USA	35.13061	-76.5102	19.60	NC
Albemarle Sound, NC, USA	36.05513	-76.0107	6.90	NC
Lamprey River, NH, USA	43.06347	-70.9078	ND	NH
Oyster River, Great Bay, NH, USA	43.12275	-70.868	ND	NH
South Slough, OR, USA	43.30852	-124.319	ND	OR
Pearl River Estuary, China	22.6124	113.7565	96.30	Pearl River
Pearl River Estuary, China	22.55339	113.7237	96.30	Pearl River
Lingdingyang Bay, China	22.48642	113.7336	96.30	Pearl River
Modamen Bay, China	22.10588	113.4292	96.30	Pearl River
Dapeng Bay, China	22.46587	114.4514	96.30	Pearl River
Pearl River Estuary, China	22.25668	114.1025	96.30	Pearl River
Douro Estuary, Portugal	41.14201	8.657858	45.00	Portugal

Table AI.5 Continued

Location	Lat	Long	N Load	Bin
Sado Estuary, Portugal	38.46184	8.785789	45.00	Portugal
San Francisco Bay, CA, USA	37.85407	-122.384	29.00	San Francisco
Westerschelde Estuary, Netherlands	51.37383	3.751369	247.07	Schelde
Scheldt Estuary, Netherlands	51.50203	3.680842	194.66	Schelde
Westerschelde Estuary, Netherlands	51.41894	3.658744	194.66	Schelde
Schelde Estuary, Netherlands	51.62258	3.859517	180.00	Schelde
Rhine Estuary, Netherlands	51.87407	3.991181	180.00	Schelde
Elbe Estuary, Germany	54.08721	8.510239	40.00	Schelde
Ems Estuary, Netherlands	53.63866	6.773767	40.00	Schelde
Tay Estuary, Scotland, UK	56.43582	-3.00096	60.68	Scotland
Thames Estuary, UK	51.49849	0.591236	102.90	Thames
Thames Estuary, UK	51.48194	2.64	98.00	Thames
Thames Estuary, UK	51.50203	0.663706	98.00	Thames
Padilla Bay, WA, USA	48.51646	-122.528	ND	WA
Changjiang Estuary, China	31.53276	121.4145	157.93	Yangtze
Changjiang Estuary, China	30.48379	121.7005	157.93	Yangtze
East China Sea, China	30.83574	122.318	157.93	Yangtze
South China Sea, China	23.41897	117.1773	157.93	Yangtze
Yellow Sea, China	34.99781	119.3249	157.93	Yellow Sea

Figure 2.1 References

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Appendix II

Supplemental Materials for Chapter 3

Table AII.1. Pearson correlation table showing all significant (**p < 0.05**, p< 0.1) linear correlations within the dark dataset. Values are shown as correlation coefficient, r, with r >0.65 considered for use in the interaction network. Abbreviations as follows: PW PO4 (porewater phosphate concentration); PWNH4 (porewater ammonium concentration); Chl a (chlorophyll-a); Pheo (pheo-pigments); %OM (% organic matter in sediment); SR (species richness); Bioturb (number of bioturbators per core).

	N2 Flux	*O2 Flux*	*NH4 flux*	*DON flux*	*DOP flux*	*PW PO4*	*Chl a*	*Pheo*	*% Mud*	*%OM*	*Individuals*	*SR*	*Bioturb*	*Bivalves*	*grazers*
N2 Flux	1														
O2 Flux	---	1													
NH4 flux	---	---	1												
DON flux	-0.579	---	-0.525	1											
DOP flux	---	---	0.667	---	1										
PW PO4	---	---	---	---	---	1									
PW NH4	-0.579	---	---	0.578	---	---									
Chl a	---	---	---	**0.671**	---	---	1								
Pheo	---	---	---	**0.666**	---	---	**---**	1							
% Mud	**-0.711**	---	---	**0.654**	**0.632**	---	**0.857**	**0.857**	1						
%OM	---	---	---	**0.751**	---	---	**0.638**	**0.630**	0.624	1					
Individuals	---	---	---	---	-0.524	**0.822**	**-0.671**	**-0.665**	-0.571	---	1				
SR	**0.736**	---	---	---	**-0.634**	---	**-0.674**	**-0.657**	**-0.785**	---	---	1			
Bioturb	---	---	---	---	-0.547	**0.816**	**-0.687**	**-0.678**	-0.578	---	**0.997**	---	1		
Bivalves	---	---	---	---	---	**0.828**	**-0.674**	**-0.669**	-0.533	---	**0.975**	---	**0.962**	1	
grazers	---	-0.565	---	---	-0.599	**0.728**	**-0.585**	-0.542	**-0.660**	-0.557	**0.940**	---	**0.929**	**0.888**	1

Table AII.2. Pearson correlation table showing all significant (**p < 0.05**, p< 0.1) linear correlations within the light dataset. Values are shown as correlation coefficient, r, with r >0.65 considered for use in the interaction network. Abbreviations as follows: PW PO4 (porewater phosphate concentration); PWNH4 (porewater ammonium concentration); Chl a (chlorophyll-a); Pheo (pheo-pigments); %OM (% organic matter in sediment); SR (species richness); Bioturb (number of bioturbators per core).

	N2 Flux	*O2 Flux*	*NH4 flux*	*DON flux*	*DOP flux*	*PW PO4*	*Chl a*	*Pheo*	*% Mud*	*%OM*	*Individuals*	*SR*	*Bioturb*	*Bivalves*	*grazers*
N2 Flux	1														
O2 Flux	---	1													
NH4 flux	---	**0.777**	1												
DON flux	**-0.643**	**-0.642**	---	1											
DOP flux	-0.504	---	---	---	1										
PW PO4	---	---	---	---	---	1									
PW NH4	-0.501	---	0.553	---	---	---									
Chl a	---	**0.652**	---	---	---	---	1								
Pheo	---	**0.650**	---	---	---	---	**0.090**	1							
% Mud	---	**0.662**	**0.768**	---	---	---	**0.857**	**0.857**	1						
%OM	-0.560	---	---	---	---	---	**0.638**	**0.630**	**0.624**	1					
Individuals	---	-0.554	---	---	---	**0.822**	**-0.671**	**-0.665**	-0.571	---	1				
SR	---	-0.587	-0.598	---	**0.764**	---	**-0.674**	**-0.657**	**-0.785**	---	---	1			
Bioturb	---	-0.545	---	---	---	**0.816**	**-0.687**	**-0.678**	-0.578	---	**0.997**	---	1		
Bivalves	---	**-0.643**	---	---	---	**0.828**	**-0.674**	**-0.669**	-0.533	---	**0.975**	---	**0.962**	1	
grazers	---	-0.540	---	---	---	**0.728**	**-0.585**	-0.542	**-0.660**	-0.557	**0.940**	---	**0.929**	**0.888**	1

Table AII.3. Averaged biogeochemical and macrofaunal data (not included in Table 3.1) from each site in the light and dark chambers (n=3).

Site	Light	N_2-N (μmol m^{-2} h^{-1})	DO (μmol m^{-2} h^{-1})	NH_4^+ (μmol m^{-2} h^{-1})	DON (μmol m^{-2} h^{-1})	DOP (μmol m^{-2} h^{-1})	[DIP] (μM)	Pheo (μg/g)	OM (%)	Bivalves (#/core)	Grazer (score)	Bioturb. (score)
1	Dark	6.99	-1587.9	369.6	-514.6	-4.09	0.96	12.73	1.96	18.6	0.8	10.2
2	Dark	-40.81	-2344.6	518.2	387.3	13.66	0.67	10.82	2.22	15.2	0.9	13.5
3	Dark	87.96	-1630.8	736.3	-1662.9	-6.51	0.31	14.68	2.38	10.2	1.8	7.86
4	Dark	59.56	-1544.5	269.9	-645.3	-134.1	0.82	8.51	1.23	20	3.8	16.5
5	Dark	95.74	-961.4	954.4	-2205.7	5.33	0.49	4.92	1.05	23	2.5	14.4
6	Dark	71.49	-1283.9	416.6	-623.3	-122.2	1.36	5.53	1.65	48	6.8	30.1
7	Dark	87.19	-855.4	227.0	-306.9	-96.21	0.22	9.08	2.70	13	0.3	9.23
1	Light	8.30	85.88	-81.0	560.0	-6.61	0.96	12.73	1.96	18.6	0.8	10.2
2	Light	27.89	1212.8	260.0	-313.9	11.42	0.67	10.82	2.22	15.2	0.9	13.5
3	Light	45.58	981.3	-58.79	89.22	-8.80	0.31	14.68	2.38	10.2	1.8	7.87
4	Light	30.80	1039.9	-425.7	132.8	-0.50	0.82	8.51	1.23	20	3.8	16.5
5	Light	52.29	957.4	-270.5	-157.7	30.75	0.49	4.92	1.05	23	2.5	14.4
6	Light	30.72	-422.2	-191.2	362.9	25.67	1.36	5.53	1.65	48	6.8	30.1
7	Light	1.48	-33.98	-276.7	369.6	128.2	0.22	9.08	2.70	13	0.3	9.23

Table AII.4. List of predictor and dependent variables used in the DistlM analysis. Predictor variables in black were used in all models, while predictors in grey (Macrofaunal variables) were only included in the second runs. DistlM's were run on light and dark data separately.

Predictor Variables	Dependent Variable
Mud Content	Net N_2 Flux
Light Intensity	
Porewater [NH_4^+]	
Porewater [NO_x]	
Porewater [DIP]	
NH_4^+ flux	
NO_x flux	
DIP flux	
DO flux	
DON flux	
DOP flux	
Individuals	
Species Richness	
Bivalve	
Grazer	
Bioturbator	

Appendix III

Supplemental Materials for Chapter 4

Flux calculation equations

$^{30}N_2$ flux:

(*E1*) $$^{30}\text{N2 flux} = \frac{\left(\frac{\Delta y}{\Delta x}\right) vol}{A}$$

Where y is the concentration of $^{30}N_2$ in µM, x is the incubation time in hours, *vol* is the volume of the overlying water column in L, and *A* is the surface area of the core in m^2. Note that the fluxes of $^{28}N_2$ and $^{29}N_2$ were calculated the same way, using the concentrations of $^{28}N_2$ and $^{29}N_2$, respectively.

Total denitrification rate:

(*E2*) $$\text{DNF} = {}^{28}\text{N2 flux} + {}^{29}\text{N2 flux}$$

Note that only the positive $^{29}N_2$ fluxes were included.

$^{30}N_2$ diffusion flux (*F*) calculated from Fick's Law:

(*E3*) $$F = \frac{-\Phi * D_a * \Delta C}{x}$$

Where Φ is sediment porosity, D_a is the apparent diffusion coefficient, ΔC is the change in N_2 concentration, and x is the thickness of the sediment layer. The apparent diffusion coefficient (D_a) of $7.7 * 10^{-6}$ cm^{-2} s^{-1} for estuarine sediments was used according to An and Joye (2001) using calculations from Boudreau (1996).

(*E4*) $$x = \sqrt{D_a * t}$$

The diffusion flux was calculated by estimating the diffusion depth (x) at each time (t).

Measured N fixation rate:

(*E5*) $$\text{Nfix} = {}^{30}N2\ flux - F$$

Proportion of N_2 pool that was labelled:

(*E6*) $$\rho = \frac{[{}^{30}N2]}{[\text{N2}_{tot}]}$$

Or, the concentration of $^{30}N_2$ at the beginning of the incubation divided by the total concentration of N_2 calculated according to the temperature and salinity of core samples (Milero and Poisson, 1981). Note that $\rho \sim 12\%$.

Potential, total N fixation rate:

(*E7*) $$\text{Nfix}_{\text{tot}} = \frac{\text{Nfix}}{\rho}$$

Net rate:

(*E8*) $$\text{Net} = \text{Nfix}_{\text{tot}} + \text{DNF}$$

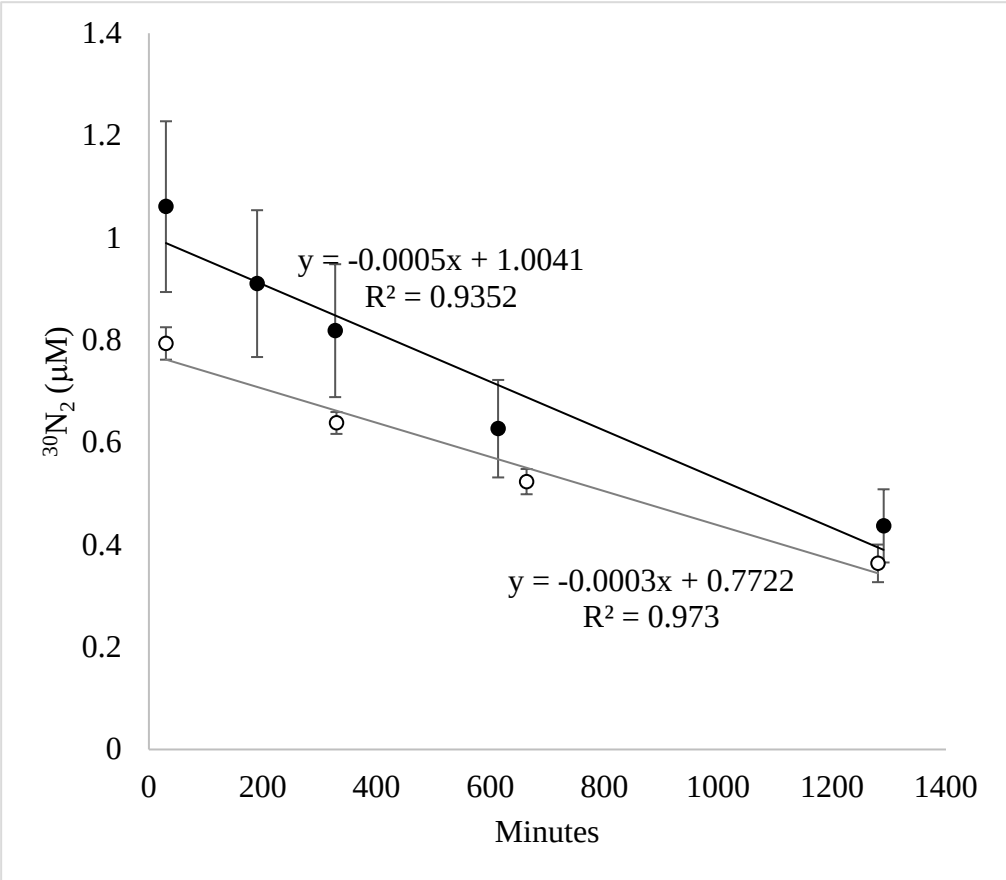

Figure AIII.1 Concentration of $^{30}N_2$ over time in the dark (dark points) and light (open points) core incubations. Darker trendline is for the dark cores (y= -0.0005x +1.0041, R^2= 0.9352) and lighter trendline is for the light cores (y= -0.0003x + 0.7722, R^2= 0.973). These are trendlines on the averaged data to demonstrate negative flux of uptake of $^{30}N_2$ by the sediment, for analysis for the chapter, each core's flux was calculated individually (R^2 >0.97) Error is standard error.

Appendix IV

Supplemental materials for Chapter 5

Table AIV.1 Macrofaunal abundances in each site in order of most to least abundant overall. jv stands for juvenile and count individuals < 6 mm.

Site Label	% Mud	*Austrovenus stutchburyi*	*Scoloplos cylindrifer*	*Macomona liliana*	*Nucula hartvigiana jv*	*Prionospio aucklandica*	*Austrovenus stutchburyi jv*	*Exospheroma planulum*	*Macomona liliana jv*	*Boccardia sp.*	*Platynereis australis*
1	0.00	2	1	7	0	3	4	0	0	0	2
2	0.00	0	6	7	4	2	5	0	2	0	0
3	0.00	7	1	7	0	1	1	0	1	1	0
4	0.00	23	0	10	3	9	0	0	0	3	0
5	0.01	7	1	10	6	0	3	0	1	0	0
6	0.02	10	5	6	0	0	7	0	0	0	0
7	1.47	7	4	14	2	0	0	0	3	0	0
8	1.62	18	4	14	8	0	0	0	0	1	0
9	2.00	9	10	4	3	0	4	32	2	0	0
10	2.13	0	3	12	0	0	2	1	0	0	2
11	2.15	10	12	4	23	0	2	0	3	2	0
12	2.26	17	9	5	1	10	2	0	0	5	1
13	2.30	4	1	12	0	0	0	0	0	1	0
14	2.36	9	3	8	1	0	0	0	0	0	1
15	2.46	3	16	4	0	1	1	0	0	0	0
16	2.64	22	15	7	5	14	3	0	3	1	1
17	2.64	13	1	11	3	0	2	13	0	0	0
18	2.84	36	14	1	1	1	2	0	0	0	0
19	3.36	10	3	11	1	5	2	0	0	0	0
20	3.89	22	5	2	0	1	2	0	0	0	0

Table AIV.1 Continued

Site Label	% Mud	*Austrovenus stutchburyi*	*Scoloplos cylindrifer*	*Macomona liliana*	*Nucula hartvigiana jv*	*Prionospio aucklandica*	*Austrovenus stutchburyi jv*	*Exospheroma planulum*	*Macomona liliana jv*	*Boccardia sp.*	*Platynereis australis*
21	4.00	17	2	7	0	1	4	0	0	0	3
22	4.15	15	7	2	11	2	1	0	0	0	0
23	4.36	11	15	2	0	2	0	0	2	0	1
24	4.74	10	4	3	2	0	1	2	0	3	0
25	5.06	13	2	**4**	14	0	2	0	0	4	0
26	7.70	5	5	1	0	4	2	0	1	0	0
27	9.11	20	5	0	0	3	3	0	1	0	2
28	10.15	6	6	0	0	0	4	0	0	0	0
29	13.77	12	7	2	0	5	1	0	3	0	0
30	13.99	4	6	1	0	0	2	0	0	1	2
31	20.57	3	3	0	3	2	0	0	0	0	3
32	23.30	0	3	1	1	2	0	0	0	0	1
33	23.43	8	2	0	1	3	1	0	1	0	1
34	28.32	2	2	2	2	0	0	0	0	0	2

Table AIV.1 Continued

Site Label	% Mud	*Nucula hartvigiana*	*Eurylana cooki*	*Aricidea sp.*	*Austrohelice crassa*	*Diloma subrostratum*	*Nemertea*	*Notoacmea scapha*	*Palaemon eupalaemon*	*Zeacumanthus sp.*	*Levinsenia gracilis*
1	0.00	1	0	0	0	0	1	0	2	0	0
2	0.00	0	0	0	0	1	0	0	0	0	0
3	0.00	0	0	0	0	0	0	0	0	0	0
4	0.00	1	0	1	0	0	1	0	0	3	4
5	0.01	0	3	0	0	0	0	1	0	0	0
6	0.02	2	1	0	0	0	0	0	0	0	0
7	1.47	0	0	0	0	0	0	0	0	0	0
8	1.62	0	0	0	0	0	0	0	0	0	0
9	2.00	1	1	0	0	0	0	0	0	0	0
10	2.13	0	0	0	0	0	0	0	0	0	0
11	2.15	0	0	0	0	0	0	0	0	0	0
12	2.26	0	0	0	0	1	0	0	0	0	0
13	2.30	0	0	0	0	2	1	1	0	0	0
14	2.36	0	1	2	0	0	0	0	0	0	0
15	2.46	0	0	6	0	2	2	0	0	0	0
16	2.64	3	2	1	0	1	0	2	0	0	2
17	2.64	0	3	0	0	0	0	1	0	0	0
18	2.84	0	0	0	0	0	0	0	0	2	0
19	3.36	1	1	1	0	1	1	2	0	1	0
20	3.89	0	2	1	0	1	0	0	1	0	0
21	4.00	1	0	0	0	1	0	0	0	0	1
22	4.15	2	1	0	1	1	0	5	0	0	0
23	4.36	0	0	0	0	0	0	0	0	0	0
24	4.74	0	3	2	0	0	0	0	0	0	0

Table AIV.1 Continued

Site Label	% Mud	*Nucula hartvigiana*	*Eurylana cooki*	*Aricidea sp.*	*Austrohelice crassa*	*Diloma subrostratum*	*Nemertea*	*Notoacmea scapha*	*Palaemon eupalaemon*	*Zeacumanthus sp.*	*Levinsenia gracilis*
25	5.06	0	0	0	0	1	1	0	0	2	2
26	7.70	0	0	1	0	0	1	0	0	1	0
27	9.11	0	0	0	1	1	0	0	0	0	0
28	10.15	1	0	0	4	2	2	0	0	0	1
29	13.77	2	0	0	2	0	0	1	0	0	0
30	13.99	2	0	1	3	1	0	0	1	0	0
31	20.57	0	0	0	2	0	0	0	6	0	0
32	23.30	2	0	1	0	0	0	0	0	0	0
33	23.43	0	0	0	3	0	0	0	0	0	0
34	28.32	0	0	0	0	0	3	0	1	1	0

Table AIV.1 Continued

Site Label	% Mud	*Phylo sp.*	*Capitella sp.*	*Perinereis vallata*	*Prionospio ehlersi*	*Copepoda sp.*	*Pseudopolydora paucibranchiata*	*Paraprionospio sp.*	*Paphies australis*	*Paradoneis lyra*	*Scaphopoda sp.*
1	0.00	1	0	0	0	0	0	0	0	0	0
2	0.00	0	0	0	2	0	2	0	0	1	0
3	0.00	0	0	0	0	0	0	0	1	0	0
4	0.00	0	0	0	0	4	0	3	0	0	0
5	0.01	0	0	0	0	0	0	0	0	0	0
6	0.02	0	0	0	0	0	0	0	0	0	0
7	1.47	0	0	0	1	0	1	0	0	0	1
8	1.62	0	0	0	0	0	0	0	0	1	0
9	2.00	0	0	0	0	0	0	0	0	0	0
10	2.13	1	0	0	0	2	0	0	0	0	0
11	2.15	0	0	0	0	0	0	0	0	0	0
12	2.26	0	0	1	0	0	0	0	0	0	0
13	2.30	0	0	0	0	0	0	0	0	1	0
14	2.36	0	1	0	0	0	0	0	0	0	0
15	2.46	0	0	0	0	0	0	0	0	0	0
16	2.64	0	1	0	0	0	0	0	0	0	0
17	2.64	0	0	0	1	0	0	0	0	0	0
18	2.84	1	0	0	0	0	0	0	0	0	0
19	3.36	0	1	0	0	0	0	0	0	0	1
20	3.89	0	0	0	0	0	2	0	0	0	0
21	4.00	1	0	1	1	0	0	2	0	0	0
22	4.15	0	0	0	0	0	0	0	3	0	0
23	4.36	0	0	1	0	0	0	0	0	0	0
24	4.74	0	0	0	0	0	1	0	0	0	0
25	5.06	0	0	0	0	0	0	0	0	0	0

Table AIV.1 Continued

Site Label	% Mud	*Phylo sp.*	*Capitella sp.*	*Perinereis vallata*	*Prionospio ehlersi*	*Copepoda sp.*	*Pseudopolydora paucibranchiata*	*Paraprionospio sp.*	*Paphies australis*	*Paradoneis lyra*	*Scaphopoda sp.*
26	7.70	0	0	0	0	0	0	0	0	0	0
27	9.11	1	2	0	0	0	0	0	0	0	1
28	10.15	2	0	0	0	0	0	0	0	1	0
29	13.77	0	0	0	0	0	0	0	0	0	0
30	13.99	1	0	0	0	0	0	0	0	0	1
31	20.57	0	0	0	0	0	0	0	0	0	0
32	23.30	0	0	0	1	0	0	0	0	0	0
33	23.43	0	1	0	0	0	0	0	0	0	0
34	28.32	0	1	3	0	0	0	0	0	0	0

Table AIV.1 Continued

Site Label	% Mud	*Macroclymenella stewartensis*	*Thoristella polychroma*	*Ceratonereis pachychaeta*	*Oligochaeta*	*Cominella adspersa*	*Halicarcinus cookii*	*Anthopleura aureoradiata*	*Ceratonereis sp.*	*Neommatocarcinus huttoni*	*Magelona dakini*
1	0.00	0	0	0	1	0	0	0	0	0	0
2	0.00	0	0	2	0	0	0	0	0	0	0
3	0.00	0	0	0	0	0	0	0	0	0	0
4	0.00	0	0	0	0	0	0	0	2	0	0
5	0.01	0	0	0	0	0	0	0	0	0	0
6	0.02	0	1	0	0	0	0	0	0	0	0
7	1.47	0	0	0	0	0	0	0	0	0	0
8	1.62	0	0	0	0	0	0	1	0	0	0
9	2.00	0	0	0	0	0	0	0	0	1	0
10	2.13	0	0	0	0	0	1	0	0	0	0
11	2.15	0	0	0	0	0	0	0	0	0	0
12	2.26	0	0	0	0	0	0	0	0	0	0
13	2.30	0	0	0	0	0	0	0	0	1	1
14	2.36	0	0	0	0	0	0	0	0	0	0
15	2.46	0	0	0	0	0	0	0	0	0	0
16	2.64	0	0	0	0	0	0	0	0	0	0
17	2.64	0	0	0	0	0	0	0	0	0	0
18	2.84	0	2	0	0	0	1	0	0	0	0
19	3.36	0	0	0	0	0	0	0	0	0	0
20	3.89	0	0	0	0	0	0	0	0	0	1
21	4.00	1	0	0	0	0	0	0	0	0	0
22	4.15	1	0	0	0	0	1	0	0	0	0
23	4.36	0	0	0	0	0	0	0	0	0	0
24	4.74	0	0	0	0	1	0	0	0	0	0
25	5.06	0	0	0	0	2	0	0	0	0	0
26	7.70	0	0	0	0	0	0	0	0	0	0
27	9.11	0	0	0	0	0	0	0	0	0	0

Table AIV.1 Continued

Site Label	% Mud	*Macroclymenella stewartensis*	*Thoristella polychroma*	*Ceratonereis pachychaeta*	*Oligochaeta*	*Cominella adspersa*	*Halicarcinus cookii*	*Anthopleura aureoradiata*	*Ceratonereis sp.*	*Neommatocarcinus huttoni*	*Magelona dakini*
28	10.15	0	0	0	1	0	0	0	0	0	0
29	13.77	0	0	0	0	0	0	0	0	0	0
30	13.99	0	0	0	0	0	0	0	0	0	0
31	20.57	0	0	0	0	0	0	0	0	0	0
32	23.30	0	0	1	0	0	0	1	0	0	0
33	23.43	2	0	0	1	0	0	0	0	0	0
34	28.32	0	0	0	0	0	0	0	0	0	0

Table AIV.1 Continued

Site Label	% Mud	*Pectinaria sp.*	*Torridoharpinia hurleyi*	*Margarella antipoda*	*Micrelenchus festivus*	*Orbinia papillosa*	*Lembos kidoli*	*Cypraea argus*	*Biffarius sp*	*Paphies australis jv*	*Lumbrineris sp.*
1	0.00	0	0	1	0	0	0	1	0	0	0
2	0.00	0	0	0	0	0	0	0	0	0	0
3	0.00	0	0	0	0	0	0	0	0	0	0
4	0.00	0	0	0	1	0	0	0	0	0	1
5	0.01	0	0	0	0	0	0	0	0	0	0
6	0.02	0	0	0	0	0	0	0	0	2	0
7	1.47	0	0	0	0	0	0	0	0	0	0
8	1.62	0	0	0	0	0	0	0	0	0	0
9	2.00	0	0	0	0	0	0	0	0	0	0
10	2.13	0	1	0	0	0	0	0	0	0	0
11	2.15	0	0	0	0	0	0	0	0	0	0
12	2.26	0	0	0	0	0	0	0	0	0	0
13	2.30	0	0	0	0	0	0	0	0	0	0
14	2.36	0	0	0	0	0	0	0	0	0	0
15	2.46	0	0	0	0	0	0	0	0	0	0
16	2.64	0	0	0	0	0	0	0	0	0	0
17	2.64	0	0	0	0	0	0	0	0	0	0
18	2.84	0	0	0	0	0	0	0	0	0	0
19	3.36	0	0	0	0	0	0	0	0	0	0
20	3.89	0	0	0	0	0	0	0	0	0	0
21	4.00	0	0	0	0	0	1	0	0	0	0
22	4.15	0	0	0	0	0	0	0	0	0	0
23	4.36	0	0	0	0	0	0	0	0	0	0
24	4.74	0	0	0	0	0	0	0	0	0	0

Table AIV.1 Continued

Site Label	% Mud	*Pectinaria sp.*	*Torridoharpinia hurleyi*	*Margarella antipoda*	*Micrelenchus festivus*	*Orbinia papillosa*	*Lembos kidoli*	*Cypraea argus*	*Biffarius sp*	*Paphies australis jv*	*Lumbrineris sp.*
25	5.06	0	0	0	0	0	0	0	0	0	0
26	7.70	0	0	0	0	0	0	0	0	0	0
27	9.11	0	0	0	0	1	0	0	0	0	0
28	10.15	0	0	0	0	0	0	0	0	0	0
29	13.77	0	0	0	0	0	0	0	1	0	0
30	13.99	0	0	0	0	0	0	0	0	0	0
31	20.57	2	0	0	0	0	0	0	0	0	0
32	23.30	0	0	0	0	0	0	0	0	0	0
33	23.43	0	0	0	0	0	0	0	0	0	0
34	28.32	0	0	0	0	0	0	0	0	0	0

Table AIV.2 Macrofaunal variables, including individual, species, trait, and function data. Ind. stands for individual. Grazer and bioturbation indices are assigned based on traits from an existing species x trait matrix (Thrush *et al.*, 2017), traits can be partial (.025, .33, .5, .75, etc.) thus indices are not necessarily whole numbers.

Site Label	% Mud	Ind.	Species	Bivalves	Crabs	Rare ind.	Surface to depth feeders	Depth to surface feeders	Surface-dwellers (epifauna)	Deep-dwellers	Permanent burrow	Tube	Motile	Predators	Grazer index	Bioturbation index
1	0.00	27	13	14	0	2	20	21	2	15	3	0	15	3	1.4	9.0
2	0.00	35	12	18	0	0	22	29	0	24	3	0	21	9	0.5	12.3
3	0.00	20	8	17	0	0	18	18	0	11	0	1	11	1	0	7.1
4	0.00	74	15	28	0	3	42	59	3	38	3	3	62	17	1	27.5
5	0.01	32	8	27	0	0	21	22	0	12	0	0	11	3	0	8.3
6	0.02	32	7	25	0	0	23	28	0	11	0	0	22	0	0	10.8
7	1.47	33	8	26	0	0	26	30	0	23	0	0	13	3	0	10.7
8	1.62	32	7	25	0	1	24	28	0	11	1	1	23	1	0	11.4
9	2.00	53	8	19	0	1	15	15	32	2	0	0	45	17.5	0	12.6
10	2.13	13	8	2	0	1	4	7	1	6	2	0	10	1	0	3.8
11	2.15	44	6	42	0	0	21	19	0	9	0	2	12	11.5	0	10.0
12	2.26	52	10	25	0	0	41	45	0	31	2	5	41	11.5	0	21.1
13	2.30	25	10	16	0	2	19	20	2	17	1	1	10	1	1.4	7.7
14	2.36	26	8	18	0	0	18	24	0	13	4	0	16	0.75	0.25	9.1
15	2.46	35	8	8	0	0	11	33	2	23	6	0	31	2	2	13.3
16	2.64	83	17	43	0	0	51	69	0	44	3	1	60	18.25	0.25	29.5
17	2.64	39	9	20	0	0	18	19	13	4	0	0	30	9	0	10.4
18	2.84	62	11	40	0	0	40	54	0	16	0	0	56	1.5	0	22.2
19	3.36	49	15	25	0	0	29	41	1	28	2	0	32	6.25	1.25	16.4
20	3.89	49	11	35	0	1	39	45	1	20	1	0	35	3.5	0.4	17.4
21	4.00	55	16	29	0	1	36	49	0	28	4	0	43	5.5	0	20.3
22	4.15	63	15	43	1	0	30	37	0	21	1	0	31	8	0	16.2
23	4.36	19	6	15	0	0	19	19	0	8	2	0	15	2.5	0	7.8
24	4.74	32	11	16	0	0	18	21	2	11	2	3	21	3	0	10.1
25	5.06	48	12	33	0	1	24	24	1	13	0	4	25	8.5	1	13.0

Table AIV.2 Continued

Site Label	% Mud	Ind.	Species	Bivalves	Crabs	Rare ind.	Surface to depth feeders	Depth to surface feeders	Surface-dwellers (epifauna)	Deep-dwellers	Permanent burrow	Tube	Motile	Predators	Grazer index	Bioturbation index
26	7.70	21	9	9	0	0	14	20	1	12	1	0	19	4	1	8.5
27	9.11	41	12	24	1	1	30	38	0	15	5	0	38	4	0.5	16.1
28	10.15	30	11	11	4	0	17	25	2	15	6	0	27	1.5	2	11.6
29	13.77	36	10	20	2	1	25	32	0	19	2	0	27	5	0	13.4
30	13.99	26	13	9	3	0	13	19	0	13	6	1	19	0.5	0.33	9.2
31	20.57	27	9	9	2	2	13	18	0	15	5	2	13	4.5	0	8.4
32	23.30	14	10	4	0	1	6	10	0	9	3	0	9	3.75	0.25	4.9
33	23.43	28	11	15	3	0	22	25	0	16	6	0	20	3.75	0.25	10.9
34	28.32	25	10	12	0	0	12	15	3	13	6	0	14	5.75	3.25	8.1

Table AIV.3 Pearson's correlations for the low mud sites

	Chl a	*Pheo*	*% Mud*	*%sand*	*N2-N*	*O2*	*Nox umol m-2 h-1*	*PO4 umol m-2 h-1*	*NH4 umol m-2 h-1*	*TN*	*TP*	*DON umol m-2 h-1*	*DOP umol m-2 h-1*	*Nox umol/L*	*PO4 umol/L*	*NH4 umol/L*	*# individuals*	*# species*	*# bivalves*	*# crabs*
Chl a	1																			
Pheo	**-0.55**	1																		
% Mud	**0.51**	0.03	1																	
% Sand	-0.01	-0.26	-0.42	1																
N2-N	0.11	0.16	0.11	**-0.74**	1															
O2	-0.22	-0.15	-0.52	**0.60**	**-0.67**	1														
Nox umol m-2 h-1	-0.23	0.24	0.05	0.19	-0.30	0.02	1													
PO4 umol m-2 h-1	0.29	0.16	0.21	-0.37	0.34	-0.24	0.24	1												
NH4 umol m-2 h-1	-0.35	**0.73**	0.13	-0.28	-0.09	0.04	0.15	0.10	1											
TN	0.03	-0.10	0.14	-0.08	0.01	-0.10	0.34	-0.11	0.04	1										
TP	0.04	0.08	0.25	-0.72	**0.79**	**-0.56**	-0.05	0.28	-0.17	0.13	1									
DON umol m-2 h-1	0.18	-0.35	0.06	0.11	-0.01	-0.26	0.21	0.27	-0.39	-0.41	-0.04	1								
DOP umol m-2 h-1	0.00	-0.12	-0.21	**0.69**	**-0.76**	**0.52**	0.02	-0.26	0.14	-0.18	-0.99	0.12	1							
Nox umol/L	-0.28	0.03	-0.18	-0.08	-0.12	0.06	-0.05	-0.11	0.07	0.13	-0.04	-0.29	-0.03	1						
PO4 umol/L	0.52	-0.05	0.22	0.13	-0.05	-0.10	-0.02	0.04	0.04	-0.31	-0.19	0.08	0.22	-0.14	1					
NH4 umol/L	0.45	-0.03	0.17	0.27	-0.19	-0.03	0.04	-0.08	0.09	-0.20	-0.26	0.03	0.27	-0.12	**0.95**	1				
# individuals	0.06	0.40	0.22	0.07	0.01	-0.13	0.15	0.24	-0.02	-0.46	-0.09	0.08	0.08	0.02	0.35	0.22	1			
# species	0.04	0.09	-0.09	0.49	-0.43	0.36	0.35	-0.03	-0.13	-0.35	-0.34	0.10	0.32	-0.20	0.21	0.18	0.66	1		
# bivalves	0.23	0.36	0.14	-0.20	0.45	-0.21	-0.07	0.41	0.02	-0.35	0.28	-0.06	-0.27	-0.05	**0.48**	0.35	**0.71**	0.27	1	
# crabs	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1
# rare individuals	-0.34	0.32	-0.26	0.17	-0.38	0.39	0.47	0.10	0.29	0.21	-0.19	-0.38	0.11	0.11	-0.19	-0.14	0.09	0.39	-0.23	ND
# major worms	-0.07	0.37	0.18	0.25	-0.16	-0.10	0.31	0.11	0.01	-0.49	-0.23	0.38	0.26	-0.50	0.17	0.09	**0.72**	**0.69**	0.29	ND
Surface to depth	0.10	0.46	0.06	0.17	0.02	0.00	0.21	0.23	0.08	-0.40	-0.15	0.06	0.15	-0.27	0.44	0.36	**0.84**	**0.69**	**0.76**	ND
Depth to surface	0.06	0.39	0.13	0.18	-0.06	-0.05	0.24	0.18	0.05	**-0.52**	-0.22	0.27	0.25	-0.39	0.44	0.34	**0.86**	**0.73**	**0.62**	ND
deep	-0.11	0.31	-0.03	0.38	-0.17	0.12	0.29	-0.03	-0.08	-0.33	-0.22	0.22	0.24	**-0.55**	0.07	0.05	**0.65**	**0.77**	0.36	ND
permanent burrow	-0.25	0.01	0.09	0.06	-0.18	0.02	0.15	-0.22	-0.08	-0.35	0.01	0.39	0.04	-0.49	-0.27	-0.30	0.08	0.39	-0.36	ND
tube structure	-0.29	**0.71**	0.05	0.06	0.12	-0.20	0.33	0.18	0.18	0.02	0.10	-0.15	-0.14	-0.20	-0.13	-0.06	0.40	0.21	0.29	ND
motile	0.04	0.37	0.26	0.12	-0.18	-0.15	0.22	0.16	0.07	-0.48	-0.29	0.19	0.29	0.06	0.42	0.31	**0.92**	**0.63**	0.47	ND
Bioturb	0.01	0.45	0.17	0.17	-0.08	-0.10	0.25	0.18	0.04	-0.50	-0.20	0.19	0.21	-0.19	0.38	0.28	**0.95**	**0.74**	**0.62**	ND
Predator	-0.17	0.33	0.11	0.11	-0.03	-0.10	0.11	0.00	-0.16	-0.17	0.04	-0.14	-0.10	0.36	-0.12	-0.17	**0.79**	**0.52**	0.43	ND
Grazer	-0.20	-0.09	-0.06	0.27	-0.46	0.42	0.28	0.00	-0.06	-0.19	-0.16	0.25	0.17	-0.35	-0.23	-0.18	-0.05	0.32	-0.39	ND
surface (epifauna)	-0.01	-0.11	0.18	-0.06	-0.26	-0.02	-0.15	-0.09	-0.07	0.08	-0.13	-0.20	0.08	**0.79**	-0.15	-0.16	0.17	-0.10	-0.16	ND
deep	-0.03	0.24	0.00	0.09	0.26	0.02	0.04	0.06	-0.17	-0.19	0.25	0.06	-0.23	**-0.62**	-0.05	-0.08	0.41	0.38	0.53	ND

Table AIV.3 Continued

	# rare individuals	*# major worms*	*Surface to depth*	*Depth to surface*	*deep*	*permanent burrow*	*tube structure*	*motile*	*Bioturb*	*Predator*	*Grazer*	*surface (epifauna)*	*deep*
# rare individuals	1												
# major worms	0.09	1											
Surface to depth	0.03	**0.72**	1										
Depth to surface	0.03	**0.88**	**0.93**	1									
deep	0.14	**0.88**	**0.77**	**0.86**	1								
permanent burrow	0.16	**0.59**	0.00	0.28	0.50	1							
tube structure	0.20	**0.51**	**0.54**	0.41	0.49	0.05	1						
motile	0.15	**0.75**	**0.74**	**0.82**	**0.57**	0.18	0.33	1					
Bioturb	0.11	**0.87**	**0.91**	**0.96**	**0.80**	0.24	0.49	**0.92**	1				
Predator	0.17	0.46	**0.51**	0.45	0.45	0.06	0.45	**0.66**	**0.65**	1			
Grazer	0.49	0.31	-0.13	0.09	0.31	0.69	-0.04	0.02	0.05	-0.09	1		
surface (epifauna)	0.16	-0.26	-0.24	-0.27	-0.39	-0.23	-0.18	0.27	-0.04	0.47	-0.10	1	
deep	-0.11	**0.53**	**0.57**	**0.57**	**0.77**	0.29	0.43	0.13	0.47	0.28	0.19	**-0.56**	1

Table AIV.4 Pearson's correlations for the higher mud sites

	Chl a	*Pheo*	*% Mud*	*%sand*	*N2-N*	*O2*	*Nox umol m-2 h-1*	*PO4 umol m-2 h-1*	*NH4 umol m-2 h-1*	*TN*	*TP*	*DON umol m-2 h-1*	*DOP umol m-2 h-1*	*Nox umol/L*	*PO4 umol/L*	*NH4 umol/L*	*# individuals*	*# species*	*# bivalves*	*# crabs*
Chl a	1																			
Pheo	-0.35	1																		
% Mud	0.26	**0.67**	1																	
% Sand	-0.29	**-0.70**	**-0.95**	1																
N2-N	0.14	-0.15	-0.02	-0.05	1															
O2	0.07	-0.65	**-0.78**	**0.76**	-0.27	1														
Nox umol m-2 h-1	-0.05	-0.18	-0.26	0.24	**0.52**	0.03	1													
PO4 umol m-2 h-1	-0.11	**0.71**	**0.63**	**-0.52**	-0.20	**-0.51**	-0.38	1												
NH4 umol m-2 h-1	-0.02	0.30	0.44	-0.27	-0.02	-0.25	-0.19	0.43	1											
TN	0.37	0.02	0.11	-0.23	-0.20	0.09	-0.08	-0.19	-0.40	1										
TP	-0.38	0.44	0.04	-0.12	-0.09	0.00	-0.12	0.03	0.27	0.18	1									
DON umol m-2 h-1	-0.46	0.13	0.01	-0.05	0.38	-0.23	-0.12	0.16	0.00	-0.34	0.07	1								
DOP umol m-2 h-1	0.39	-0.44	-0.03	0.11	0.10	0.00	0.12	-0.03	-0.25	-0.18	**-1.00**	-0.05	1							
Nox umol/L	**-0.66**	0.42	-0.03	0.10	0.14	-0.35	0.40	0.21	0.33	**-0.51**	0.15	0.09	-0.16	1						
PO4 umol/L	-0.11	-0.28	-0.36	0.43	0.35	0.14	**0.90**	-0.39	-0.03	-0.19	-0.20	-0.28	0.20	**0.53**	1					
NH4 umol/L	-0.25	0.00	-0.17	0.23	0.37	-0.11	**0.87**	-0.17	0.05	-0.27	-0.14	-0.21	0.13	**0.71**	**0.94**	1				
# individuals	-0.33	-0.34	**-0.58**	**0.64**	0.05	0.40	**0.51**	-0.41	-0.24	0.04	0.20	0.01	-0.21	0.20	**0.55**	0.40	1			
# species	-0.08	-0.31	-0.28	0.39	0.00	0.31	**0.59**	-0.22	-0.01	0.17	-0.06	-0.08	0.05	0.12	**0.62**	0.48	**0.74**	1		
# bivalves	-0.21	-0.43	**-0.62**	**0.65**	0.13	0.42	0.35	-0.48	-0.23	-0.02	0.25	0.00	-0.26	0.08	**0.39**	0.22	**0.92**	0.48	1	
# crabs	0.09	0.44	0.33	-0.45	-0.08	0.01	-0.04	0.31	0.12	0.21	0.42	0.06	-0.40	-0.16	-0.23	-0.18	-0.21	0.00	-0.36	1
# rare individuals	-0.41	0.49	0.09	-0.12	0.36	-0.36	0.33	0.21	-0.09	-0.20	0.22	0.29	-0.23	**0.52**	0.20	0.39	0.27	0.02	0.25	-0.08
# major worms	-0.18	-0.31	-0.39	0.44	0.03	0.35	**0.50**	-0.27	-0.30	0.05	-0.40	0.14	0.40	0.16	**0.56**	0.46	**0.57**	**0.75**	0.30	-0.11
Surface to depth	-0.21	-0.39	**-0.59**	**0.55**	0.17	0.37	**0.57**	-0.58	-0.36	0.07	0.08	0.10	-0.08	0.17	0.56	0.38	**0.89**	**0.64**	**0.81**	-0.13
Depth to surface	-0.27	-0.14	-0.33	0.37	-0.04	0.22	0.49	-0.30	-0.16	0.18	0.03	0.00	-0.03	0.31	**0.58**	0.47	**0.80**	**0.77**	**0.59**	-0.04
deep	0.21	0.46	**0.62**	**-0.64**	-0.02	-0.46	0.23	0.40	0.17	0.15	0.06	-0.11	-0.05	0.05	0.03	0.14	-0.27	0.12	-0.47	**0.66**
permanent burrow	-0.46	0.08	-0.18	0.33	-0.08	0.16	-0.09	0.31	0.19	-0.34	0.24	0.12	-0.26	0.15	-0.03	0.05	0.15	0.03	0.17	-0.12
tube structure	-0.14	-0.48	**-0.63**	**0.58**	0.14	0.46	**0.62**	**-0.64**	-0.43	0.08	0.07	0.01	-0.07	0.05	**0.57**	0.38	**0.89**	**0.69**	**0.79**	-0.07
motile	-0.22	-0.39	**-0.59**	**0.57**	0.13	0.40	**0.60**	**-0.55**	-0.34	0.07	0.13	0.03	-0.13	0.15	**0.59**	0.42	**0.95**	**0.71**	**0.86**	-0.11
Bioturb	-0.20	0.01	-0.05	0.22	-0.32	0.06	0.02	-0.02	0.02	0.00	0.03	-0.27	-0.06	0.16	0.19	0.18	**0.51**	**0.27**	**0.53**	**-0.52**
Predator	0.20	0.05	0.29	-0.25	-0.54	-0.18	-0.30	-0.01	-0.11	**0.55**	0.09	-0.49	-0.11	-0.31	-0.25	-0.27	-0.06	0.03	-0.11	0.00
Grazer	-0.16	-0.12	0.05	0.03	-0.39	-0.13	-0.34	-0.09	-0.01	0.16	0.11	-0.13	-0.14	-0.16	-0.23	-0.24	0.00	-0.02	-0.02	-0.16
surface (epifauna)	-0.39	-0.14	-0.33	**0.50**	-0.06	0.14	0.34	-0.09	0.08	0.03	0.16	-0.09	-0.18	0.38	**0.51**	0.44	**0.82**	**0.67**	**0.74**	-0.41
deep	-0.21	-0.39	**-0.59**	**0.55**	0.17	0.37	**0.57**	-0.58	-0.36	0.07	0.08	0.10	-0.08	0.17	0.56	0.38	**0.89**	**0.64**	**0.81**	-0.13

Table AIV.4 Continued

	# rare individuals	*# major worms*	*Surface to depth*	*Depth to surface*	*deep*	*permanent burrow*	*tube structure*	*motile*	*Bioturb*	*Predator*	*Grazer*	*surface (epifauna)*	*deep*
# rare individuals	1												
# major worms	0.14	1											
Surface to depth	0.20	0.45	1										
Depth to surface	0.19	**0.64**	**0.96**	1									
deep	0.16	**0.78**	**0.73**	**0.85**	1								
permanent burrow	-0.12	-0.12	-0.29	-0.18	-0.02	1							
tube structure	0.23	-0.14	-0.13	-0.25	-0.27	-0.31	1						
motile	0.14	**0.61**	**0.91**	**0.96**	**0.76**	-0.12	-0.17	1					
Bioturb	0.21	**0.62**	**0.95**	**0.98**	**0.84**	-0.16	-0.12	**0.97**	1				
Predator	0.35	0.21	0.30	0.24	0.36	-0.43	0.32	0.21	0.33	1			
Grazer	-0.40	-0.27	-0.22	-0.18	-0.03	0.27	-0.15	-0.11	-0.12	0.20	1		
surface (epifauna)	-0.45	-0.28	-0.16	-0.17	-0.10	0.02	0.15	-0.10	-0.11	0.12	**0.80**	1	
deep	0.23	0.42	**0.59**	**0.57**	**0.69**	-0.29	0.34	0.49	**0.64**	**0.69**	0.08	0.15	1

Appendix V

Supplemental Materials for Chapter 6

Figure 6.1 References (in addition to the chapters of this dissertation)

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Appendix VI

Table AVI.1 Summary of denitrification rates reported from intact intertidal sediments world-wide and in New Zealand

Location	Denitrification Rate	Measurement Method	Study
Global			
Humber Estuary, UK	10 – 1007	Acetylene block	Barnes & Owens 1900
Taugus estuary, Portugal	0.0 – 250.0	Isotope Pairing	Cabrita and brotas 2000
Huon Estuary, Australia	0.3 – 3	Isotope Pairing	Cook et al. 2004
Gold Coast Australia	~20	N_2/Ar	Eyre and Ferguson 2011
Virgina Coast Reserve, VA, USA	9.09-275.8	N_2/Ar	Gonzalez et al. 2013
Wadden Sea, Netherlands	1.0 – 55.0	Acetylene block	Kieskamp et al. 1991
River Torridge, UK	1.04 – 11.56	Acetylene block	Koch et al. 1992
Wadden Sea, Germany	6.0 – 24	^{15}N tracer	Marchant et al. 2014
San Fransico Bay, CA, USA	1.6 – 2.4	Acetylene block	Oremland et al. 1984
Ago Bay, Japan	4.0 – 40.0	Gas Chromatograph	Patel 2008
Bogue Sound, NC, USA	13.1 – 92.5	N_2/Ar	Piehler & Smyth 2011
Western Port, Victoria, Australia	0.0 – 35	Isotope Pairing	Russel et al. 2016
Thames River, UK	0 – 19,616	Isotope Pairing	Trimmer et al. 2000
Yangtze Estuary, China	18.71 – 35.87	Acetylene block	Wang et al. 2007
Arcachon Bay, Fance	2.0 – 6.0	Isotope Pairing	Welsh et al. 2000

Table AV.1 Continued

New Zealand			
Tuapiro estuary, North Island	1.0 - 620	Acetylene block	Douglas et al.
Tokomaririo estuary, South Island	0.0 - 45	Isotope Pairing	Gongol and Savage 2016
Delaware Inlet, South Island	4.76 - 23.5	Acetylene block	Kaspar 1983
Mahurangi Harbour, North Island	38.03 - 127	N_2/Ar	O'Meara et al. 2020
Whangateau Harbour , North Island	0.0 - 320	N_2/Ar	Schenone & Thrush 2020
Whangateau Harbour , North Island	26.5 - 135.4	^{15}N tracer	Chapter 4
Mahurangi Harbour, North Island	1.2-110	N_2/Ar	Chapter 3
Okura Estuary, North Island	2.3 - 313	N_2/Ar	Chapter 5

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