Response and resilience of estuarine benthic ecosystems to anthropogenic pressures

A thesis
submitted in fulfilment
of the requirements for the degree
of
Doctor of Philosophy in Biological Sciences
at
The University of Waikato
by
TARN PAIGE DRYLIE

2020

"The first law of ecology is that everything is related to everything else"

- Barry Commoner

Abstract

Human activities are altering coastal environments at local and global scales with largely unknown consequences. Catchment land-use changes increase the rates of delivery of fine sediments and nutrients to estuaries, resulting in increased suspended sediment concentrations (SSC) and eutrophication; this thesis investigates the response and resilience of soft-sediment intertidal ecosystems to these two anthropogenic stressors.

Increased SSC elevates water-column turbidity and reduces the light available for benthic primary producers that sustain estuarine food webs. In intertidal habitats, this turbidity stressor is removed during low tide as the water-column uncovers the seafloor. In other words, turbidity is a temporally displaced stressor in intertidal environments. Photosynthesis during low tide periods of emergence may therefore provide resilience against elevated turbidity during periods of submergence. However, the role of low tide primary production (PP) in estuarine benthic foodwebs has largely been overlooked. Emerged and submerged benthic PP was measured in adjacent seagrass and microphytobenthos-dominated (sandflat) soft-sediment habitats at three locations along a turbidity gradient. Benthic chambers were used to measure the flux of CO_2 across emerged sediments, and dissolved O_2 across submerged sediments to derive net (NPP) and gross (GPP) PP. Emerged NPP and GPP were higher (2–16 times) than submerged in all instances ($p < 0.01$), and when standardised by mean incident photosynthetically active radiation, the difference between emerged and submerged seagrass PP increased with site turbidity (from 2 to 26 times greater). Emerged PP may be crucial for providing resilience against benthic productivity losses in turbid environments.

Eutrophication results in the deposition of excess organic material to soft-sediments and stimulates microbial respiration; this consumes O_2, potentially leading to hypoxia, and releases CO_2, causing localised acidification. I enriched intertidal sediments with increasing quantities of organic matter to identify the effects of eutrophication-induced acidification on benthic structure and function, and assessed whether biogenic calcium carbonate ($CaCO_3$) would alter the response. Declines in macrofauna biodiversity (abundance and species richness), reduced benthic NPP and impaired nutrient cycling occurred along the eutrophication gradient. $CaCO_3$ did not alter the macrofaunal response, but significantly reduced negative effects on function (e.g. net autotrophy occurred at higher levels of organic enrichment in $CaCO_3$-treated plots than controls (1400 vs 950 g dw m^{-2})). This study represents a crucial step forward in understanding the ecological effects of coastal acidification and the role of biogenic $CaCO_3$ in moderating responses.

Ecological theory states that the resilience of ecosystem functions to environmental disturbance depends on the biodiversity that underpins them, but field validations of this are lacking. I explored the shifts in sandflat macrofaunal community composition following organic matter enrichment, and the consequences for the representation and composition of functional groups (FG). Taxa-specific sensitivities to enrichment resulted in significant response diversity which provided resilience to FG, but substantial declines in the abundance of dominant taxa meant they became functionally extinct nevertheless. Density compensation occurred in one FG, but overall community abundance decreased by 80 % between low and high levels of enrichment. Evidence of density-dependent relationships between ecosystem function and biodiversity were discovered, highlighting the need to conserve macrofaunal biodiversity to preserve function despite the existence of stabilising mechanisms.

This thesis demonstrates the existence of mechanisms that provide resilience against increased SSC and eutrophication in benthic ecosystems, but highlights that the structure and function of these systems remain sensitive nevertheless.

Preface

The main body of this thesis comprises three research chapters (Chapters 2–4) which have been published, or are currently in preparation for publication, in peer-reviewed scientific journals. As the chapters are written as stand-alone articles there is some repetition in the methods (specifically in Chapters 3 and 4). I assumed responsibility for the fieldwork, laboratory analyses, data analysis and writing of this thesis and the material within was produced from my own ideas except where referenced. This work was undertaken under the supervision of Professor Conrad Pilditch, Dr Hazel Needham and Dr Adam Hartland from the University of Waikato, and Dr Andrew Lohrer from the National Institute of Water and Atmospheric Research Ltd. (NIWA).

Chapter 2 has been published by *Journal of Sea Research* (2018) under the title "Benthic primary production in emerged intertidal habitats provides resilience to high water column turbidity" by T.P. Drylie, A.M. Lohrer, H.R. Needham, R. H. Bulmer and C.A. Pilditch. DOI: 10.1016/j.seares.2018.09.015

Chapter 3 has been published by *Scientific Reports* (2019) under the title "Calcium carbonate alters the functional response of coastal sediments to eutrophication-induced acidification" by T.P. Drylie, H.R. Needham, A.M. Lohrer, A. Hartland and C.A. Pilditch. DOI: 10.1038/s41598-019-48549-8

Chapter 4 is currently in preparation for publication by *New Zealand Journal of Marine and Freshwater Research*, under the title "Resilience of benthic macrofauna to experimental organic enrichment: Consequences for ecosystem function" by T.P. Drylie, A.M. Lohrer, H.R. Needham and C.A. Pilditch.

I also contributed to a publication that accompanies Chapter 2 and is not included in this thesis, published by *Frontiers in Marine Science* (2018) under the title "Elevated turbidity and the nutrient removal capacity of seagrass" by R.H. Bulmer, M. Townsend, T.P. Drylie and A. M. Lohrer. DOI: 10.3389/fmars.2018.00462

Acknowledgements

Ehara taku toa, he takitahi, he toa takitini

My success should not be bestowed onto me alone, as it was not individual success but success of a collective

There are so many people who have contributed to the completion of this thesis, but no one more than Professor Conrad Pilditch. Thank you for your academic guidance, personal encouragement and for building such a welcoming, supportive and positive research environment. Thank you also to Dr Hazel Needham, aside from your academic knowledge and unwavering commitment to helping me produce great quality work, you have been a fantastic female mentor and have supported me at all times. Huge thanks to Dr Drew Lohrer, your expertise has been invaluable throughout the development and execution of my research. Thank you for always being available and adding a much-needed calm to my PhD experience with your chilled vibes! Thank you to Dr Adam Hartland for explaining the basics of biogeochemistry to me, and for always offering valuable words of advice and feedback. I could not have hoped for a better supervisory panel.

I express my gratitude to everyone who assisted me in the field, especially those who worked through not one but two cyclones! Particularly I would like to thank Dean Sandwell, Dudley Bell, Warrick Powrie and the NIWA Benthic Ecology team for your wealth of knowledge and instilling in me a confidence in my practical capabilities. Thanks also go to Rebecca Gladstone-Gallagher, Bradley Monahan, Steph Mangan, Vera Rullens, Grady Petersen, Jane Cope, Ryan Koverman, Kate Rogers and Georgina Flowers.

My sincerest thanks go to the team 'behind-the-scenes': Gloria, Fiona and Vicki in the Science Administrators office, you always have the answers to my questions and it has been a pleasure getting to know you all; Anthea Kivell in the Research and Enterprise office, who made the stressful topic of money completely stress-free; Tanya Mete, for all your assistance in making my enrolments and changes to conditions run smoothly; and Cheryl Ward, without whom I would have spent days and tears formatting this thesis! You are all so valued.

Thank you to all of the benthic ecology students and post-docs, you have been an overflowing source of optimism, knowledge and laughs and I cannot imagine getting to this point without you. Special mentions are required for Dr Rebecca Gladstone-Gallagher (I’m lucky to have ‘roomed’ with someone who has the same internal temperature and is as equally addicted to tea as me) and Dr Emily Douglas, for mentoring me through my PhD.

To Mum, Dad and Ayla. No words can do justice to how grateful I am to always have your love and support behind me. You are my personal cheer-leaders yet you keep me grounded, and nothing has been more testing throughout this journey than undertaking it so far from you. Thank you for providing me with the foundations that have allowed me to reach this milestone. To my wonderful partner Matt, you’ve been everything I’ve needed at all times; an ocean of calm, my Mr Motivator and a happy distraction. I am so appreciative of all the support you have given me.

I would lastly like to acknowledge and show my gratitude to the National Institute of Water and Atmospheric Research (NIWA) for awarding me the doctoral scholarship that allowed me to undertake this study and also supporting Dr Drew Lohrer’s time (Coasts and Oceans Research Programme 5, Managing Marine Ecosystems, COME1902), and the Ministry for Business, Innovation and Employment (MBIE) for supporting Prof. Conrad Pilditch and Dr Adam Hartland’s (MBIE contract UOWX1602).

Table of Contents

List of Figures

List of Tables

CHAPTER 1: General Introduction

1.1 Background

Estuaries represent one of the most ecologically, socially and economically important environments in the world, despite their 180 million ha area making up less than 1 % of the global marine environment (Costanza *et al.*, 2014). The biotic communities associated with these environments contribute to the provision of human-centred benefits, 'ecosystem services', including climate regulation, food production and cultural values (Snelgrove *et al.*, 2014). Ironically, the continued delivery of these services is threatened by human activities that degrade the underlying ecosystem functions and jeopardise their stability (Millennium Ecosystem Assessment, 2005).

Ecological stability refers to the ability of an ecosystem to resist change, or return to equilibrium conditions, following a disturbance (Connell & Sousa, 1983). Research at the turn of the Century highlighted the complexity of interactions within ecological systems as a key factor determining stability (Polis, 1998). Biological diversity (biodiversity) represented a key component of this complexity (Yachi & Loreau, 1999). Biodiversity may contribute to ecological stability by increasing the resistance of the ecosystem to a disturbance, defined as the ability of the ecosystem to experience a disturbance without exhibiting any loss in its functionality. This is because as the biodiversity underpinning ecosystem functions increases, so too does the probability that at least one taxon will be resistant to a given disturbance. Alternatively, biodiversity may increase the resilience of the ecosystem, i.e. its ability to recover from a disturbance after incurring losses in functionality (Lake, 2016). The spatial scales over which functionally important taxa exist are likely to be a key factor affecting the resilience of an ecosystem (Peterson, Allen & Holling, 1998). Collectively, theories that link biodiversity and ecosystem functions are known as biodiversity–ecosystem functioning (B–EF) hypotheses (Naeem et al. 1994; 1995).

Ecological systems are additionally dependent upon interactions between the biological, chemical and physical elements they encompass. For example, in estuarine sediments, the resident macrofauna (macroscopic animals >500 µm; Schlacher & Woolridge, 1996), benthic autotrophs and microbial communities control the movement and transformation of oxygen, organic material and nutrients, and connect sedimentary processes to the overlying seawater (benthic–pelagic coupling) (Hargrave, 1973; Sullivan *et al.*, 1991; Welsh, 2003). These biotic components of the ecosystem interact in numerous fundamental ways; for example, benthic microphytes (microphytobenthos; MPB) produce oxygen that supports all aerobic lifeforms and labile organic material that feeds macrofaunal grazers (Miller *et al.*, 1996) (Figure 1.1a). This oxygen additionally fuels the microbial remineralisation of dissolved and particulate organic matter into ammonium (ammonification) and nitrate (nitrification), which may then be converted by anaerobic microbes into biologically unavailable nitrogenous gas (denitrification) (Seitzinger, 1988; An & Joye, 2001) or be assimilated by MPB. Through their maintenance of burrows and churning of sediments, macrofauna can enhance the primary productivity (PP) of MPB by promoting the release of porewater nutrients (Lohrer *et al.*, 2010), and stimulate coupled nitrification–denitrification processes by substantially increasing habitat complexity and the surface area of oxic–anoxic interfaces (Binnerup *et al.*, 1992; Laverock *et al.*, 2011) (Figure 1.1b).

The nature of ecological interactions are often difficult to predict due to their context-specificity; for example, the influence of a burrowing mud crab on benthic biogeochemical processes varies depending on the sediment type it inhabits (Needham *et al.*, 2011), and the effects of key macrofaunal taxa on ecosystem functions may depend on the composition of the rest of the assemblage (Rossi *et al.*, 2008). Actually, any modifications may alter the complex interaction networks that govern the entire ecosystem (Thrush *et al.*, 2012). Disturbances (i.e. changes to environmental conditions) that are sufficiently strong to alter the interactions within benthic ecosystems and result in the crossing of critical ecological boundaries, i.e. ‘tipping points’, can push the system into an alternative state, termed a regime shift, that is difficult or impossible to return from (Scheffer &

Carpenter, 2003; Thrush *et al.*, 2014). The accumulation of anthropogenic stressors in estuarine ecosystems means the potential for regime shifts is increasing.

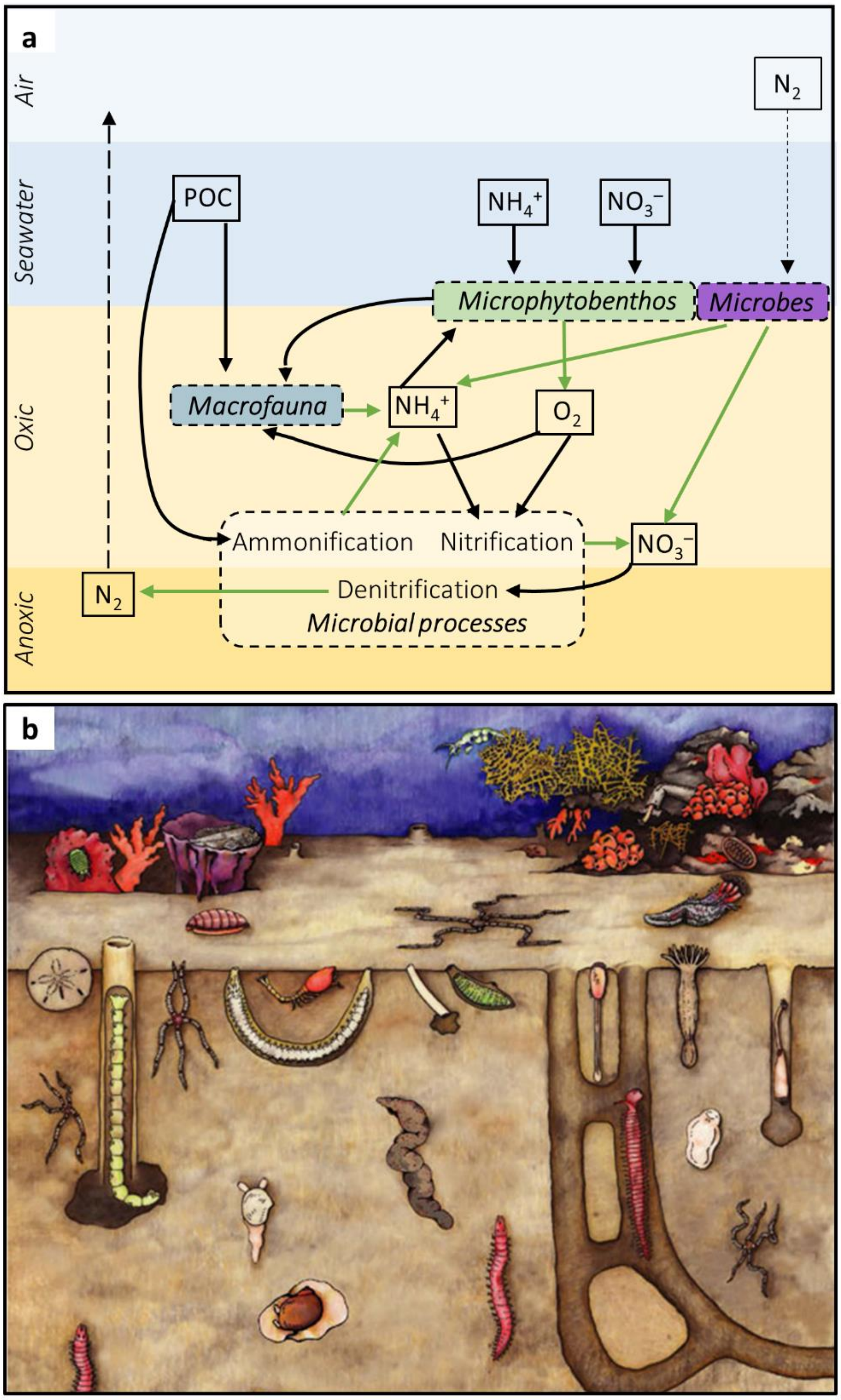


Figure 1.1. a) Simplified diagram of interactions occurring in estuarine sediments (oxic and anoxic layers) and overlying seawater. Black arrows = consumption, green arrows = production, dashed arrows = diffusion. POC = particulate organic carbon. b) Cross-section of estuarine sediments depicting the habitat complexity created by benthic macrofauna (adapted from NOAA, https://graysreef.noaa.gov).

1.1.1 Anthropogenic stressors

Anthropogenic stressors can be defined as environmental and biotic factors that exceed their natural ranges of variation due to human activities (Sanderson *et al.*, 2002). Evidence is mounting that every ecosystem on Earth, from the highest mountain peak (Figueroa, 2013) to the deepest ocean trench (Chiba *et al.*, 2018) has been impacted by humans. The tendency for human populations to concentrate in coastal areas means negative anthropogenic effects on the marine environment are felt most strongly in transitional estuarine habitats than any other (Dolbeth *et al.,* 2007). For example, the growth of coastal human populations requires greater infrastructure and urban development, the consequences of which include habitat modification, chemical contamination, toxic metal pollution, increased nutrient and sediment loading, and intensified fishing activity, among others (Turner *et al.*, 1999; Lotze & Milewski, 2004; Halpern *et al.*, 2008). The impacts of humans are, however, not constrained to these local geographical scales, as anthropogenic perturbations of global biogeochemical cycles mean broad-scale stressors such as sea level rise (SLR), global warming, ocean acidification (OA) and increased storm events are also of growing concern (Doney *et al.*, 2012). In New Zealand, the loading of terrestrially-derived sediments and nutrients are the greatest anthropogenic stressors of estuarine ecosystems (Thrush *et al.*, 2004; Heggie & Savage, 2009).

Increased input rates of terrestrial fine silts and clays (otherwise referred to as mud) have resulted from increased erosion due to deforestation and large-scale pastoral farming in New Zealand (Page *et al.*, 2000; Swales *et al.*, 2002; Thrush *et al.*, 2004). The short-term smothering of estuarine sediments by even minor depositions of mud (≤ 5 mm) can impact the structure of benthic macroinvertebrate communities and alter sediment biogeochemistry, for example decreasing subsurface oxygenation (Rodil *et al.*, 2011; Hohaia *et al.*, 2014). In the longer term, the gradual shift in grain size distribution to finer, more muddy sediments has well-documented consequences for benthic community composition, generally resulting in lower diversity and an increasing dominance of short-lived taxa over those that are large and functionally important (Beukema, 1976; Thrush *et al.*, 2003; Anderson, 2008). Furthermore, increases in suspended sediment concentrations (SSC) can interfere directly with suspension feeding animals, reducing physiological condition (Ellis *et al.*, 2002). Through reducing the penetration of light due to scattering by suspended particles (i.e. elevating turbidity), increases in SSC also limit the light available for photosynthesis at the seafloor by seagrasses, macroalgae and MPB (Joint & Pomroy, 1981; Colijn *et al.*, 1987; Ruiz & Romero, 2003).

Seagrasses represent a visible primary producer in temperate estuaries, forming meadows that are valued for their carbon storage properties (Fourqurean *et al.*, 2012; Spivak *et al.*, 2019), provision of nursery areas (Heck *et al.*, 2003) and their high productivity (Hillman *et al.*, 1989). Wide-spread losses of subtidal seagrass meadows in New Zealand have been attributed to light-limitation caused by increased SSC (Park, 1999; Bulmer *et al.*, 2015a), and similar trends have been observed worldwide (Longstaff & Dennison, 1999; Newell & Koch, 2004). Macroalgae do not constitute a spatially dominant/important habitat in New Zealand estuaries and have therefore not been a focus of my thesis.

Microphytobenthos are a central component of estuarine ecosystems that influence numerous ecological functions (see Figure 1.1a). They account for between 50 and 80 % of overall estuarine PP (Underwood & Krompkamp, 1999; Jones *et al.*, 2017), constituting a major labile food source for deposit-feeding grazers and suspension feeders alike, and can play a significant role in supporting distant food webs through their export (Duarte & Cebrián, 1996; Miller *et al.*, 1996). On intertidal sandflats elevated turbidity reduces MPB PP, which can result in a significant efflux of nutrients from the sediment into the overlying seawater (Pratt *et al.*, 2014b). These nutrients may fuel water column PP (Moore *et al.,* 1996).

The overloading of nutrients to coastal systems can result in eutrophication, the phenomenon of excessive water-column algal production and subsequent delivery of organic carbon to the benthos (Nixon, 1995). The deposition of dead algae stimulates sediment microbial respiration, thus increasing the concentration of carbon dioxide (CO_2) and driving coastal acidification (Wallace *et al.*, 2014). Hypoxia (defined as dissolved O_2 levels ≤ 2 ml L^{-1}) usually accompanies this rise in CO_2 as a consequence of aerobic respiration (Howarth *et al.*, 2011). As the continued absorption of atmospheric CO_2 drives down seawater pH globally (OA; Eisler (2012)) and the frequency of eutrophic events increases, resultant coastal acidification will become more pervasive (Melzner *et al.*, 2013). What this means for estuarine sediments and their resident communities is unclear, as early assumptions that infaunal taxa would be tolerant to conditions of lower pH owing to their inhabiting naturally low O_2, high CO_2 environments have not been widely verified. Additionally, only a few studies have focussed on the response of non-calcifying organisms (see review by Bhadury (2015)). However, a mesocosm study by Widdicombe *et al.* (2009) revealed significant alterations in the structure and diversity of polychaete and nematode assemblages under different levels of acidified seawater. According to the biodiversity–ecosystem function hypothesis, alterations in community composition are likely to affect ecosystem function, as biodiversity and function are intrinsically linked (Nixon, 1995; Hooper *et al.*, 2005). Despite this, coastal eutrophication has only recently been studied in the context of biodiversity–ecosystem function relationships, and a focus on the associated hypoxic stress dominates (Diaz & Rosenberg, 2008; Villnäs *et al.*, 2011; Gammal *et al.*, 2017; Norkko *et al.*, 2019).

1.1.2 Ecosystem resilience

The occurrence of anthropogenic pressures undermines the ability of ecosystems to function in a way that ensures delivery of the services humans obtain from them. However, benthic ecosystems are inherently resilient owing to the naturally high levels of disturbance they experience (Thistle, 1981; Hall, 1994), and mechanisms through which estuarine habitats may exhibit resilience to the pervasive stressors of elevated turbidity and eutrophication-induced acidification have been identified. In intertidal ecosystems the pressures associated with elevated turbidity are only experienced by the benthos when the tide is in, as this stressor is displaced at low tide. Relatively little is understood about the functioning of benthic communities during such periods of emergence compared to submergence, with only a handful of studies investigating the carbon budget and productivity of specific locations using CO_2 flux measurements (Migné *et al.*, 2004; Spilmont *et al.*, 2007; Ouisse *et al.*, 2011; Kwon *et al.*, 2014; Bulmer *et al.*, 2015b). However, the indication from these studies is that productivity in intertidal sediments may vary according to tidal state, and that emerged sediments can be highly productive (Hargrave *et al.*, 1983; Schwarz, 2004). To fully comprehend the implications of elevated turbidity for benthic ecosystems a more thorough understanding of how they function when emerged is required, as the regular displacement of this stressor may allow compensatory autotrophic processes to take place.

A characteristic of coastal zones that may naturally afford them resilience against eutrophication-induced acidification is the prevalence of carbonate sediments: the coastal ocean contains 50 % of oceanic $CaCO_3$ deposits, despite covering only 7 % of its total area (Gattuso *et al.*, 1998). Calcium carbonate tends towards dissolution under acidic conditions due to a decrease in the concentration of carbonate ions (CO_3^{2-}) as they react with the increasing concentrations of hydronium ions (H_3O^+) formed by the rapid dissociation of carbonic acid (H_2CO_3) (Equations 1–3). This reduces the $CaCO_3$ saturation state (Ω) according to Equation 4 (where K_{SP} is the solubility product for the given $CaCO_3$ polymorph (calcite, aragonite or magnesian-calcite)), below which dissolution is favoured. When $CaCO_3$ dissolution occurs, there is an increase in the capacity of porewaters to buffer H_3O^+ owing to an increase in HCO_3^- (Equation 5), thus $CaCO_3$-rich sediments may be able to buffer fluctuations in pH (Equation 6) (Stumm & Morgan, 2012).

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \qquad \text{(Equation 1)}$$

$$\leftrightarrow HCO_3^- + H_3O^+ \qquad \text{(Equation 2)}$$

$$\leftrightarrow CO_3^{2-} + H_3O^+ \qquad \text{(Equation 3)}$$

$$\Omega_{CaCO3} = \frac{[Ca^{2+}][CO_3^{2-}]}{K_{SP}} \qquad \text{(Equation 4)}$$

$$CaCO_3 + H_3O^+ \leftrightarrow HCO_3^- + H_2O + Ca^{2+} \qquad \text{(Equation 5)}$$

$$pH = -\log[H_3O^+] \qquad \text{(Equation 6)}$$

If so, sedimentary conditions may be improved (e.g. made less acidic) for the resident biotic communities allowing the interactions that underpin ecosystem functions to be maintained (Clements & Chopin, 2017). Thus far, the potential for $CaCO_3$ buffering to provide resilience to benthic ecosystems against acidification has only been investigated by Rodil *et al.* (2013)), with inconclusive results, despite an early recognition that sediment $CaCO_3$ dissolution occurs as a result of metabolic CO_2 production (Walter & Burton, 1990; Hales *et al.*, 1994).

In addition to the potential resilience afforded by $CaCO_3$ dissolution, eutrophication -induced stress to benthic ecosystems may not result in declines in ecosystem function if the macrofaunal community exhibit resistance and/or redundancy. Individually, taxa may exhibit resistance to a stressor (they are able to withstand the disturbance) or sensitivity (they are excluded from the area when the disturbance occurs); however even if some taxa are sensitive, the community as a whole may be resilient if it contains redundancy. In theory, diverse ecosystems contain multiple taxa that perform the same function; therefore, some of these taxa can be lost without any declines in functionality being observed (i.e. there is redundancy) (Naeem, 1998). The implication is that ecosystem functions become more stable as the biodiversity of an ecosystem increases. Empirical evidence has come to support this (Tilman *et al.*, 2006; Worm *et al.*, 2006), though the mechanisms that underpin this phenomenon are still debated (Loreau & de Mazancourt, 2013).

Macrofauna contribute to ecosystem functions directly (e.g. they are a food source for higher trophic levels and initiate the decomposition of organic matter (Virnstein *et al.*, 1983; Welsh, 2003)) and indirectly (e.g. by facilitating microbial processes responsible for transforming organic matter into nutrients (Kristensen & Kostka, 2013)), and their loss from soft-sediments has been related to a decrease in ecosystem function (Kristensen & Blackburn, 1987; Danovaro *et al.*, 2008; Lohrer *et al.*, 2010; Norkko *et al.*, 2013). A long history of study has enabled a thorough understanding of how benthic macrofaunal communities respond to organic matter enrichment, a consequence of eutrophication, with declines in diversity and a proliferation of small opportunistic taxa demonstrated almost globally (Pearson

& Rosenberg, 1978; Gray *et al.*, 2002). The consequences of such shifts for the functional composition of the macrofaunal assemblage are not well-understood, however, which limits the ability to predict the resilience of ecosystem functions to eutrophication.

1.2 Rationale

The anthropogenic degradation of estuaries is threatening the invaluable number of provisioning, regulatory, supporting and cultural services they provide, and is set to accelerate in the future. The response of benthic ecosystems to elevated SSC during submergence are well-documented; however, the period of emergence has been largely overlooked in benthic ecology research and represents a potential pathway providing resilience to benthic PP in turbid locations. Future world scenarios predict that eutrophication and associated decreases in porewater pH will occur more frequently and occur against a backdrop of global OA; biogenic $CaCO_3$ may provide a mechanism that reduces the negative effects of acidification on benthic ecosystems, though the effects of eutrophication-induced acidification on temperate carbonate sediment ecosystems are unknown. The aim of my thesis was therefore to develop an understanding of the ecological functioning of benthic ecosystems when emerged and determine the effects of eutrophication-induced acidification on their structure and function.

Benthic processes such as photosynthesis and macrofaunal bioturbation exert large controls on the flux of dissolved nutrients and O_2 across the sediment–seawater interface (Miller *et al.*, 1996; Sundback *et al.*, 2000; Braeckman *et al.*, 2010). As such, solute fluxes are often measured to indicate benthic ecosystem functions pertaining to productivity, metabolism and nutrient recycling (Berelson *et al.*, 1998; Webb & Eyre, 2004; Karlson *et al.*, 2007; Lohrer *et al.*, 2013). I used this approach in Chapter 2 to investigate the contributions of submerged and emerged PP to total benthic PP, and as I expected that $CaCO_3$ would alter the response of ecosystem functions to eutrophication-induced acidification, solute fluxes were also measured in Chapter 3.

1.3 Thesis overview

The main body of this thesis comprises three studies of the response and resilience of estuarine benthic ecosystems to two major stressors: elevated turbidity from increased sediment loading and organic matter over-enrichment from nutrient-induced eutrophication (Figure 1.2). The functional importance of emerged intertidal sediments in alleviating the temporally displaced stressor of turbidity through autotrophic pathways is investigated through field observations (Chapter 2), and the buffering of sediment pH by $CaCO_3$ deposits (Chapter 3) and the resilience of macrofaunal functional diversity (Chapter 4) against organic enrichment is explored via a manipulative field experiment.

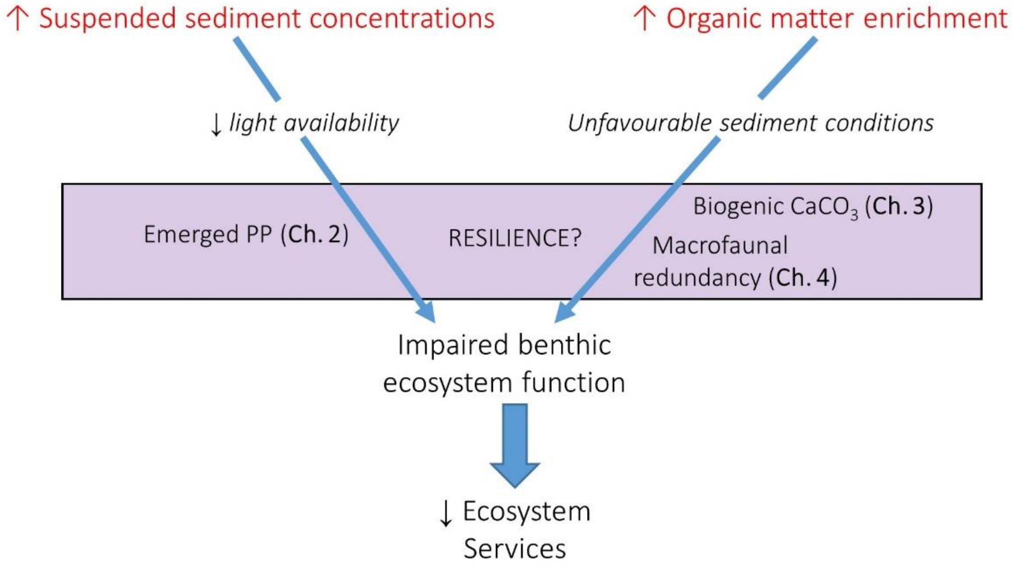


Figure 1.2. Conceptual diagram illustrating the linkages between local anthropogenic stressors (increasing suspended sediment concentrations and organic matter enrichment) and ecosystem services, with the potential resilience mechanisms that form the basis for each research chapter highlighted. PP = primary production.

1.3.1 Chapter 2

In Chapter 2, the effect of increasing water column turbidity on submerged and emerged benthic PP was investigated in sandflat habitats dominated by MPB and seagrass (*Zostera muelleri*) to determine whether the period of emergence could offer resilience to intertidal habitats. The development of a method that enabled comparable measurements to be taken during both tidal states was required, which is detailed in Appendix 1. Fluxes of gaseous CO_2 (emergence) and dissolved O_2

(submergence) were measured at three locations within a single estuary that varied in turbidity levels using benthic incubation chambers that enabled the whole community (shoots, roots and rhizomes) to be considered. From these measurements the relative importance of emerged PP was determined, and trends between PP and site turbidity were highlighted for both tidal states. This study reported comparable measurements of benthic community PP from intertidal habitats during emerged and submerged conditions for the first time, and contributed to bridging the gap in understanding emerged benthic ecosystem functioning.

1.3.2 Chapter 3

In Chapter 3, I conducted a field experiment investigating the ability of biogenic $CaCO_3$ to maintain intertidal benthic ecosystem structure and function along a gradient of eutrophication-induced acidification. Organic matter enrichment treatments (0–2250 g dw m^{-2}, increasing in 250 g increments) formed the eutrophication gradient, and enriched plots were allocated one of two $CaCO_3$ treatments (with or without the addition of 2000 g m^{-2}). After 62–70 d of enrichment treatment effects on sediment properties, macrofauna community indices and key ecosystem functions (e.g. NPP and nutrient processing), derived from solute fluxes measured in benthic incubation chambers, were assessed. This study enabled me to demonstrate the complex linkages between eutrophication, sediment biogeochemistry and ecosystem function *in situ* and highlighted the variable ability of $CaCO_3$ to preserve function.

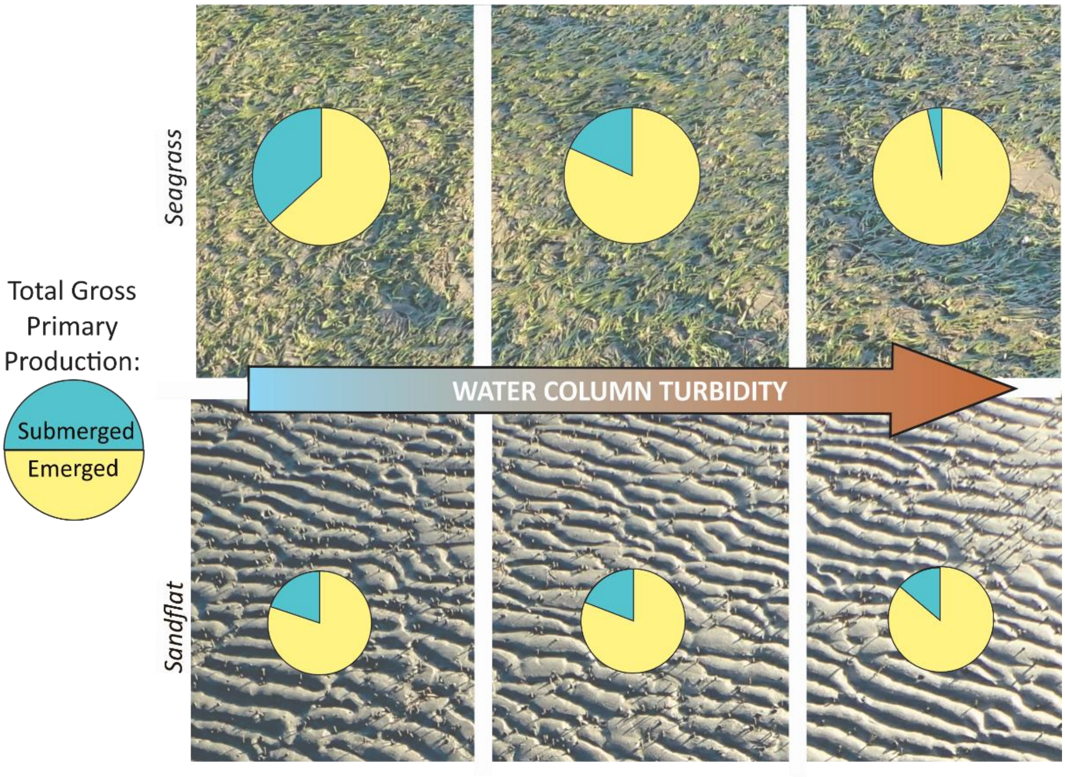
1.3.3 Chapter 4

Chapter 4 drew from the field experiment conducted in Chapter 3 and examined the shifts in macrofaunal community composition along the gradient of organic matter enrichment. The sensitivities of common individual taxa were modelled and analyses of the taxonomic and functional composition of the whole community identified assemblage shifts in response to this stressor. Response diversity and density compensation within functional groups were explored and found to provide resilience to functional diversity, despite taxa-specific sensitivities driving declines in ecosystem functions. This study builds on previous laboratory-based research focussed predominantly on single-taxa responses to decreases in sediment pH, and contributes empirical evidence of the link between biodiversity and ecosystem function.

CHAPTER 2:

Benthic primary production in emerged intertidal habitats provides resilience to high water column turbidity

2.1 Graphical abstract



2.2 Introduction

Estuarine ecosystems are highly dynamic and experience natural fluctuations in water clarity due to changes in suspended particle concentrations. Riverine inputs of sediment following rainfall events, wind and wave driven resuspension, and tidal transport of sediments from one location to another can all alter suspended sediment concentrations (SSC) over short time scales (Green & Coco, 2007; Talke & Stacey, 2008; Seers & Shears, 2015). Anthropic catchment development has however led to elevated inputs of fine terrigenous sediments into waterways (Thrush *et al.*, 2004; Seers & Shears, 2015), resulting in consistently high levels of turbidity in some estuaries. Elevated turbidity can significantly reduce light penetration through the water column thus limiting benthic primary productivity (Gameiro *et al.*, 2011). In New Zealand, and many temperate estuaries, the dominant benthic primary producers are MPB and seagrass meadows (e.g. Turner and Schwarz (2006a) and Jones *et al.* (2017)). Significant decreases in primary production (PP) in sandflat habitats (Pratt *et al.*, 2014b) and biomass losses in seagrass habitats (Longstaff & Dennison, 1999) have been associated with elevated turbidity, which has negative implications for the benthic food webs they underpin. Nevertheless, films of microphytobenthos (MPB) and seagrass meadows continue to inhabit persistently turbid intertidal environments, emphasising the potential role of PP during periods of tidal exposure (emergence) in sustaining these habitats (Schwarz, 2004).

Microphytobenthos communities reside in the upper millimetres of sediments and often dominate intertidal sandflats (Stephenson *et al.*, 2019). Where present, they can contribute up to 80 % of total benthic PP (Underwood & Krompkamp, 1999; Jones *et al.*, 2017) and play important biological and physical roles through oxygenating surface sediments (e.g. Baillie (1986) and Larson and Sundback (2008)), retaining nutrients within the benthos (e.g. Bautista and Paerl (1985) and Sundback *et al.* (2000)) and stabilising sediments (e.g. Yallop *et al.* (1994) and Lelieveld *et al.* (2003)). Additionally, benthic consumers exhibit a strong preference for MPB as a food source over macrophyte detritus (originating from seagrass, mangrove or saltmarsh), owing to a higher quality of organic material and greater palatability (Miller *et al.*, 1996), such that benthic secondary production can be largely supported by MPB (Cahoon & Safi, 2002; Evrard *et al.*, 2012; Jones *et al.*, 2017).

Seagrass meadows may also thrive in temperate estuarine environments, providing high rates of carbon storage and habitat for fish, bird and invertebrates via their structural characteristics (Heck *et al.*, 2003; Fourqurean *et al.*, 2012; Parsons *et al.*, 2013). Seagrass habitats are recognised as one of the most ecologically and economically valuable marine ecosystems (Costanza *et al.*, 2014), however anthropogenic pressures such as eutrophication, sedimentation and climate change continue to drive a worldwide decline in meadow area of approximately

7 % yr^{-1} (Waycott *et al.*, 2009). In intertidal regions, seagrass beds often exhibit higher PP than MPB due to a greater biomass of photosynthesising tissues, in addition to associated epiphytes and benthic MPB within seagrass habitats (Moncreiff *et al.*, 1992). *Zostera muelleri* is the only species of seagrass found in New Zealand and is most abundant in estuarine intertidal regions following large declines of subtidal populations between 1959 and 1996, likely in response to reductions in water clarity (Park, 1999; Turner & Schwarz, 2006b).

The distribution of marine autotrophs is influenced by light availability, depending specifically on whether photosynthesising tissues consistently receive light intensities allowing for photosynthetic gains in excess of metabolic costs. The minimum light requirements of intertidal MPB and *Z. muelleri* as reported in the literature are highly variable (Gattuso *et al.*, 2006; Lee *et al.*, 2007), possibly because of site-specific adaptations to varying light regimes (via physiological (Kromkamp *et al.*, 1998; Kohlmeier *et al.*, 2014b), morphological (Park *et al.*, 2016) and/or behavioural (Mitbavkar & Anil, 2004) adaptations). Despite this potential for adaptation, short-term increases in water-column turbidity caused MPB net primary productivity (NPP) during submergence to decrease three-fold in field experiments, with even greater reductions when photosynthetic efficiency (NPP µg Chl a^{-1}) was considered (Pratt *et al.*, 2014b). Similarly, losses in seagrass biomass have frequently been associated with reductions in light availability (Longstaff & Dennison, 1999; Ruiz & Romero, 2001).

A tipping point from one ecosystem state to another can occur if turbidity is frequently high and submerged benthic PP is lost, such that a shift from a clear, ecologically diverse system dominated by benthic PP towards a turbid system dominated by water column PP arises (Fisher *et al.*, 1988). This is often characterised by a loss of submerged vegetation and benthic diversity, resulting in negative feedbacks as nutrients are not intercepted at the sediment-water interface and thus continue to fuel water column PP (Moore *et al.*, 1996; Pratt *et al.*, 2014b). In addition, sediment mud content (particles < 63 µm) and water-column turbidity are often positively correlated, as the fine particles are easily resuspended by wind and wave action. Evidence from New Zealand suggests that high sediment mud content is associated with lower Chl *a* content (a proxy for MPB abundance) (Cahoon *et al.*, 1999), and reduced rates of biomass-corrected gross primary productivity (GPP) in MPB-dominated sediments (Pratt *et al.*, 2014a). To obtain meaningful data on the impact water column turbidity has on benthic PP it is therefore key that the effects of turbidity and sediment mud content are separated.

Despite the negative effects observed during submergence, turbidity in intertidal environments may be considered a temporally displaced stressor, as during periods of emergence the light attenuating effect of suspended sediments is removed. This period of emergence may therefore be critical for benthic PP should light levels during submergence consistently fall below minimum requirements for photosynthesis. It is predicted that the relative contribution of emerged PP to total benthic PP would increase in significance along a gradient of increasing water column turbidity, as contributions from submerged PP decrease. In such cases, highly turbid locations would exhibit great dependence on emerged PP to maintain function. Indeed, Vermaat & Verhagen (1996) demonstrated positive net photosynthesis occurred in seagrass habitats primarily during daylight low tides in turbid estuaries, and predictions that emergence may provide a mechanism to avoid negative impacts of turbidity were made (Vermaat *et al.*, 1997). More recently, Schwarz (2004) showed in a New Zealand estuary that during winter almost all seagrass PP occurred whilst plants were exposed, but concluded that emergence was not essential to the maintenance of these beds due to the presence of a subtidal fringe. However, few direct comparisons of *in situ* submerged and emerged seagrass community PP have been made (Ouisse *et al.*, 2011). Moderate information does exist on PP of MPB communities during emergence, originating mainly from studies in Europe (Migné *et al.*, 2004; Spilmont *et al.*, 2006; Spilmont *et al.*, 2007; Migné *et al.*, 2009), however direct comparisons of *in situ* submerged and emerged community PP are again scarce (Migné *et al.*, 2018). To my knowledge, in neither seagrass nor sandflat habitats has there been a simultaneous investigation of submerged and emerged PP in response to increasing site turbidity; such studies are crucial to understand the role of emerged sediments as potential refuges for benthic PP in light of globally increasing SSC.

In this study, the effect of increasing water column turbidity on submerged and emerged benthic PP was investigated in sandflat habitats dominated by MPB and *Z. muelleri* beds in a temperate New Zealand estuary. The PP of the whole community is considered through use of benthic incubation chambers that encompass patches of seagrass habitat (containing shoots, roots and rhizomes), and all associated organisms. The relative importance of emerged PP was determined, providing insight into whether the period of emergence can offer resilience to intertidal habitats against increases in site turbidity, and trends between PP and site turbidity were highlighted for both tidal states. If compensating for increased turbidity, I expected emerged PP to be appreciably greater in turbid locations than at clear locations.

2.3 Methods

The study was conducted in the southern branch of the Kaipara Harbour, New Zealand. The harbour is one of the largest estuaries in the world (947 km^2) of which 43 % is intertidal sand and mudflat (Heath, 1975). Water quality sampling has indicated turbidity in the harbour can range from < 5 to ~150 NTU (Cumming, 2016). Unvegetated sediments dominate the intertidal, with extensive meadows and patches of seagrass also present that at times extend into the subtidal (Hewitt & Funnell, 2005). Based on information gathered during routine monitoring of multiple mid-intertidal sites in the harbour (Hailes & Carter, 2016) and access to catchment sediment dispersion models (Lohrer, 2017), three sites were selected to span an expected gradient in turbidity. Mairetahi Creek (MAI; 36°32’51” S, 174°21’39” E) was chosen to have the highest turbidity, Kakaraia Flat (KKF; 36°25’9” S, 174°23’37” E) an intermediate level and Tapora Bank (TPB; 36°23’37.6” S, 174°17’34.5” E) being the least turbid (Figure 2.1). Sites experienced a diurnal tidal cycle with similar emersion periods (4–5 h) and depths at high tide (~1.2–1.6 m) and were selected for two habitat types: unvegetated sandflat and immediately adjacent dense seagrass (*Zostera muelleri*) meadows (at least 1,000 m^2). Seagrass plots were chosen by eye to have similar seagrass coverage, aiming for a minimum of 80 %. Both habitat types were selected to have comparable sediment properties (fine organically-poor sands (1–4 % organic matter content (OC)), with a low average mud (silt/clay particles < 63 µm) content (3–12 %)).

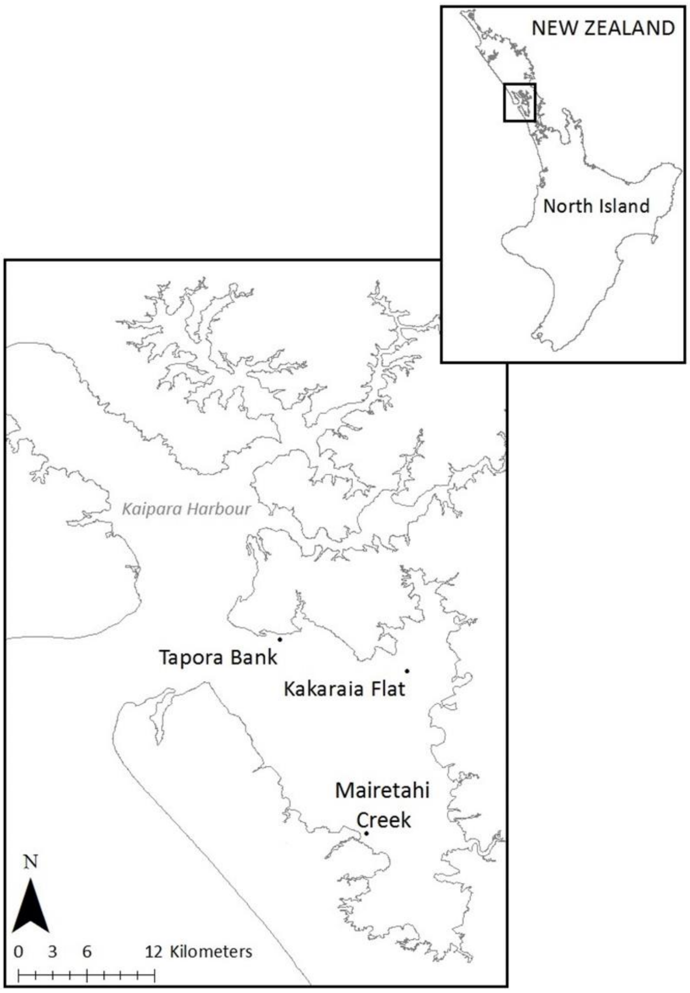


Figure 2.1. Location of the Kaipara Harbour (upper map) and the three study sites (lower map). TPB = Tapora Bank (least turbid), KKF = Kakaraia Flat (intermediate turbidity) and MAI = Mairetahi Creek (most turbid).

2.3.1 Field sampling

Sites were visited on two occasions to measure submerged (26th–29th February 2016, austral summer) and emerged (13th–15th April 2016, late austral summer) primary productivity (PP) in the two habitat types (unvegetated sediment and seagrass beds). Sites were sampled on consecutive days, with measurements occurring around midday to encompass the period when irradiance is naturally highest. This resulted in a temporal separation between the submerged (midday high tide) and emerged (midday low tide) PP measurements, which extended to six weeks due to unsuitable weather. At each site, five replicate pairs of light and dark benthic incubation chambers (L50 x W50 x H15 cm) were deployed in each habitat (~1.5 m apart), running parallel to the seagrass meadow fringe (seagrass and sandflat plots were directly opposing). Light chambers were fitted with transparent domed lids to measure net primary production (NPP) in ambient light, whilst dark chamber lids were covered with shade cloth to measure benthic respiration (i.e. sediment oxygen consumption (SOC)) in the absence of light. Measurements made inside dark chambers with HOBO loggers have confirmed this method effectively omits all light (V. Rullens, unpublished data). Once sealed, chambers encapsulated ~30 L of water/air over a sediment area of 0.25 m^2.

2.3.1.1 Submerged primary production

Chamber bases were deployed at low tide and equipped with a D-Opto water temperature logger (5 min sampling frequency), an Odyssey photosynthetically active radiation (PAR) logger (5 min sampling frequency) and a Seabird CTD pump that ran for 5 s every 40 s to provide intermittent non-directional stirring of the water. Nylon tubing (2 m length, 3.2 mm dia.) was connected to a sampling port in the chamber lids for seawater extraction; an inlet on the opposing side allowed ambient water to be drawn into the chamber to maintain constant water volume. As bases became submerged on the incoming tide (water depth ~0.3 m), lids were positioned and any air bubbles removed before clamping in place. Double-layered 1 m^2 pieces of shade cloth were clamped over dark chambers.

Immediately after submergence, 1 x 60 mL seawater sample was collected from each chamber to provide initial DO concentrations, with further samples collected at the middle (~2 h after initial) and final stages of the incubation (~4 h after initial). In addition, 3 x 50 mL ambient seawater samples were collected as chambers were sealed, and three pairs of light and dark 1 L bottles were filled and secured at the seafloor for sampling at the end of incubations, allowing water column PP to be accounted for. Seawater DO levels were measured immediately on-site using an optical DO probe (PreSens FIBOX 3 LCD trace v7).

2.3.1.2 Emerged primary production

Chamber bases were deployed once seawater had drained completely from sites. A battery-powered fan was placed against an interior wall of the chamber to promote the gentle mixing of air, and prevent the formation of any 'dead spaces' which could influence the diffusion of gases from the sediment (Holmes & Mahall, 1982; Eklund, 1992). An Odyssey PAR logger was staked and inserted into the sediment inside the chamber prior to each incubation (1 min sampling frequency), at which point sediment temperature was also measured.

To conduct incubations, a single modified chamber lid was sequentially clamped to bases that housed a thermocouple measuring air temperature, and an air in and air out port for connection to a LI-COR 8100A Automated Soil CO_2 Flux System (following the approaches of Migné *et al.* (2002) and Streever *et al.* (1998)). The concentration of CO_2 (ppm) and moisture content (% humidity) was measured every second in a continuous stream of air circulated through nylon tubing from the chamber to the LI-COR 8100A. Incubations were 5 min in duration based on existing considerations and protocols for terrestrial soils (Healy *et al.*, 1996), marsh habitats (Streever *et al.*, 1998) and intertidal mudflats (Clavier *et al.*, 2011), and preliminary tests conducted in intertidal habitats similar to the study sites (see Appendix 1). This duration was long enough for CO_2 diffusion to stabilise, but not so long that changes in the humidity (a maximum 10 % change was allowed) and CO_2 concentration within the chamber would start to influence gas diffusion. There was an average decrease in relative humidity of 0.7 % across all incubations. Light incubations were conducted first, following which chamber bases were covered with shade cloth and allowed to acclimate for a minimum of 30 min prior to carrying out dark incubations. This period was chosen as trials in seagrass-dominated sediments took this long to become dark-acclimated (i.e. for photosynthetic processes to cease, see Appendix 1).

2.3.1.3 Site characteristics

Site turbidity was estimated using a Conductivity–Temperature–Depth (RBR XR-620; CTD) profiler with an integrated turbidity sensor (Seapoint OBS) deployed in a nearby channel at all sites for the duration of submerged incubations, along with calculations of water column light attenuation (% surface light received at the seafloor determined by a surface reference sensor and two sensors at the seafloor outside of chambers (i.e. without the attenuation of the chamber lid)). Photographs were taken of seagrass chambers for calculation of live seagrass cover using random count analysis (CPCe v 4.1); 100 points were selected per photo and manually assessed according to whether they fell on live seagrass blades, dead blades or unvegetated sediment (Kohler & Gill, 2006). Following incubations (both submerged and emerged), four small sediment cores (2.6 cm diameter x 2 cm depth) were collected per chamber and pooled, frozen and stored in the dark for later analysis of sediment properties (median grain size, chlorophyll *a* (Chl *a*) concentration, phaeopigment concentration and OC). An additional large core (13 cm diameter x 15 cm depth) was taken from the centre of each chamber and sieved *in situ* over a 500 µm mesh to retain the macrofaunal portion of the benthic fauna (James *et al.*, 1995), preserved in 70 % isopropyl alcohol and stored for later identification and enumeration of macrofauna species and determination of seagrass total biomass.

In the laboratory, sediment samples were thawed, homogenised and divided for grain size, OC and Chl *a* and phaeopigment analyses. Grain size samples (~10 g) were digested in 10 % hydrogen peroxide until all organic matter had been removed (indicated by the ceasing of bubble formation), before measuring using a Malvern Mastersizer 2000 (particle size range 0.05–2000 µm). OC was determined according to percent weight loss on ignition of 5 g sediment samples dried at 100 ^{o}C until constant weight, then subsequently combusted at 550 ^{o}C for 4 h (Heiri *et al.*, 2001). Chl *a* samples were freeze-dried to standardise sample water content prior to homogenising; 5 g subsamples were then boiled in 90 % ethanol for pigment extraction and analysed spectrophotometrically (UV-1800 Shimadzu UV spectrophotometer) using an acidification step (1 M hydrochloric acid) to separate Chl *a* and phaeopigments. Macrofauna samples were stained with Rose of Bengal, sorted and identified to species level where possible, and all seagrass roots and shoots were removed and dried at 80 ^{o}C until constant weight for estimation of dry weight biomass.

2.3.2 Data analysis

Dissolved O_2 and CO_2 fluxes (µmol m^{-2} h^{-1}) were determined by calculating the slope of the regression of discrete DO measurements (submerged) and continuous CO_2 measurements (emerged) ($r^2 > 0.8$ for most calculations) over the incubation duration. A respiratory quotient of 1 was used to convert CO_2 consumption to O_2 production during photosynthesis in light chambers (Hopkins, 2006) and, CO_2 production to O_2 consumption via aerobic respiration in dark chambers (Hargrave, 1969). This value is justified as all incubated sediments were well-oxygenated with low mud and organic contents, such that oxidation of reduced solutes was likely to be negligible (Hargrave, 1969), and is typical of values reported for similar conditions (Boucher *et al.*, 1994; Findlay & Watling, 1997). Light chamber fluxes were equivalent to NPP, and dark chambers to SOC: summing SOC and NPP from paired chambers provided an estimate of gross primary productivity (GPP). Because of my focus on PP, SOC is not referred to again, but rates and statistical analyses for differences between sites, habitat and tidal stage are presented in Appendix 2 for completeness. Water column PP from phytoplankton accounted for < 5 % of submerged benthic PP and was therefore not removed from flux values.

Two measurements of PAR were used for normalising rates of PP: incident and ambient. Incident refers to PAR received at the water surface (during submerged incubations) and sediment surface (during emerged incubations), whereas ambient refers to PAR received at the sediment surface inside chambers i.e. after water column and chamber lid attenuation. GPP was initially standardised by mean incident PAR received over the course of the incubation (GPP_{IL}) to account for the variability in light intensities between sites (see Results). Submerged GPP was standardised by mean incident PAR at the water surface as opposed to the seafloor to retain the light attenuation effects of water-column turbidity. Where available, these values were taken from an Odyssey PAR logger placed on-site logging every five minutes, or at TPB when submerged and all emerged instances, from a LICOR quantum sensor at a nearby weather station (logging for 2 min at 10 min intervals). A measure of photosynthetic efficiency (GPP_{AL+B}), was determined by standardising GPP by mean *ambient* PAR at the seafloor during light incubations (both emerged and submerged) and the photosynthesising biomass within chambers (seagrass total biomass or sediment Chl *a* content i.e. productivity per unit of photosynthesising biomass (Pratt *et al.*, 2014b)). In this instance, seafloor PAR was used to provide an accurate measure of how effectively the primary producers utilised the energy available.

The PERMANOVA+ package for PRIMER v7 was used for all statistical analyses, and Euclidean distance matrices were the basis for all tests. Normalised sediment descriptors (median grain size, mud content and OC) and univariate measures of macrofauna community diversity (species richness and abundance) showed little variation between submerged and emerged sampling and were therefore pooled for analysis. Two-way PERMANOVAs were used to identify significant differences ($p(perm) < 0.05$) in sediment characteristics (a multivariate measure combining grain size, mud content and OC) and measures of macrofauna diversity as a function of site (three levels: TPB, KKF and MAI) and habitat (two levels: seagrass and sandflat). The remaining site characteristic variables, photosynthesising biomass and incident PAR, were analysed using two-way PERMANOVAs to highlight any significant effect of site and tidal state (two levels: submerged and emerged).

Due to the variation in incident PAR between sites and tidal states, NPP and GPP were compared only between habitats within each site and tidal state using one-way PERMANOVAs (habitat as a fixed factor). After standardising by incident PAR, three-way PERMANOVAs were performed on GPP_{IL} to illustrate any individual or interactive effects of site, habitat and tidal state. Finally, because photosynthetic efficiency (GPP_{AL+B}) was derived by standardising GPP by different measures of biomass, two-way PERMANOVAs were performed separately on seagrass and sandflat values to investigate differences in GPP_{AL+B} as a function of site and tidal state. Where significant interactions between factors occurred the results of main effects tests were ignored (two-way interactions were ignored in the presence of significant three-way interactions), and post-hoc pairwise tests then identified which pairs of levels differed within each factor.

2.4 Results

2.4.1 Site characteristics

A water-column turbidity gradient across sites, increasing from TPB to MAI, was confirmed by turbidity sensor and light attenuation measurements (Table 2.1). Water-column turbidity as indicated by nephelometric turbidity units (NTU) at TPB (33) was approximately half that at MAI (68), with only a minor difference between MAI and KKF (60). Light attenuation measurements indicated a more gradual increase in turbidity, with light penetration through the water-column decreasing by 33 % between TPB and KKF, and 79 % between TPB and MAI. Sediment characteristics did not exhibit a consistent relationship with water-column turbidity. The least turbid site, TPB, showed the highest average mud content (10.5 % compared to 3.6 % at KKF and 7.8 % at MAI), whilst MAI, the most turbid site, had an average median grain size comparable to TPB (194 and 195 µm respectively) with KKF possessing the smallest average median particle size (172 µm) (Table 2.1). All sites differed significantly from one another with respect to a multivariate measure of sediment characteristics (median grain size, mud content and OC), and between seagrass and sandflat habitats within sites (Table 2.2). Despite significant site and habitat effects, the absolute differences between these variables were minor; for example, average OC and median grain size showed maximum ranges of 0.5 % and 30 µm across sites (Table 2.1). Univariate indicators of macrofaunal community diversity also differed between sites, with total abundance decreasing significantly with increasing site turbidity. In addition, species richness was lowest at the most turbid site MAI (16), when compared to TPB and KKF (both 21) (Tables 2.1 & 2.2). Seagrass habitats had a greater species richness than sandflat habitats (21 versus 18), but total abundance did not vary (Table 2.2).

Table 2.1. Site characteristics during submerged (February 26th–29th, 2016) and emerged (April 13th–15th, 2016) primary production incubations. Means are displayed with standard deviation in parentheses. Incident PAR is equivalent to the light received at the water/sediment surface outside chambers, whereas ambient PAR is that received at the seafloor inside chambers (i.e. after water column and/or lid attenuation). Dw = dry weight.

	TPB				KKF				MAI			
	Submerged		Emerged		Submerged		Emerged		Submerged		Emerged	
Light regime												
Turbidity (NTU)	33	(16)	-		60	(9)	-		68	(6)	-	
Incident PAR (μmol photon m^{-2} s^{-1})	1769	(418)	1365	(103)	606	(437)	591	(349)	263	(332)	246	(95)
Ambient (seafloor) PAR (μmol photon m^{-2} s^{-1})	321	(28)	447	(102)	93	(63)	195	(50)	10.7	(8.1)	99	(29)
Light attenuation (% surface PAR at seafloor)	19.8	(8.4)	-		13.2	(5.8)	-		4.1	(1.1)	-	
Sediment												
Grain size (μm)	197	(15)	193	(10)	168	(8)	175	(11)	189	(9)	198	(11)
Mud content (%)	9.9	(5.7)	11.1	(3.2)	4	(3)	3.1	(2.8)	8.1	(1.9)	7.4	(3.2)
Organic matter content (%)	1.9	(0.8)	2.2	(0.5)	2.1	(0.4)	1.8	(0.2)	1.7	(0.3)	1.7	(0.4)
Seagrass												
Cover (%)	99	(1)	95	(3)	-		85	(6)	91	(7)	81	(4)
Total biomass (dw g m^{-2})	290	(66)	182	(11)	308	(97)	186	(13)	161	(32)	201	(53)
Microphytobenthos												
Sand												
Chl *a* (dw μg g^{-1} sediment)	7.3	(1.4)	5.6	(0.5)	11.2	(0.9)	7.3	(0.8)	11.2	(0.9)	7.3	(0.4)
Phaeophytin (dw μg g^{-1} sediment)	2.3	(0.5)	2.5	(0.3)	3.3	(0.7)	2.9	(0.3)	3.2	(0.3)	2.8	(0.1)
Seagrass												
Chl *a* (dw μg g^{-1} sediment)	7.5	(1.1)	5.5	(0.6)	13.4	(1.2)	6.9	(0.5)	11.9	(2.6)	8.5	(0.5)
Phaeophytin (dw μg g^{-1} sediment)	2.9	(0.4)	3.2	(0.7)	3.3	(0.7)	2.6	(0.4)	2.6	(0.5)	2.9	(0.7)
Macrofauna ($core^{-1}$)												
Total abundance	314	(89)	283	(65)	128	(54)	249	(107)	75	(16)	193	(102)
Species richness	21	(4)	21	(4)	21	(6)	22	(3)	14	(4)	18	(3)

Table 2.2. Results of two-way PERMANOVAs comparing multivariate sediment characteristics (median grain size, organic matter content and mud content) and univariate indicators of macrofaunal community diversity as a function of site (fixed factor: 3 levels; TPB, KKF and MAI) and habitat (fixed factor: 2 levels; seagrass and sandflat). Photosynthesising biomass (seagrass and sandflat MPB) is compared as a function of site and tide (fixed factor: 2 levels; submerged (Sub.) and emerged (Em.)) in each habitat. Significant effects (p(perm) < 0.05) are given in bold, and post-hoc pairwise tests are shown for significant interactions. Main effects are ignored in the presence of a significant interaction.

				Pairwise tests	
Term	df	Pseudo-F	p (perm)	Site	Habitat
Sediment characteristics					
Site	2	33.3	<0.01		
Habitat	1	31.9	<0.01		
Site x Habitat	2	4.30	**<0.01**	**Seagrass**	**TPB**
				TPB ≠ KKF	Seagrass ≠ Sand
				TPB ≠ MAI	
				KKF ≠ MAI	**KKF**
				Sand	Seagrass ≠ Sand
				TPB ≠ KKF	
				TPB ≠ MAI	**MAI**
				KKF ≠ MAI	Seagrass ≠ Sand
Macrofauna					
Species richness					
Site	2	22.9	**<0.01**	TPB = KKF > MAI	
Habitat	1	22.5	**<0.01**		Seagrass > Sand
Site x Habitat	2	2.25	0.1		
Total abundance					
Site	2	38.7	**<0.01**	TPB > KKF > MAI	
Habitat	1	0.63	0.4		
Site x Habitat	2	1.52	0.2		
Seagrass biomass					
Site	2	0.85	0.4		
Tide	1	1.76	0.2		
Site x Tide	2	1.08	0.3		
Sandflat MPB biomass (Chl *a*)					
Site	2	60.3	<0.01		
Tide	1	169	<0.01		
Site x Tide	2	12.1	**<0.01**	**Submerged**	**TPB**
				TPB < KKF = MAI	Em. < Sub.
				Emerged	**KKF**
				TPB < KKF < MAI	Em. < Sub.
					MAI
					Em. < Sub.

Live seagrass cover was > 80 % during all benthic incubations, with TPB exhibiting the greatest coverage during both submerged and emerged sampling (Table 2.1). Although seagrass data were not able to be collected from submerged incubation chambers at KKF, field notes indicated a level of coverage at this site somewhere between that at TPB and MAI (99 and 91 %, respectively). The site with the highest turbidity (MAI) had the lowest seagrass cover and biomass, although among-site differences in seagrass cover and biomass were either not significant (seagrass biomass) or not tested (missing seagrass cover data at KKF) (Table 2.1 & 2.2). In sandflat habitats, a significant interactive effect of site and tidal state on Chl *a* content was observed (Table 2.2). Chl *a* was lowest at TPB during both submerged and emerged sampling, and consistently higher concentrations were found during submerged sampling at all sites (on average 1.4 times greater) (Tables 2.1 & 2.2).

Incident PAR varied between sites during incubations due to varying weather conditions during sampling, with TPB experiencing the highest mean PAR during both submerged (3 and 7 times higher than KKF and MAI respectively) and emerged (2 and 6 times higher than KKF and MAI) sampling (Figure 2.2). The incident light regime did not differ greatly between submerged (late February) and emerged (mid-April) sampling within sites (range < ± 10 % of the mean of the two sampling dates). These variations in incident PAR were accounted for in GPP estimates by standardising by the mean incident PAR experienced during incubations (see below).

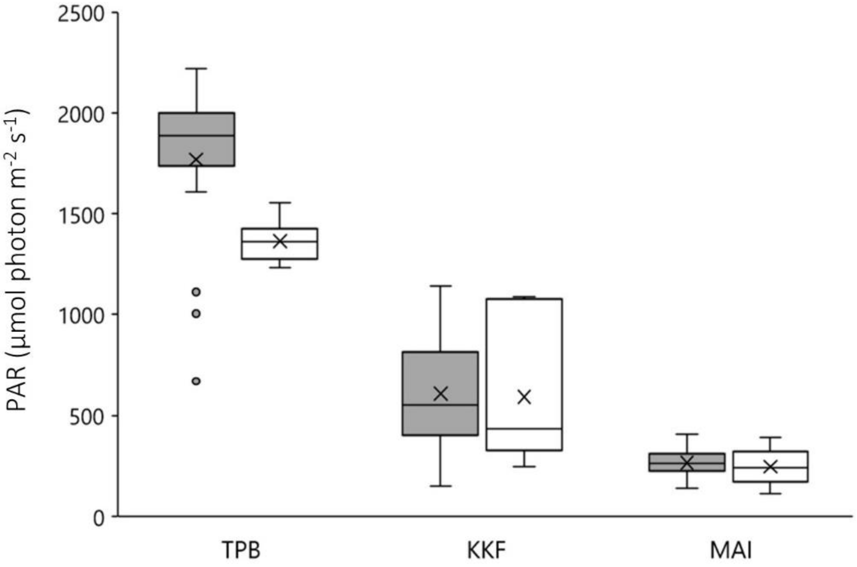


Figure 2.2. Surface incident photosynthetically active radiation (PAR) during primary production measurements. Sites were visited on consecutive days during submerged (February 26th–29th 2016; grey bars) and emerged (April 13th–15th 2016; white bars) sampling campaigns. Whiskers indicate maximum and minimum values with circles marking outliers. Box limits represent 25th and 75th percentiles, solid lines within boxes are median values and crosses indicate means.

2.4.2 Primary Production

Submerged seagrass and sandflat NPP differed significantly at TPB (the clearest site) but not at KKF and MAI (the more turbid sites) (Figure 2.3a, Table 2.3). Additionally, net heterotrophy (i.e. an uptake of O_2 in light chambers) occurred in all but TPB seagrass habitats (Figure 2.3a). During emergence, net autotrophy occurred throughout and seagrass NPP was significantly greater than sandflat NPP at all sites, being almost 3 times more productive at TPB, 4 times at KKF, and 6 times at MAI (Figure 2.3b, Table 2.3). The pattern of NPP across sites during emergence (Figure 2.3b) generally reflected the pattern in incident light availability (Figure 2.2).

Significant effects of habitat on GPP were found only at TPB during submergence, with seagrass habitat being 4 times more productive than sandflat habitat (Figure 2.3c, Table 2.3). However when emerged, seagrass was significantly more productive than sandflat at all sites (Table 2.3), having a 3 times greater GPP on average (Figure 2.3d). In both seagrass and adjacent sandflat, and during submergence and emergence, trends in GPP across sites were again reflective of incident light variability. Despite this, incident light varied little between tidal states within sites (Figure 2.2); therefore, it is notable that emerged GPP appeared greater than submerged at all sites in both habitats (Figure 2.3c & 2.3d).

Once standardised by mean incident PAR, emerged GPP_{IL} was significantly greater than submerged at all sites in both habitats (Figures 2.3e & 2.3f, Table 2.3). The magnitude of difference between tidal states increased with increasing site turbidity and was most evident in seagrass habitats: at TPB, emerged GPP_{IL} was 1.3 times greater than submerged, at KKF it was 5.1 times greater, and at MAI it was 26 times greater. When submerged and emerged GPP_{IL} are combined to provide a measure of total GPP_{IL}, the proportion that PP during emergence contributes is 64 % at TPB, 84 % at KKF and 96 % at MAI. Seagrass habitats were again more productive than sandflat habitats, with the largest difference occurring during emergence at MAI, where sandflat GPP_{IL} was just 11 % of seagrass (Figures 2.3e & 2.3f). Trends with turbidity were dependent on tidal state in seagrass habitats, as GPP_{IL} decreased with increasing turbidity when submerged (Figure 2.3e) and increased with increasing turbidity when emerged (Figure 2.3f). Sandflat habitats however showed no trend with turbidity, as GPP_{IL} was greatest at KKF (intermediate turbidity) during both tidal states (Figures 2.3e & 2.3f, Table 2.3).

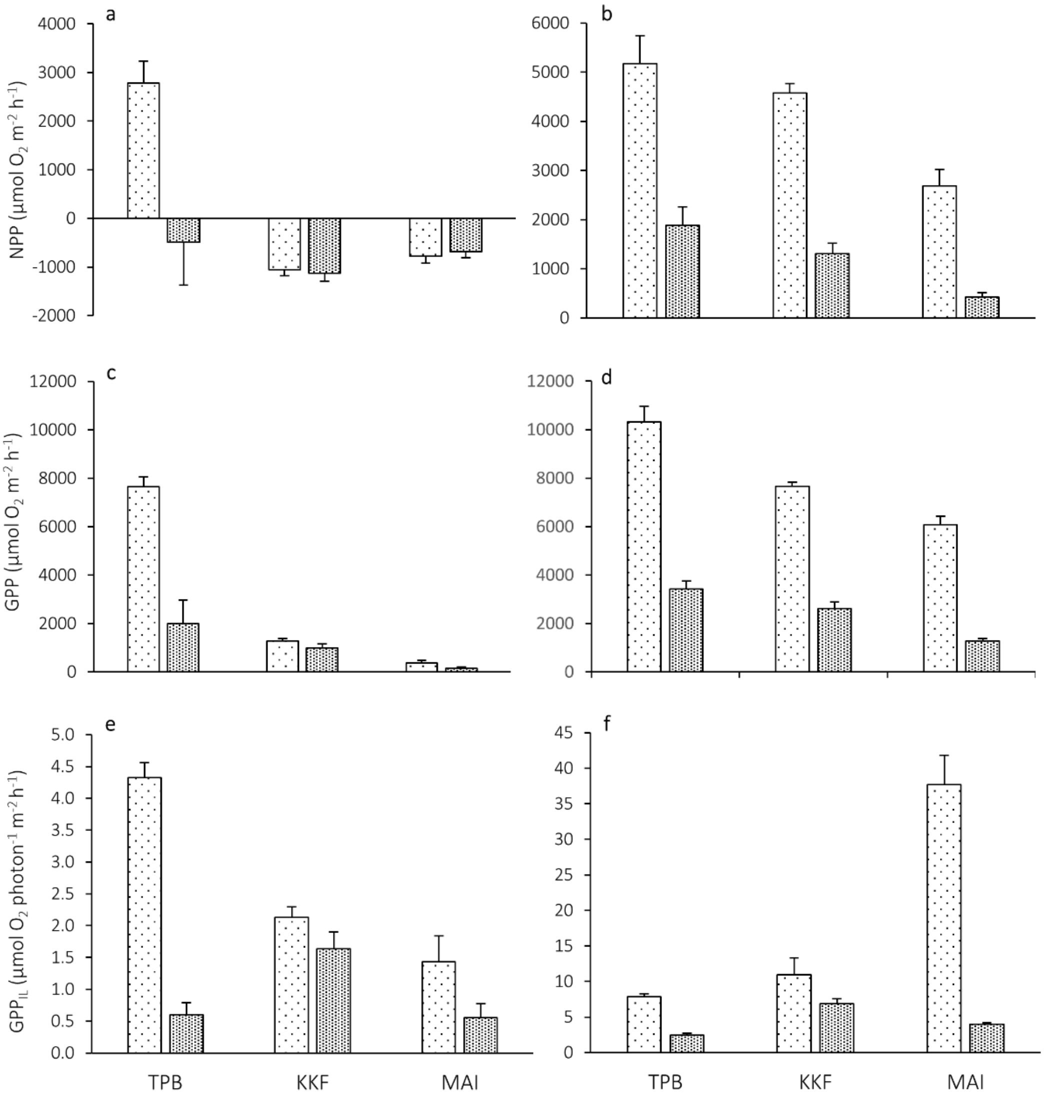


Figure 2.3. Net primary production (NPP) (a, b), gross primary production (GPP) (c, d) and mean incident PAR-corrected GPP (GPP_{IL}) (e, f) during submerged (left panel) and emerged (right panel) tidal conditions in seagrass (sparsely dotted bars) and sandflat (densely dotted bars) habitats. TPB was the least turbid site and MAI the most turbid (see Table 2.1). Bars represent means (n = 5) and standard error bars are displayed. Note the change in scale between figures a) and b) and also e) and f).

2.4.3 Photosynthetic efficiency (GPP_{AL+B})

Correcting GPP by seafloor ambient PAR and photosynthesising biomass provides a measure of photosynthetic efficiency (GPP_{AL+B}), and little difference was found between emerged and submerged conditions in either habitat (Figure 2.4). Sandflat habitats at MAI showed the greatest apparent difference, with submerged efficiency being 2.2 times greater than emerged (Figure 2.4b); however, no significant effect of tidal state on GPP_{AL+B} was found in either habitat at any site (Table 2.3). Seagrass habitats showed an increased GPP_{AL+B} at the most turbid site (MAI) during both tidal conditions, with the average efficiency at TPB (least turbid) and KKF (intermediate turbidity) being 27 % and 23 % of that at MAI, respectively (Figure 2.4a, Table 2.3). Contrastingly, sandflat GPP_{AL+B} did not show a consistent trend with site turbidity overall, although during submergence efficiency seemingly increased with increasing turbidity (Figure 2.4b), despite no significant effect of site (p(perm) = 0.08) (Table 2.3).

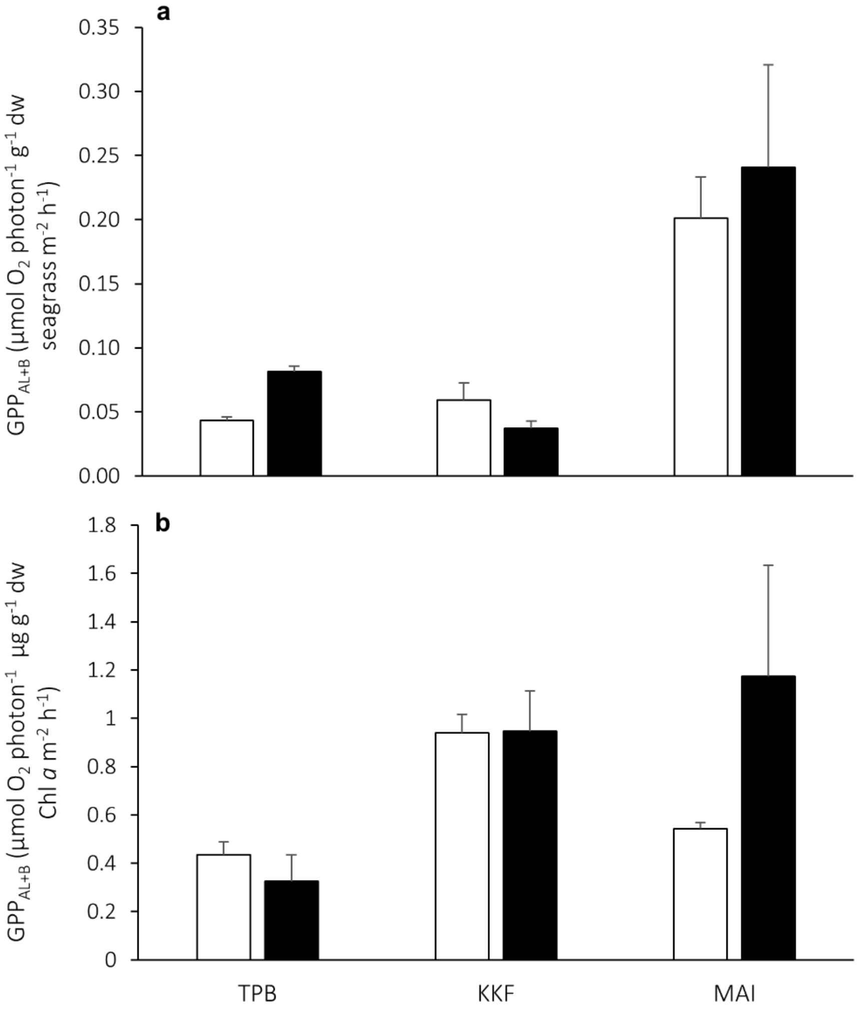


Figure 2.4. Gross primary productivity (GPP) corrected by mean ambient photosynthetically active radiation (PAR) at the sediment surface during submergence (filled bars) and emergence (clear bars) and photosynthetic biomass (seagrass total biomass or sediment Chl *a* content) to provide a measure of photosynthetic efficiency (GPP_{AL+B}) in a) seagrass and b) sandflat habitats. Bars represent means (n = 5) and standard error bars are displayed.

Table 2.3. PERMANOVA results comparing measures of primary production as a function of different factors (site, habitat, tide). Due to variations in incident light levels, one-way PERMANOVA compared net primary productivity (NPP) and gross primary productivity (GPP) between habitats (fixed factor, 2 levels: seagrass & sandflat) within site and tidal state. A three-way PERMANOVA was used to compare GPP standardised by mean incident PAR (GPP_{IL}) between site (fixed factor, 3 levels: TPB, KKF & MAI), habitat and tide (fixed factor, 2 levels: submerged and emerged). Within each habitat type, a two-way PERMANOVA compared photosynthetic efficiency (GPP_{AL+B}) (GPP standardised by mean ambient photosynthetically active radiation (PAR) and photosynthesising biomass). Significant effects (p(perm) < 0.05) are given in bold, and post-hoc pairwise tests are given for significant interactions. Significant interactions are prioritised over main effects, and three-way interactions over two-way interactions.

Term	df	Pseudo-F	p (perm)	Pairwise tests Site	Habitat	Tide
NPP						
Habitat						
TPB, emerged	1	23.3	**<0.01**		Seagrass > Sand	
KKF, emerged	1	127	**<0.01**		Seagrass > Sand	
MAI, emerged	1	41.6	**<0.01**		Seagrass > Sand	
TPB, submerged	1	58.2	**<0.01**		Seagrass > Sand	
KKF, submerged	1	0.13	0.7		Seagrass = Sand	
MAI, submerged	1	0.26	0.6		Seagrass = Sand	
GPP						
Habitat						
TPB, emerged	1	90.1	**<0.01**		Seagrass > Sand	
KKF, emerged	1	272	**<0.01**		Seagrass > Sand	
MAI, emerged	1	170	**<0.01**		Seagrass > Sand	
TPB, submerged	1	140	**<0.01**		Seagrass > Sand	
KKF, submerged	1	2.46	0.1		Seagrass = Sand	
MAI, submerged	1	3.58	0.1		Seagrass = Sand	
GPP_{IL}						
Site x Habitat	2	38.7	<0.01			
Site x Tide	2	42.6	<0.01			
Habitat x Tide	1	68.4	<0.01			
Site x Habitat x Tide	2	29.1	**<0.01**	**Seagrass, emerged**	**TPB, emerged**	**TPB, seagrass**
				TPB = KKF < MAI	Seagrass > Sand	Em. > Sub.
						TPB, sand
						Em. > Sub.
				Seagrass, submerged	**TPB, submerged**	**KKF, seagrass**
				TPB > KKF = MAI	Seagrass > Sand	Em. > Sub.
				Sand, emerged	**KKF, emerged**	**KKF, sand**
				TPB < MAI < KKF	Seagrass = Sand	Em. > Sub.
				Sand, submerged	**KKF, submerged**	**MAI, seagrass**
				MAI = TPB < KKF	Seagrass = Sand	Em. > Sub.
					MAI, emerged	**MAI, sand**
					Seagrass > Sand	Em. > Sub.
					MAI, submerged	
					Seagrass = Sand	

Table 2.3 continued.

Term	df	Pseudo-F	p (perm)	Pairwise tests Site	Habitat	Tide
GPP_{AL+B}						
Seagrass						
Site	2	14.39	**<0.01**	**TPB = KKF < MAI**		
Tide	1	0.39	0.5			
Site x Tide	2	0.63	0.6			
Sand						
Site	2	2.88	0.08			
Tide	1	2.65	0.1			
Site x Tide	2	1.03	0.4			

2.5 Discussion

Comparable submerged and emerged primary productivity (PP) measurements were obtained across a gradient in site turbidity in two intertidal habitats: sandflat dominated by MPB and seagrass (*Zostera muelleri*) beds. Sediment characteristics were similar between sites and a turbidity gradient was confirmed, ensuring site effects could be correlated with this factor. When variations in incident light availability were accounted for (GPP_{IL}), emerged habitats were highly productive, consistently exceeding submerged productivity at all sites. Additionally, the difference between submerged and emerged productivity increased as sites became more turbid, highlighting an elevated dependence on emerged PP in turbid locations. No trends between site turbidity and sandflat productivity or photosynthetic efficiency (GPP_{AL+B}) were found; however, the relationship between seagrass habitat GPP_{IL} and site turbidity depended on tidal state: increased site turbidity was associated with decreased GPP_{IL} during submergence, and with increased GPP_{IL} during emergence. These varying responses were likely driven by differing degrees of photo-acclimation between sites, illustrated in part by significantly greater GPP_{AL+B} at the most turbid site (MAI) during both tidal states.

The temporal separation of submerged and emerged sampling may have increased the chance of making Type 1 errors (rejecting the null hypothesis when it is true) in statistical analyses. This is because the variance between groups may have been over-estimated, which could increase the F-ratio (mean square between groups divided by the mean square within groups; Underwood, 1997). I do not consider this to be problematic, however, as all statistical tests of differences between submerged and emerged PP returned p-values < 0.01, i.e., they were highly significant and not close to the limit of significance. Also, emerged PP was measured later in the year than submerged, so environmental conditions should have favoured submerged PP, yet emerged PP was substantially greater than submerged.

Seagrass habitat PP (net and gross) significantly exceeded sandflat PP (as expected owing to the greater photosynthesising biomass associated with seagrass habitats (Moncreiff *et al.*, 1992), only when light conditions were unimpeded by water column turbidity, i.e. during emergence at all sites and during submergence at the least turbid site (TPB). At these times, seagrass NPP was up to six times greater than sandflat NPP, demonstrating the dominant role seagrass habitats can have in carbon fixation in these systems. These findings are consistent with those of Ricart *et al.* (2015) and Fourqurean *et al.* (2012), who have highlighted the importance of seagrass meadows as blue carbon sinks, but contrast those of Migné *et al.* (2016) who documented a greater drawdown of CO_2 in unvegetated sediments. No difference between habitats was detected during submergence at the intermediate and highest turbidity locations (KKF and MAI), indicating a possible light-limitation of photosynthesis that prevented productive differences between habitats from manifesting. The average light intensity inside chambers during submergence was 93 and 11 µmol m^{-2} s^{-1} at KKF and MAI, respectively, whilst the minimum light requirements for subtidal *Z. muelleri* in Kaipara Harbour is ~57 µmol photon m^{-2} s^{-1} in summer (Bulmer *et al.*, 2015a), and is > 37 for temperate intertidal MPB (Migné *et al.*, 2002). Although light requirements of intertidal autotrophs are known to exhibit great intraspecific variability and site specificity (Lee *et al.*, 2007; Kohlmeier *et al.*, 2016), these values suggest that light availability within chambers was close to the minimum required, and thus low rates of photosynthesis likely occurred at KKF and MAI, despite positive GPP values at both sites. Productive differences between habitats were therefore not observed at mid and high turbidity locations during submergence. However, seagrass habitats were considerably more productive than sandflat in all other instances where light was abundant.

Differences in PP (GPP_{IL}) between tidal states became more pronounced as site turbidity increased, due to the decreasing ability of either habitat to photosynthesise during submergence. This culminated in seagrass habitats at the most turbid site where emerged PP contributed 96 % of the total GPP_{IL}. Differences in photosynthetic biomass were not responsible for the increased productivity during emergence, as Chl *a* concentration decreased between submerged and emerged measurements within sites and tide had no significant effect on seagrass biomass. In sandflat habitats, vertical migration of the MPB community to the sediment surface during emergence in response to changes in irradiance and temperature (Pinckney & Zingmark, 1991; Mitbavkar & Anil, 2004; Du *et al.*, 2012) may be driving increased PP, with previously reported differences between emerged and submerged PP being attributed to this phenomenon (Holmes & Mahall, 1982). In both habitats the physical characteristics of emergence, i.e. being surrounded by air rather than seawater, could also contribute to the discrepancy between tidal states. Diffusion of gases occurs more rapidly through air than water, and this potentially enables greater rates of photosynthesis during emergence (Silva *et al.*, 2005). When combined with light levels that exceed minimum requirements, PP during emergence can far exceed that during submergence.

The contribution of emerged PP to total PP is therefore great at all sites, but becomes crucially important as site turbidity increases and light capture during submergence becomes impaired. This is further highlighted by the fact that net heterotrophy dominated during submergence, with only seagrass habitats at TPB exhibiting net autotrophy, whilst all sites and habitats were characterised by net autotrophy during emergence. In the intermediate (KKF) and high (MAI) turbidity locations photosynthetic gains were consequently restricted to periods of emergence in both habitats, suggesting that submerged benthic food webs in these locations are supported by emerged PP (Vermaat & Verhagen, 1996; Vermaat *et al.*, 1997). Allochthonous inputs may also partly support these benthic food webs if autochthonous resources are limited (Bartels *et al.*, 2018); however, it has previously been demonstrated in shallow New Zealand estuaries with extensive intertidal areas that MPB PP far outweighs any other source, accounting for 90 %

of the organic matter needed to sustain secondary and tertiary consumers (Jones *et al.*, 2017).

The relationship between GPP_{IL} and site turbidity differed depending on tidal state in seagrass habitats: during submergence an elevation in turbidity resulted in a decrease in GPP_{IL}; however, an increase in GPP_{IL} was observed with increasing site turbidity during emergence. These trends were not driven by seagrass biomass, as there were no significant differences between sites or tidal states. Whilst live seagrass cover seemingly decreased with increasing site turbidity, potentially explaining the decrease in GPP_{IL} when submerged, it cannot explain the reversed trend during emergence. The ability of seagrass species to adapt physiologically and morphologically to site-specific light regimes has been demonstrated widely (reviewed by Lee *et al.* (2007)), and may partially explain the differing trends. Pigment contents of *Z. muelleri* leaves may adapt to suit ambient light settings, such that populations from low light environments (e.g. highly turbid locations) are characterised by high contents of light-harvesting pigments and low contents of photoprotective pigments (Kohlmeier *et al.*, 2014b), and a reduction in the light saturation point of photosynthesis (I_k) has also been demonstrated (Park *et al.*, 2016). Such photo-acclimation may have developed at the more turbid locations (KKF and MAI) as the turbidity conditions measured during submerged incubations are believed to be representative of the sites year-round (Hailes & Carter, 2016; Lohrer, 2017), indicating persistently low light levels. It is therefore suggested that the content of light-harvesting pigments in seagrass leaves increased as site turbidity increased, and that I_k was potentially lower at the more turbid locations. Such adaptations would allow greater photosynthetic gains to be made at the more turbid locations than at less turbid locations when light levels were high (during emergence).

Despite emerged productivity being greater than submerged, photosynthetic efficiency did not differ between tidal states. This provides evidence that photoinhibition and desiccation did not greatly reduce efficiency during emergence, as protective mechanisms of both seagrasses and MPB when exposed to these stressors results in decreased photosynthetic efficiency, among other physiological effects (Franklin *et al.*, 2003; Coelho *et al.*, 2009; Kohlmeier *et al.*, 2016). As light levels were characteristically high at the least turbid site (TPB), efficiency was expectedly low in both habitats during both tidal states. No further relationship was found in sandflat habitats between efficiency and turbidity, supporting the findings of Pratt *et al.* (2014b) that MPB photosynthetic efficiency is impaired with increasing SSC. In *Z. muelleri* beds, however, there was a substantial increase in efficiency at the most turbid location (MAI) under both tidal conditions compared to the other locations, highlighting an adaptation of the seagrasses here to both low (during submergence) and high (during emergence) light intensities. Previously discussed adaptations (increased light-harvesting pigment content (Kohlmeier *et al.*, 2014b) and decreased I_k (Park *et al.*, 2016)) act to increase efficiency during submergence, whereas efficiency during emergence is mostly determined by adaptations against desiccation and photoinhibition.

Seagrass self-shading can reduce productivity during submergence, but can also act as a protective mechanism against photoinhibition during emergence, as only the leaves that rest on top of the bed are exposed to the greatest light intensities (Clavier *et al.*, 2011). Schwarz (2004) demonstrated in *Z. muelleri* beds in New Zealand that only 25 % of incident irradiance filtered through to the bottom leaves when seagrasses were flat on the sediment surface; thus, leaves beneath the upper layer may receive optimum light levels to maintain high photosynthetic efficiency. Desiccation stress was also expected to be minor during the short duration measurements; all emerged incubations were conducted within two hours of sites becoming uncovered; therefore, plots were not completely dried out at the time PP was measured. It is predicted that photosynthetic rates and efficiency would decrease with increasing periods of emergence due to elevated desiccation stress, as has previously been demonstrated in other *Zostera* species (Leuschner *et al.*, 1998); however, this is likely to depend on site-specific characteristics. Additionally, morphological characteristics may change in response to desiccation stress, with higher seagrass shoot densities and broader leaves conveying desiccation tolerance (Park *et al.*, 2016; Manassa *et al.*, 2017). Although not reported, shoot area was greatest at the most turbid location (MAI), suggesting greater desiccation tolerance that may have enabled greater photosynthetic efficiency during emergence (unpublished data).

The variability of incident PAR between sites represents a potentially confounding factor in this study and limits the comparisons that can be made between raw values of NPP and GPP. To enable comparisons across sites, tidal states and habitats light variability was normalised by either surface incident ($_{IL}$) or ambient light ($_{AL}$) availability (µmol O_2 photon^{-1} m^{-2} s^{-1}). This approach assumes linear relationships between photosynthesis and irradiance for both *Z. muelleri* and MPB during incubations (i.e. photosynthesis was light-limited). Photosynthesis–irradiance (P–I) relationships are, however, only linear until a certain irradiance, beyond which photosynthesis becomes light saturated and the rate of photosynthesis plateaus and/or declines (Henley, 1993). Mean ambient PAR inside chambers ranged from 447 µmol m^{-2} s^{-1} (TPB during emergence) to 11 µmol m^{-2} s^{-1} (MAI during submergence), whilst previous studies in temperate estuaries have illustrated that saturation of photosynthesis occurs ~440 µmol m^{-2} s^{-1} in MPB communities (Barranguet *et al.*, 1998), and beyond ~242 in *Z. muelleri* (Schwarz, 2004) during summer months. Based on these values, it would be reasonable to assume a linear relationship between MPB productivity and PAR at all sites, and for *Z. muelleri* productivity at KKF and MAI, but perhaps not at TPB (where light intensities were greater than 242 µmol m^{-2} s^{-1}).

Literature light saturation values were, however, determined via pulse amplitude modulated (PAM)-fluorometry, which obtains measurements from a single seagrass blade, and is likely to underestimate the saturating irradiance for photosynthesis of intact seagrasses (roots, rhizomes and leaves), as whole plant oxygen demand is greater than that for photosynthetic tissues alone (Lee *et al.*, 2007). The self-shading effect of seagrasses during emergence also means that whilst the uppermost/outer leaves may have experienced light levels >242 µmol m^{-2} s^{-1}, most of the seagrass bed would have received only a fraction of this (Schwarz, 2004; Clavier *et al.*, 2011). Furthermore, MPB communities have been observed migrating away from the sediment surface to avoid damaging light levels (Barranguet *et al.*, 1998). Whilst it may be an oversimplification to assume linear P–

I relationships when normalising light variability generally, light conditions during this study indicate it was an appropriate approach.

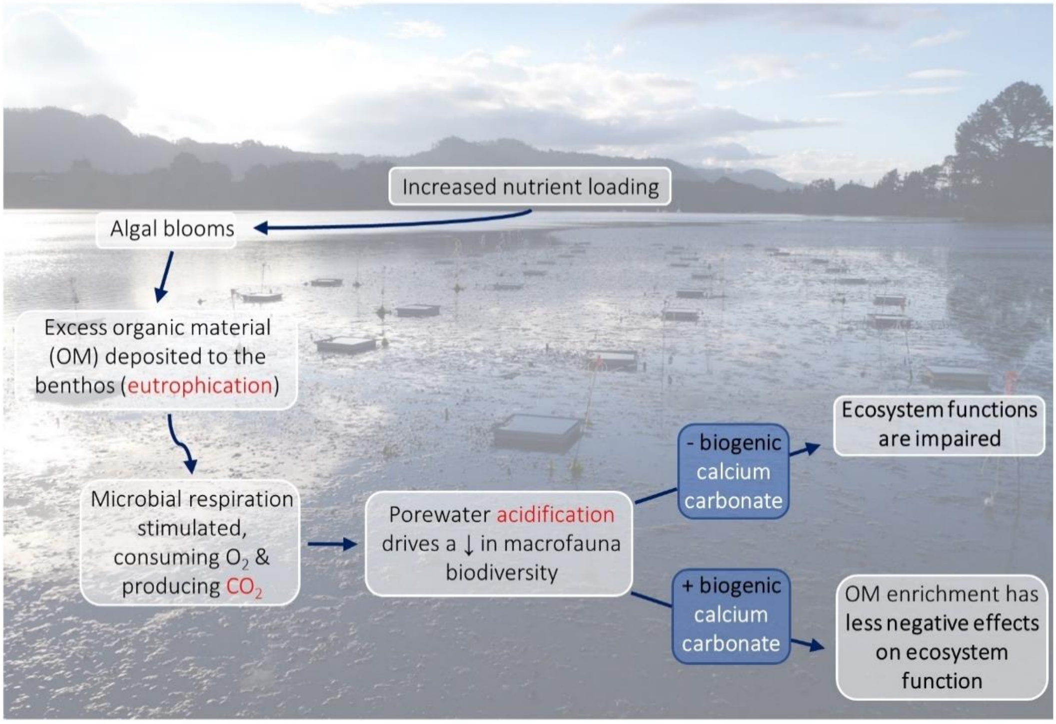
2.6 Conclusions

The findings of this study begin to fill an appreciable gap in the literature concerning comparisons of submerged and emerged PP. Although my study is not replicated seasonally and is restricted to sites within a single estuary, the reported trends are likely to be applicable to similar intertidal systems where primary producers persist despite minimal light intensities during submergence. I have highlighted that significant differences occur in the way MPB and *Z. muelleri* habitats respond to changes in light climate caused by water column turbidity, and that the responses within *Z. muelleri* habitats may vary with tidal state. Seagrass habitats were consistently more productive than MPB-dominated sands, and showed more pronounced responses to changing light levels, likely owing to their physical position on, as opposed to within, the sediment surface. The importance of emerged PP in both habitats cannot be overstated. In all but one of the locations surveyed net autotrophy occurred only during emergence, indicating a potential dependence of submerged food webs on emerged PP, especially in turbid locations. Emerged PP may, therefore, provide resilience to benthic intertidal ecosystems against increasing coastal turbidity. However, this likely depends on the duration of emergence (sufficient to support submerged food webs, yet not so long that desiccation limits PP), and whether suspended sediments settle out and smother primary producers during emergence. To verify and expand on this study, future research should include temporal replication of paired submerged and emerged measurements, as well as at different tidal elevations, to determine how environmental variability affects the productivity trends observed here. It would also be valuable to conduct measurements across a greater range of turbidity levels and at finer scales, to determine if thresholds in the relationship between productivity and turbidity exist.

CHAPTER 3:

Calcium carbonate alters the functional response of coastal sediments to eutrophication-induced acidification

3.1 Graphical abstract



3.2 Introduction

Acidification of seawater via the increasing absorption of atmospheric CO_2 (ocean acidification; OA) is a key contemporary issue for the marine environment (Mason, 2010). However, eutrophication-induced acidification (Wallace *et al.*, 2014) has received comparatively little attention, despite affecting vast portions of the world's coastal zones (Diaz & Rosenberg, 2008). Eutrophication is defined as an increase in the rate of supply of organic carbon to an ecosystem and occurs in coastal waters primarily via the anthropogenic input of excess nutrients (Nixon, 1995). Excess nutrients promote short-lived algal blooms which, upon collapsing, are deposited to the benthos (Teichberg *et al.*, 2010) where microbially-driven aerobic respiration of organic matter releases CO_2 in approximate equivalence to O_2 consumption (Körtzinger *et al.*, 2001), causing localised acidification (Cai *et al.*, 2011). In highly productive estuarine environments, eutrophication-induced acidification adds variation to background fluctuations in pH driven by changing rates of respiration and photosynthesis (Wootton *et al.*, 2008; Baumann *et al.*, 2015) and watershed effects (Duarte *et al.*, 2013), and occurs against a backdrop of global OA that is set to reduce seawater pH 0.3–0.4 units by 2100 (IPCC, 2014b). The co-occurrence of these acidification pathways means coastal environments may experience decreases in seawater pH far exceeding those predicted from OA alone (Sunda & Cai, 2012; Melzner *et al.*, 2013). Additionally, biogeochemical changes associated with aerobic (i.e., O_2 depletion) and anaerobic (i.e., dissimilatory N and S reduction) pathways of benthic organic matter degradation mean that acidification rarely acts in isolation, but is rather a single component in a multi-stressor setting also comprising hypoxia and increased concentrations of toxic solutes (Howarth *et al.*, 2011; Fitch & Crowe, 2012).

Coastal benthic macrofauna communities exhibit predictable responses to organic matter enrichment, such as reduced abundance and diversity at high levels of loading (Pearson & Rosenberg, 1978). These responses are usually attributed to the onset of hypoxia (Diaz & Rosenberg, 2008); however, empirical evidence suggests some organisms respond more strongly to fluctuations in pH than O_2 (e.g. juvenile clams (Ringwood & Keppler, 2002)). Whilst it is generally accepted that calcifying organisms will fare poorly under acidification due to calcium carbonate ($CaCO_3$) under-saturation and increased likelihood of dissolution (Orr *et al.*, 2005; Gazeau *et al.*, 2013; Thomsen *et al.*, 2015), the consequences for non-calcifying organisms are less certain and vary interspecifically (Birchenough *et al.*, 2015). Understanding the response of benthic communities to decreasing pH is critical, as numerous ecosystem services (e.g. food production and nutrient cycling) are underpinned by functions (e.g. primary production and organic matter mineralisation) that are strongly influenced by resident macrofauna (Norling *et al.*, 2007; Braeckman *et al.*, 2010). Evidence from laboratory experiments suggests that processes such as denitrification may be disrupted by decreases in seawater pH, owing to a breakdown in the facilitative interactions between macrofauna and denitrifying bacteria (Donohue *et al.*, 2012; Laverock *et al.*, 2013). Naturally occurring gradients of acidification have been utilised to investigate the effects on benthic communities in the field, for example by monitoring communities with increasing distance from shallow volcanic CO_2 vents (Hall-Spencer *et al.*, 2008; Dahms *et al.*, 2018; González-Delgado & Hernández, 2018). Whilst these studies have provided insights into potential changes in community structure, including decreases in the abundance of calcareous organisms, and even evolutionary adaptations, there are frequently additional environmental variables that co-vary along the gradient (such as temperature and toxic trace elements) making it difficult to attribute the responses to acidification alone (Vizzini *et al.*, 2013).

The difficulty in conducting controlled acidification experiments *in situ* has meant benthic OA research has tended towards laboratory-based studies often using simplified biological communities or individual species. Of the 324 studies published by 2014 on marine organisms' responses to reduced coastal pH, three quarters were conducted in the laboratory (Wahl *et al.*, 2015). Whilst laboratory-based studies provide a controlled mechanistic understanding of acidification effects, they cannot predict *in situ* ecological responses given the complexity of natural ecosystems. For example, the ability for mobile organisms to disperse from impacted sediments (Günther, 1992) is inhibited when entire experimental units (e.g. cores or mesocosms) are acidified, but this is a crucially important behavioural trait providing resilience to benthic communities when considered at landscape or ecosystem scales (Pilditch *et al.*, 2015; Greenfield *et al.*, 2016). Recent OA field studies have also highlighted how functional redundancy in benthic communities may provide resilience against acidification which would otherwise be masked in low diversity laboratory studies. Baggini *et al.* (2015) documented a switch in the main grazer of macroalgae from sea urchins to fishes along a gradient of increasing CO_2 concentration, preventing a phase shift from an ecosystem dominated by encrusting algae to macroalgae. Numerous laboratory studies have previously identified positive responses of marine autotrophs to increased CO_2 (see Koch *et al.* (2013)), but this may not be observed *in situ* if species replacement maintains grazing pressure.

Furthermore, laboratory experiments frequently neglect the multi-stressor nature of benthic acidification (the usual mechanism for reducing seawater pH is via the bubbling of CO_2 gas (Widdicombe *et al.*, 2013; Vopel *et al.*, 2018), shifting water column pH only), and the natural buffering capacity of the coastal environment may be overlooked or poorly replicated in artificial settings. To make empirical studies more relevant to the real-world, Hewitt *et al.* (2007) advocate conducting field experiments across environmental gradients to address scaling issues between the understanding gleaned from simplified small-scale laboratory experiments and the response of diverse large-scale ecosystems (Snelgrove *et al.*, 2014; Lohrer *et al.*, 2015).

The complex biogeochemistry of the coastal zone is likely to affect ecological responses to acidification. Coastal ocean sediments contain approximately 50 % of oceanic $CaCO_3$ deposits, originating in temperate regions mostly from bivalve shells and bryozoan skeletons (Gattuso, 1998). $CaCO_3$ minerals dissolve in response to increased acidity from CO_2 hydrolysis, according to equations 1 and 2.

$$CO_{2(g)} + 2H_2O_{(aq)} \leftrightarrow HCO_3^-{}_{(aq)} + H_3O^+{}_{(aq)} \qquad \text{(Equation 1)}$$

$$CaCO_{3(s)} + H_3O^+{}_{(aq)} \leftrightarrow HCO_3^-{}_{(aq)} + H_2O_{(aq)} + Ca^{2+}{}_{(aq)} \qquad \text{(Equation 2)}$$

When CO_2 hydrolysis and $CaCO_3$ dissolution are coupled in this way, there is no net change in hydronium ion (H_3O^+) concentration and thus no change in pH (pH = -log[H_3O^+]), although buffering capacity, i.e. the ability to neutralise H_3O^+, increases according to the increase in HCO_3^- (Stumm & Morgan, 2012). The porewaters of $CaCO_3$-rich coastal sediments may therefore possess high buffering capacities (Rao *et al.*, 2014; Pickett & Andersson, 2015) which reduce the potential for significant fluctuations in pH. $CaCO_3$ has even been suggested as a tool to prevent the negative effects of localised benthic acidification (Clements & Chopin, 2017). Whilst Green *et al.* (2013) observed a three-fold increase in bivalve recruitment in cores buffered with crushed shell hash, Greiner *et al.* (2018) observed no such effect in similarly buffered plots, despite significant increases in porewater pH. To date, the only field study investigating the link between benthic acidification, $CaCO_3$ buffering and ecosystem function was inconclusive due to heterogeneity in the environment

(Rodil *et al.*, 2013). It is necessary to clarify the nature of these linkages as intensifying coastal acidification may remove the key species that consolidate $CaCO_3$ into sediments (Green *et al.*, 2009) and lead to dissolution of existing $CaCO_3$ deposits (Sulpis *et al.*, 2018), such that any resilience provided by $CaCO_3$ buffering to ecosystem functioning will be lost.

In the present study, the ability of biogenic $CaCO_3$ to maintain intertidal benthic ecosystem structure and function along a gradient of eutrophication-induced acidification was investigated *in situ*. The presumed mechanism by which $CaCO_3$ would increase resilience was through reducing the range of pH variability via buffering of CO_2 (equations 1 & 2). As such, it was hypothesised that sediments with abundant $CaCO_3$ would experience less extreme fluctuations in pH than those without when mineralising excess organic matter to CO_2. If fluctuating pH was the primary eutrophication-induced stressor, the macrofauna community was expected to be less affected by organic matter enrichment when there was excess $CaCO_3$, helping to maintain ecosystem function. Conversely, a lack of a positive effect of $CaCO_3$ could indicate that additional eutrophication-induced stressors (hypoxia, H_2S toxicity) were more important regulators.

3.3 Methods

3.3.1 Study site and experimental setup

The experiment was conducted in the mid-intertidal zone of Tuapiro estuary (37°29.406', 175°57.074') within Tauranga Harbour, New Zealand. The site experiences a diurnal tidal cycle with inundation periods of ~5 h and a mean water depth ~1.2 m upon maximum inundation. Plots were established within a 12 m x 30 m (360 m^2) area of homogeneous fine sand (median grain size 191 µm) with low mud (silt/clay particles < 63 µm) (< 5 %), $CaCO_3$ (< 1 %) and organic content (1.6–2.2 % in un-enriched plots) (Table 3.1).

In austral summer (31st January 2017), forty 1.44 m^2 plots were established (2 m apart) in a 10 column by 4 row array. Four replicates of each of the following three treatments were randomly allocated: ambient (no treatment), procedural control (PC; sediment disturbed mimicking treatment setup) and plots that received $CaCO_3$

(pure crushed oyster shell, fragments < 5 mm) but no organic matter (+$CaCO_3$ (–OM)). The remaining 28 plots were randomly assigned one of nine organic matter enrichment treatments created using 250–2250 g dw m^{-2} of organic plant-derived slow-release garden fertiliser (treatments increasing in 250 g increments in a gradient design). Enriched plots were also assigned a $CaCO_3$ treatment (with or without the addition of 2000 g dw m^{-2}). Replication was carried out for the lowest (250 g m^{-2} (n = 3)), middle (1250 g m^{-2} (n = 2)) and highest (2000 (n = 2) and 2250 g m^{-2} (n = 3)) organic matter treatments. The materials used to create organic matter and $CaCO_3$ treatments were chosen to mimic natural inputs to estuarine systems, whilst slow-release fertiliser ensured sustained organic matter degradation would result in alterations to the biogeochemical properties of the sediment.

Plots were excavated to 5 cm depth, taking care to minimise disruption to the sediment matrix, before evenly distributing organic matter and $CaCO_3$ (in the +$CaCO_3$ treatments) across plots and replacing sediment. Treatment materials were incorporated at 5 cm depth to minimise the risk of resuspension with the flooding tide, whilst ensuring they remained within the range of macrofaunal bioturbation. The organic matter enrichment range was chosen to create a gradient in sediment OC that would elicit a response typical of eutrophically-stressed systems (i.e. acidified porewaters and reduced biodiversity), and represents a one-off depositional event such as a macroalgal bloom collapse. Such events are common globally (e.g. Kennison and Fong (2014) and Quillien *et al.* (2017)) and in the wider estuary due to seasonal *Ulva* spp. blooms (de Winton *et al.*, 1998). The level of $CaCO_3$ enrichment elevated the average ambient content at the study site by 10x, which is representative of the use of $CaCO_3$ as an acidification mitigation tool rather than a natural increase in $CaCO_3$ content. Comparisons of sediment characteristics, macrofaunal community indices and benthic solute fluxes between ambient, procedural and $CaCO_3$ control plots confirmed there was no significant effect associated with the presence of the added $CaCO_3$ or the procedure of adding it to the sediment (see Appendix 3).

3.3.2 *In situ* sampling

Benthic solute fluxes and sediment properties were measured *in situ* after nine weeks (3rd–4th April 2017, early austral autumn), and macrofauna communities were sampled the following week (11th April 2017), allowing time for changes in the sediment biogeochemistry and macrofauna community to occur. Benthic flux incubations were conducted over two days with midday high tides. Fluxes of dissolved solutes across the sediment–water interface were measured under light and dark conditions in each plot, i.e. in the presence and absence of photosynthesis. As rates of photosynthesis vary with light intensity (Eilers & Peeters, 1988), all light measurements were conducted on day one (and dark measurements on day two) so that chambers experienced the same light regime (Anthony *et al.*, 2004). One ambient plot was dropped from the analysis due to a chamber malfunction.

The operation of the incubation chambers used for measuring benthic fluxes has previously been described (Lohrer *et al.*, 2013; Thrush *et al.*, 2017; Drylie *et al.*, 2018). Briefly, plots were divided into quarters and chamber bases (L50 x W50 x H15 cm) were positioned in a randomly chosen quarter ~10 cm from plot edges, and inserted 5 cm into the sediment. On day 2 (dark incubations), chambers were placed diagonally opposite to the day 1 position to minimise disturbance effects. HOBO Pendant temperature and light loggers (Onset) were installed in 16 of the chambers dispersed across the site, with an additional three secured on the seafloor (10 s sampling frequency). Domed Perspex lids were clamped onto bases on the incoming tide, encapsulating ~34 L of water. Lids were transparent for light incubations, whilst double-layered shade cloth covered lids for dark incubations. Previous measurements using HOBO loggers have recorded zero light inside chambers covered using this method (V. Rullens, unpublished data). Syringe-drawn water samples (60 mL) were collected in triplicate at the start and end of the ~4-h incubation. Ambient seawater samples (n = 3) were collected as chambers were sealed, and 3 light and dark pairs of 1 L bottles were filled and secured at the seafloor for the duration of the incubations; sampling the bottles at the end of incubations allowed determination of the contribution of water column processes to solute fluxes. Water column processes accounted for < 7 % of benthic solute fluxes in both light and dark conditions and were therefore ignored. Seawater dissolved oxygen (DO) was measured immediately using an optical probe (PreSens FIBOX 3 LCD trace v7); a two-point calibration was performed prior to measurements in a Na_2SO_4 O_2-free (0 %) solution and air-saturated water (100 %) following the manufacturer's instructions. Samples were then filtered through 0.8 µm glass fibre filters (Whatman GF/F) and stored frozen, in the dark prior to analysis of dissolved nutrient concentrations.

Weather conditions varied substantially between days of benthic flux measurements; day 1 was sunny with little wind wave activity whereas day 2 was cloudy with intermittent rain and some wind wave activity. Although the cloudier conditions experienced on day 2 were irrelevant in terms of incident sunlight (day of dark incubations), mean water temperature inside chambers differed between day 1 (23 ^{o}C) and 2 (19 ^{o}C), which could have impacted solute fluxes.

Sediment cores were collected from within each plot to characterise sediment properties. Six small cores (2.6 cm diameter x 2 cm depth) were pooled per plot and stored frozen, in the dark for analysis of sediment grain size and indicators of MPB biomass (chlorophyll *a* (Chl *a*) and phaeopigments (phaeo)). Four medium cores (5 cm diameter x 7 cm depth) were collected and 0–2 cm and 2–5 cm sections were pooled for OC analysis at both depth intervals and $CaCO_3$ content at 2–5 cm (depth of burial). Two large cores (13 cm diameter x 15 cm depth) for macrofaunal analysis were collected and sieved *in situ* over a 500 µm mesh and preserved in 70 % isopropyl alcohol. Finally, porewater pH was measured in each plot using a digital field probe (Eutech pHTestr1, 2; accurate to ± 0.2 pH units with a tip diameter of 3 mm) at ~2 cm depth following calibration using pH 4 and 7 standard solutions.

Sediment samples were thawed, homogenised and divided for analysis of sediment properties. Grain size samples (~10 g) were digested in 10 % hydrogen peroxide to remove organic matter before measuring using a Malvern Mastersizer 2000 (particle size range 0.05–2000 µm). Sediment pigment samples (Chl *a* and phaeo) were processed using standard procedures (Arar & Collins, 1997) and analysed on a Turner 10–AU fluorometer using an acidification step. Percent organic and $CaCO_3$ contents were determined from 5 g dried sediment samples (100 ^{o}C until constant weight) according to standard loss on ignition (LOI) procedures for OC (Heiri *et al.*, 2001) and $CaCO_3$ (Wang *et al.*, 2011). Macrofauna samples were stained with Rose of Bengal and identified to lowest possible taxonomic level (usually species). Filtered water samples were thawed and analysed for dissolved inorganic nutrient species (ammonium (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-)) using standard colorimetric and fluorometric techniques (Astoria 2 Pacific autoanalyser).

3.3.3 Data analysis

Dissolved oxygen and nutrient fluxes (µmol m^{-2} h^{-1}) were calculated by subtracting the final concentration (µmol L^{-1}) of the given solute from the initial concentration and standardising by the incubation duration (h), chamber water volume (L) and enclosed sediment surface area (m^2). These fluxes underpinned the quantification of key ecosystem functions: light chamber DO flux was equivalent to net PP (NPP) and dark chamber DO flux was equivalent to sediment oxygen consumption (SOC; a proxy for community metabolism), while fluxes of nitrogenous compounds indicated rates of nutrient cycling. Concentrations of NO_2^- and NO_3^- were frequently below detection limits and are thus combined and represented as NO_x.

In all analyses, organic matter treatment (i.e. the quantity of organic matter initially added to plots) was used as the continuous independent variable against which responses were measured. Using organic matter treatment better represented the initial stress experienced by the benthic ecosystem, whereas OC (measured at the time of flux incubations) alternatively indicated the degree of organic matter degradation that had occurred since plot establishment, which may vary according to the natural heterogeneity of the environment.

To determine whether organic matter and $CaCO_3$ treatments (incorporated at 5 cm sediment depth) had altered sediment characteristics as intended, OC (2–5 cm) and porewater pH were regressed against organic matter treatment, and a *t*-test was performed to detect differences in $CaCO_3$ content (2–5 cm) between +$CaCO_3$ and –$CaCO_3$ plots. Macrofauna abundance data were averaged from two cores per plot, and total abundance and total number of taxa were determined per core (133 cm^2).

Simple linear regression was used to identify significant ($\alpha < 0.05$) responses of the macrofauna community and ecosystem functions to organic matter treatment (using Statistica v13).

To simultaneously account for the gradient in organic matter treatment and assess the effects of $CaCO_3$ addition on sediment characteristics, macrofauna community and ecosystem functions, PERMANCOVA (using PERMANOVA+, Primer v7) were performed based on Euclidean distances with $CaCO_3$ treatment considered a fixed factor and organic matter treatment a continuous co-variable. Where a significant interaction ($p(perm) < 0.05$) occurred $–CaCO_3$ and $+CaCO_3$ treatments were considered separately for subsequent analyses, in the absence of a significant interaction the treatments were pooled. As PERMANCOVA obtains p-values through permutation and does not assume normality of residuals or homogeneity of variances, response variables were untransformed.

Some ecosystem functions were variable yet significantly correlated with OM treatment; therefore, multiple regression (using distance-based linear models; DistLM in Primer v7) was used to determine the drivers of this variability. Marginal tests first identified significant ($p(perm) < 0.1$) predictors of response variables when considered alone, and full models then determined the best combination of predictor variables after accounting for the variance attributed to organic matter treatment (organic matter treatment was always fitted first). Full models used a backward elimination procedure with the corrected Akaike's Information Criterion (AICc) and 9999 permutations to obtain the most parsimonious model (Anderson *et al.*, 2008). Predictor variables were normalised and highly correlated variables (Pearson's $r > 0.8$) were identified; the variable explaining the lesser proportion of variance was excluded from model selection to avoid collinearity (Dormann *et al.*, 2013).

3.4 Results

Sixty-two days after manipulation, surface sediment characteristics (0–2 cm) were similar between plots (Table 3.1) and any effects of the disturbance caused by treatment establishment had subsided (see Appendix 3 for ambient and control treatment comparisons). Only phaeopigments, a product of chlorophyll degradation, showed a relatively large range (2–27 µg g^{-1} dw sediment) driven by high values in organically enriched plots. At 2–5 cm sediment depth (organic matter and $CaCO_3$ were added at a depth of 5 cm), a significant elevation of $CaCO_3$ content in +$CaCO_3$ treatments (1.4 %) versus –$CaCO_3$ treatments (0.5 %) (F(1, 33) = 12.81, p = 0.001; Figure 3.1a) was achieved. There was no significant interactive effect between $CaCO_3$ and organic matter treatments on organic content (OC) or porewater pH, but there was a significant effect of organic matter treatment (Table 3.2). A linear increase was observed in OC along the organic matter treatment gradient ($r^2 = 0.44$, $p < 0.001$; Figure 3.1b) with mean OC doubling from 2 % in 0 g dw m^{-2} plots to 4 % in 2250 g dw m^{-2} plots, and a linear decrease in porewater pH from 7.3 to 6.6 ($r^2 = 0.47$, $p < 0.001$; Figure 3.1c). Both OC and porewater pH exhibited high variability, particularly at the highest levels of organic matter treatment.

Table 3.1. Surface sediment (0–2 cm) characteristics in ambient, procedural control (PC) and treatment plots 62 d after manipulation. Medians (and ranges) are given.

Treatment	Median grain size (µm)	Mud content (%)	Organic content (%)	Chlorophyll *a* (µg g^{-1} dw sediment)	Phaeopigment (µg g^{-1} dw sediment)
Ambient (n = 3)	189 (187–190)	2.6 (1.7–4.7)	1.7 (1.6–1.9)	10.3 (10.1–14.2)	5.1 (4.4–5.6)
PC (n = 4)	191 (185–195)	4.5 (2.3–5.7)	2.1 (1.9–2.2)	10.2 (8.2–14.5)	3.6 (3.4–5.7)
+$CaCO_3$ (-OM) (n = 4)	193 (190–197)	3.1 (1.3–4.5)	1.8 (1.7–1.9)	11.5 (9.5–12.5)	3.6 (2.9–4.8)
+$CaCO_3$ (+OM) (n = 14)	192 (177–199)	2.6 (1.1–6.1)	1.9 (1.7–3.0)	11.8 (8.9–20.3)	6.0 (4.3–22.7)
-$CaCO_3$ (+OM) (n = 14)	191 (182–198)	2.8 (1.3–3.5)	2.0 (1.7–2.4)	11.6 (6.6–15.2)	5.7 (2.2–27.1)

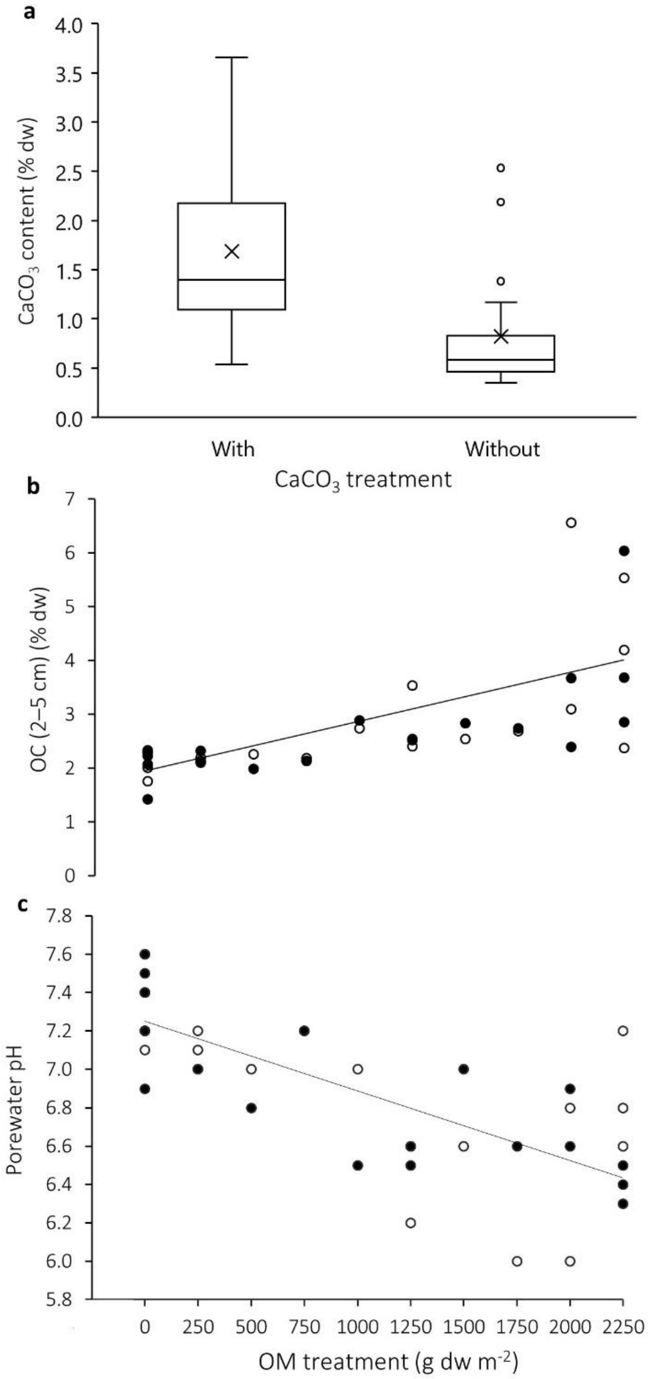


Figure 3.1. Effects of treatments ($CaCO_3$ and organic matter (OM) addition) on sediment a) $CaCO_3$ content (t-test, $p = 0.001$) b) organic content (OC) ($r^2 = 0.44$, $p < 0.001$) and c) porewater pH ($r^2 = 0.47$, $p < 0.001$) 62 d after manipulation. Closed symbols represent $–CaCO_3$ treatments and open symbols represent $+CaCO_3$ treatments. Regression lines are fitted to the pooled data set (see Table 3.2). Boxplot whiskers indicate the non-outlier range, with circles marking outliers. Box limits represent 25th and 75th percentiles, lines intersecting boxes are median values and crosses within boxes are means.

Table 3.2. Results of PERMANCOVA testing the direct and interactive effects of organic matter (OM, continuous covariable) and $CaCO_3$ (fixed factor) treatments on sediment characteristics, macrofauna community and ecosystem functions. Bolded values indicate significant (p(perm) < 0.05) and italicised values indicate marginally significant (p(perm) < 0.1) effects. OC = organic content, NPP = net primary production, SOC = sediment oxygen consumption, ln NH_4^+ = natural log-transformed ammonium and NO_x = nitrate + nitrite. Degrees of freedom = 1 for all tests.

			Source	Pseudo-F	p (perm)
Sediment characteristics					
	OC (2–5 cm)		OM	26.1	**<0.01**
			$CaCO_3$	0.61	0.45
			OM x $CaCO_3$	0.26	0.62
	Porewater pH		OM	28.9	**<0.01**
			$CaCO_3$	0.03	0.87
			OM x $CaCO_3$	0.003	0.96
Macrofauna					
	Total abundance		OM	100	**<0.01**
			$CaCO_3$	0.49	0.49
			OM x $CaCO_3$	1.30	0.27
	Total species		OM	38.6	**<0.01**
			$CaCO_3$	1.15	0.29
			OM x $CaCO_3$	1.98	0.17
Ecosystem functions					
	NPP		OM	39.4	**<0.01**
			$CaCO_3$	0.26	0.66
			OM x $CaCO_3$	8.91	**<0.01**
	SOC		OM	0.52	0.48
			$CaCO_3$	0.17	0.68
			OM x $CaCO_3$	1.56	0.23
	ln NH_4^+ flux	Light	OM	62.8	**<0.01**
			$CaCO_3$	0.001	0.98
			OM x $CaCO_3$	1.69	0.21
		Dark	OM	0.61	0.44
			$CaCO_3$	0.89	0.35
			OM x $CaCO_3$	11.4	**<0.01**
	NO_x flux	Light	OM	1.07	0.31
			$CaCO_3$	3.12	*0.08*
			OM x $CaCO_3$	0.04	0.84
		Dark	OM	0.06	0.80
			$CaCO_3$	0.30	0.59
			OM x $CaCO_3$	0.98	0.33

Macrofauna community indices were only affected by organic matter treatment; $CaCO_3$ treatment and its interaction with organic matter were both insignificant (Table 3.2). Mean total abundance decreased by 72 % ($r^2 = 0.74$, $p < 0.001$; Figure 3.2a) and total species by 31 % ($r^2 = 0.52$, $p < 0.001$; Figure 3.2b) along the organic matter treatment gradient.

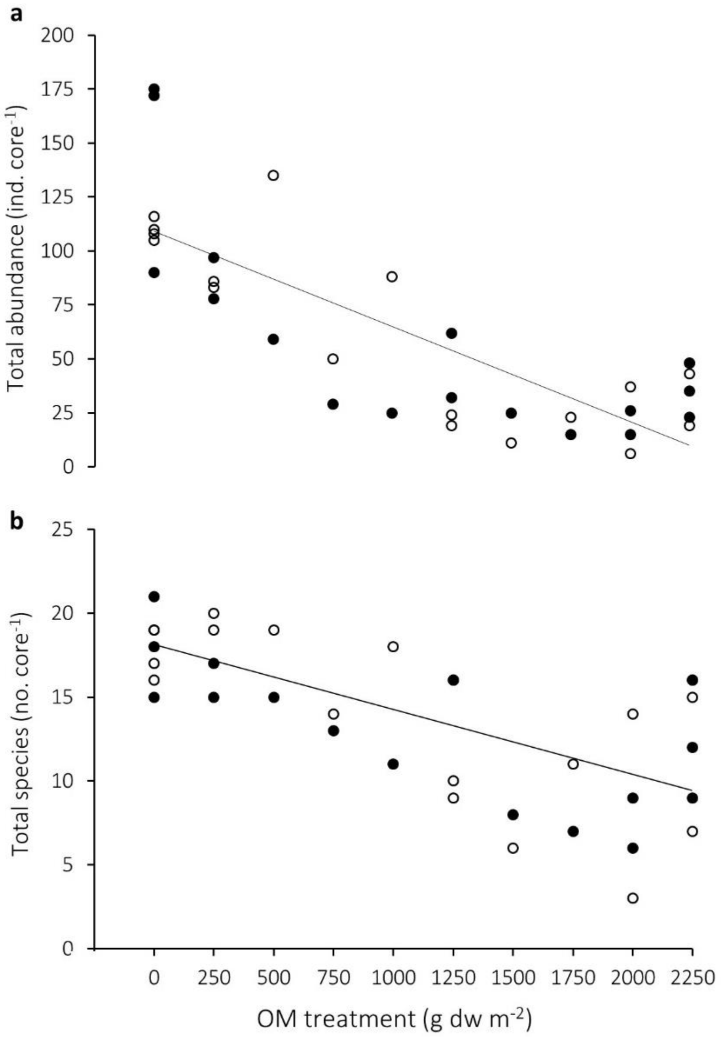


Figure 3.2. Macrofauna community response to organic matter (OM) treatment: a) total abundance ($r^2 = 0.74$, $p < 0.001$) and b) total species ($r^2 = 0.52$, $p < 0.001$). Closed symbols represent –$CaCO_3$ treatments and open symbols represent +$CaCO_3$ treatments, and regression lines are fitted to the pooled data set (see Table 3.2).

Treatment effects were not consistent across ecosystem functions; NPP and NH_4^+ flux exhibited significant relationships with organic matter treatment, whereas SOC and NO_x did not (Table 3.2). Chambers at low levels of enrichment were initially autotrophic (PP > SOC) during light incubations and became heterotrophic (SOC > PP) at higher levels of organic matter treatment (Figure 3.3a). $CaCO_3$ treatment significantly altered the response of NPP to organic matter enrichment (Table 3.2), with +$CaCO_3$ plots exhibiting net autotrophy to higher levels of organic matter treatment than –$CaCO_3$ plots (~1400 versus ~950 g dw m^{-2}). NPP decreased significantly with increasing organic matter treatment in –$CaCO_3$ plots ($r^2 = 0.77$, $p < 0.001$), whereas the loss in productivity in +$CaCO_3$ plots was less steep, more variable and was only marginally correlated with organic matter treatment (r^2 = 0.24, p = 0.05). SOC showed no relationship with organic matter or $CaCO_3$ treatments (Figure 3.3b, Table 3.2) with high variability across the range of organic matter treatments.

Under light conditions natural log-transformed NH_4^+ flux ($NH_4^+{}_{light}$) showed a significant response to organic matter treatment, but not $CaCO_3$ or its interaction with organic matter treatment (Table 3.2). As organic matter enrichment increased, the rate of $NH_4^+{}_{light}$ efflux from sediments increased exponentially (r^2 = 0.65, p = < 0.001; Figure 3.3c), such that the rate of $NH_4^+{}_{light}$ efflux was more than 500 times greater in 2250 versus 0 g dw m^{-2} treatments. In light chambers, NO_x ($NO_{x\ light}$) showed a marginally significant response to $CaCO_3$ addition (but not organic matter treatment; Table 3.2), with a slight decrease in net uptake (~15 µmol m^{-2} h^{-1}) in +$CaCO_3$ treatments (Figure 3.3e). Under dark conditions (i.e. in the absence of microphytobenthos (MPB) uptake), there was a significant relationship between organic matter treatment and natural log-transformed NH_4^+ ($NH_4^+{}_{dark}$) efflux which changed with $CaCO_3$ treatment, as indicated by the significant interaction term (Table 3.2). In –$CaCO_3$ treatments, efflux of $NH_4^+{}_{dark}$ increased exponentially as organic matter treatment increased (r^2 = 0.35, p = 0.01; Figure 3.3d); however, this relationship was absent in +$CaCO_3$ treatments and efflux rates were generally reduced. There was far greater variability in nutrient cycling measurements obtained under dark versus light conditions.

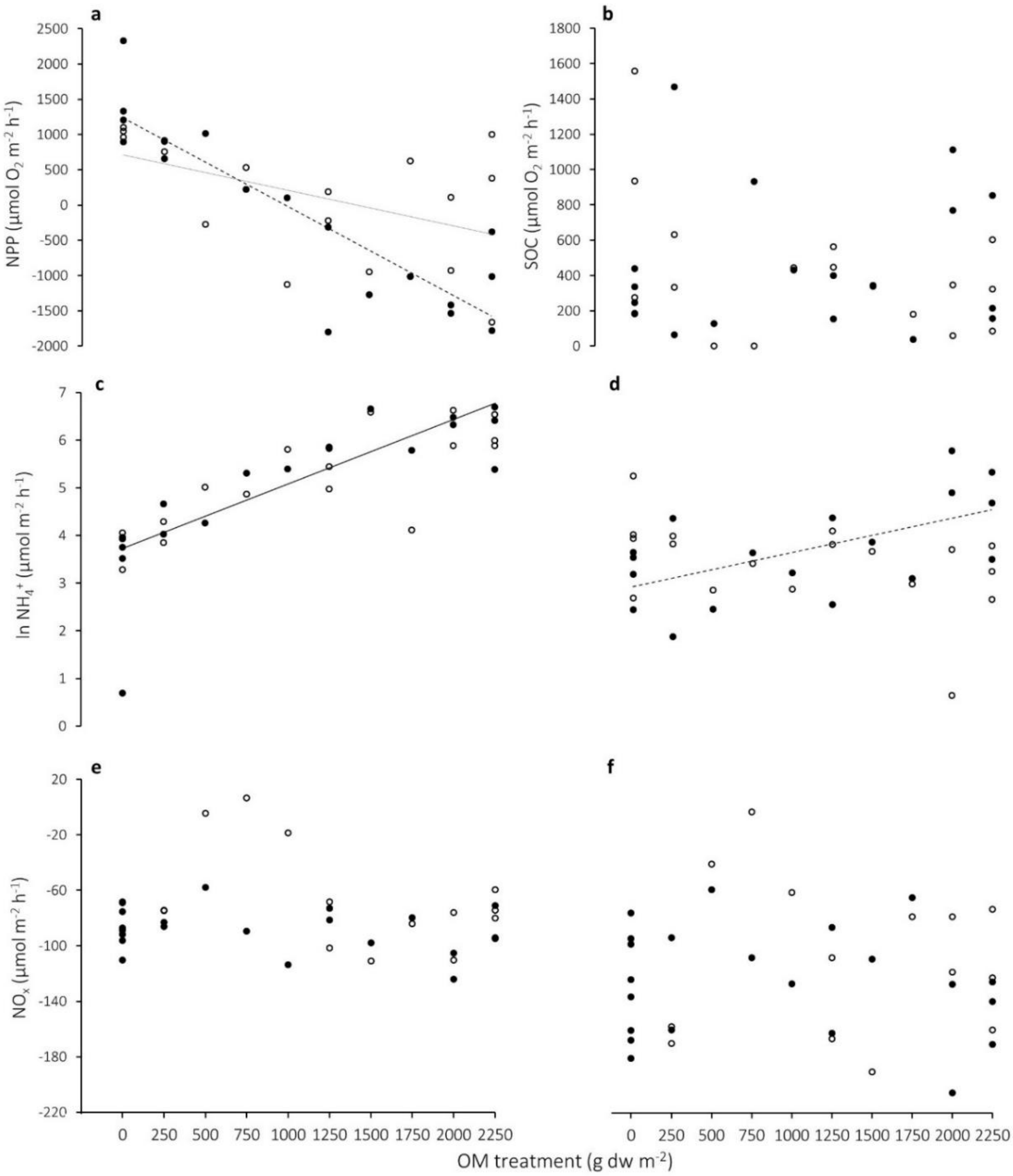


Figure 3.3. Response of ecosystem functions to organic matter (OM) and $CaCO_3$ addition: a) net primary productivity (NPP); b) sediment oxygen consumption (SOC); c) natural log-transformed ammonium (NH_4^+) efflux in the light and d) dark; e) combined nitrite and nitrate (NO_x) uptake in the light and f) dark. Closed symbols represent $–CaCO_3$ treatments and open symbols represent $+CaCO_3$ treatments. Significant ($\alpha < 0.05$) linear regressions are indicated (see text for test statistics): dashed line = $–CaCO_3$ treatment; solid grey = $+CaCO_3$ treatment; solid black = combined $CaCO_3$ treatments (i.e. no interaction between OM and $CaCO_3$ treatments; see Table 3.2).

In marginal tests, variability in ecosystem functions was correlated with several variables including organic matter treatment, sediment characteristics and macrofauna community indices (Table 3.3). For functions that exhibited a significant interaction between organic matter and $CaCO_3$ treatments (NPP and $NH_4^+{}_{dark}$), the $–CaCO_3$ treatment had a greater number of significant predictors that individually explained more of the variation than the +$CaCO_3$ treatment. When organic matter treatment was fitted first, it was the only predictor included in backwards elimination linear regression models of NPP for both $CaCO_3$ treatments, but the amount of variance explained decreased from 76 % in –$CaCO_3$ treatments to 21 % in +$CaCO_3$ treatments. Although macrofauna total abundance and porewater pH explained large amounts of variance in NPP (–$CaCO_3$ treatments) in marginal tests, both variables were highly correlated with organic matter treatment ($r > 0.8$) and were excluded from the model. The variables included in the full model of $NH_4^+{}_{dark}$ flux differed depending on $CaCO_3$ treatment. A combination of organic matter treatment and sediment characteristics ($CaCO_3$ content and phaeopigments) together explained 68 % of the variance in –$CaCO_3$ treatments, whereas only 17 % of the variance was explained in the full model for +$CaCO_3$ treatments by organic matter treatment. In marginal tests total species explained more of the variation in +$CaCO_3$ treatments than organic matter treatment but was excluded from the full model. For $NH_4^+{}_{light}$ flux, despite numerous variables being identified as significant predictors when considered alone, only two were included in the full model; organic matter treatment contributed most of the total variance explained (66 of 71 %), with the remainder attributed to chlorophyll *a* (Chl *a*), an indicator of MPB abundance.

Table 3.3. Results of DistLM determining best combinations of predictor variables (backwards elimination procedure) explaining ecosystem function response to organic matter (OM) treatment. OM treatment was fitted first in all models and $CaCO_3$ treatments are analysed separately where significant interactions between OM and $CaCO_3$ treatments occur. Proportion gives the amount of variability explained by each variable when considered alone (marginal tests). Variables in bold were included in the best full models and total shows the total variation explained by full models. Level of significance: *p < 0.1, ** p < 0.05, *** p < 0.01.

Function	Treatment	Variable	Pseudo-F	Proportion	Total
NPP	-$CaCO_3$	**OM treatment**	**60.3**	**0.76*****	
		Total abundance	20.8	0.52***	
		Porewater pH	18.0	0.49***	
		Phaeopigments	8.63	0.31***	
		OC 2-5 cm	7.38	0.28***	
		Total species	7.46	0.28**	
		Mud content	4.74	0.2**	
					76%
	+$CaCO_3$	**OM treatment**	**7.01**	**0.21*****	
		Total abundance	10.6	0.4***	
		Porewater pH	3.64	0.19*	
					21%
Ln Dark NH_4^+ flux	-$CaCO_3$	**OM treatment**	**9.76**	**0.34*****	
		Phaeopigments	**10.1**	**0.35*****	
		$CaCO_3$ content	**5.86**	**0.24****	
		Total abundance	4.93	0.21**	
		Total species	3.39	0.15*	
					68%
	+$CaCO_3$	**OM treatment**	**3.34**	**0.17 ***	
		Total species	6.61	0.29**	
		Total abundance	4.1	0.2*	
					17%
Ln Light NH_4^+ flux		**OM treatment**	**73.1**	**0.66*****	
		Total abundance	51.7	0.58***	
		Porewater pH	18.4	0.33***	
		OC 2-5 cm	15.6	0.3***	
		Total species	14.4	0.28***	
		Phaeopigments	5.19	0.12**	
		Chl *a*	4.89	0.12**	
					71%

3.5 Discussion

Natural fluctuations in pH may be extreme in the benthos of productive estuarine environments (Baumann *et al.*, 2015); however, coastal eutrophication and global OA are set to intensify acidification across varying scales of magnitude, space and time such that benthic ecosystem functions may be compromised. For the first time an *in situ* manipulation of porewater pH and investigation of the ability of $CaCO_3$ to prevent negative change has shown that $CaCO_3$ deposits may increase the functional resilience of benthic ecosystems to localised eutrophication-induced acidification.

The decline of benthic biodiversity with increasing inputs of organic matter is well-documented and usually attributed to the inducement of hypoxic conditions (Pearson & Rosenberg, 1978; Riera *et al.*, 2013); however, the potential for co-occurring porewater acidification to drive these declines has been comparatively under-studied. The highest organic matter treatment resulted in a porewater pH of 6.6, and the loss of one third of macrofauna species and almost three quarters of total individuals. No increase in porewater pH could be detected in $+CaCO_3$ treatments, nor any difference in the relationship between macrofauna community indices and organic matter treatment. This suggests that $CaCO_3$ did not buffer porewater pH sufficiently to decrease the physiological stress of the organisms, or that other consequences of organic matter degradation such as hypoxia or increased $[H_2S]$ were more important in driving the macrofaunal response. The latter seems most likely, given $CaCO_3$ dissolution under open-system conditions would serve to maintain pH within a given range, rather than drive it higher than typical porewater values (Stumm & Morgan, 2012).

These biodiversity losses were associated with a predictable decrease in function, given biodiversity–ecosystem functioning relationships are frequently density-dependent (Sandwell *et al.*, 2009) and/or underpinned by key species (Thrush *et al.*, 2006; Woodin *et al.*, 2016). NPP decreased significantly along the organic matter treatment gradient whilst $NH_4^+{}_{light}$ efflux increased, indicating a decrease in the utilisation of nutrients by the MPB community. Chl *a* biomass, a proxy for MPB abundance, showed no relationship with organic matter treatment and therefore cannot explain trends in NPP. It has been hypothesised that increased $pCO_{2(aq)}$ would result in increased productivity of photoautotrophs, and although most research has focussed on seagrasses and macroalgae (Koch *et al.*, 2013; Celis-Plá *et al.*, 2015), increases in the PP of MPB have been observed in the laboratory following exposure to CO_2-acidified seawater (Johnson *et al.*, 2013; Vopel *et al.*, 2018). The unfavourable sediment conditions (low pH, low $[O_2]$) created here may have stressed the MPB community beyond being able to take advantage of excess CO_2. The decrease in abundance of macrofauna, whose activities are known to stimulate MPB PP (Lohrer *et al.*, 2004), may also have played a role. However, $CaCO_3$ addition increased the resilience of NPP to these conditions. Significant increases in pH, total alkalinity (TA) and saturation states have been measured previously as a result of biogenic $CaCO_3$ dissolution (Green *et al.*, 2013; Pickett & Andersson, 2015; Sulpis *et al.*, 2018); so the coarse methods used to measure pH changes here (pH field probe with ± 0.2 pH units accuracy) may have masked finer scale alterations to porewater biogeochemistry which benefitted the MPB community. Importantly, $CaCO_3$ addition had the effect of maintaining autotrophy of the benthic ecosystem to a considerably higher level of organic matter treatment, and this effect was strong enough to be detected amid the complexity of the field.

The response of light and dark nutrient cycling to organic matter treatment (increasing efflux of NH_4^+ and no response of NO_x) indicates a limitation or breakdown of microbially-mediated nitrification pathways that oxidise NH_4^+ to NO_2^- and NO_3^-. There are several ways that organic matter treatment may have limited nitrification. For example, a decrease in bioturbation and bioirrigation activity driven by the decrease in macrofaunal abundance may have reduced the availability of aerobic sediments (Solan *et al.*, 2004), which are required by microbial nitrifiers (i.e. ammonia- and nitrite-oxidisers). However, Braeckman *et al.* (2014) have previously shown that in the short-term (14 d), decreases in benthic nitrification rates under acidified conditions are mediated predominantly by changes in microbial community activity, rather than macrofaunal facilitation. Inhibition of nitrifiers at low pH may therefore be a factor, although it is expected that microbes are tolerant to considerable fluctuations in pH and Kitidis *et al.* (2011) previously found no significant decrease in nitrification rates at pH 6.1. Substrate-limitation is perhaps the most likely cause as NH_3 is the true substrate for ammonia-oxidisers, yet below pH ~8.0 NH_3 is present almost exclusively as NH_4^+ (Suzuki *et al.*, 1974; Beman *et al.*, 2011). Alternatively, biogeochemical processes such as dissimilatory NO_x reduction to NH_4^+ may have increased [NH_4^+], a process which can be quantitatively more important than denitrification in organically-rich sediments (Binnerup *et al.*, 1992; Kessler *et al.*, 2018).

$CaCO_3$ addition altered the relationship between nutrient cycling and organic matter treatment (in the dark only), such that there was no trend of increasing NH_4^+ efflux in +$CaCO_3$ treatments. As porewater O_2 would have been scarce in dark conditions (absence of photosynthesis) and $CaCO_3$ addition had no effect on the macrofaunal community, it is unlikely that the improvement to sediment conditions that helped maintain nitrification rates was due to increasing availability of aerobic sediments. Similarly, there was no detected increase in pH with $CaCO_3$ addition, therefore NH_3 availability should be equally limiting. It seems most likely, therefore, that subtle changes to porewater biogeochemistry that were not measured played a role in improving conditions for nutrient cycling microbes. In future research, it will be important to monitor a larger suite of carbonate chemistry variables (pH, TA, dissolved inorganic carbon (DIC)) to identify the mechanisms underlying these functional responses. However, the aim of this study was to investigate whether laboratory-based theory could be applied in the field, and indeed, $CaCO_3$ addition successfully dampened the negative effects of organic matter enrichment on nutrient cycling. This justifies further investigation of these interactions *in situ* and has important consequences for the functioning of coastal ecosystems, given the conversion of biologically-available nitrogen to nitrogenous gas provides resilience against further eutrophication (Seitzinger & Nixon, 1985; Cosby *et al.*, 2003).

Whilst some ecosystem functions responded strongly to organic matter treatment, SOC showed no relationship with enrichment, OC or porewater pH. Since SOC is the net result of biological (BOD) and chemical oxygen demand (COD) (Hargrave, 1969), it was hypothesised that SOC would increase with organic matter treatment due to an increased BOD of the stimulated microbial community and COD from increasing abundance of reducing substances, as has previously been reported for organically enriched sediments (e.g. Hargrave *et al.* (1997), Valdemarsen *et al.* (2010) and Bannister *et al.* (2014)). The lack of a relationship may be explained by a 'compensation' in O_2 consumption along the organic matter treatment gradient, whereby the gradual decrease in respiration and O_2 consumption facilitated by macrofauna (due to decreasing abundance and stress-induced change in activity levels) was offset by a gradual increase in microbial respiration and COD, such that overall SOC remained constant.

In $–CaCO_3$ treatments combinations of organic matter treatment, sediment characteristics and macrofauna community variables were important predictors of NPP and $NH_4^+{}_{dark}$ flux. In $+CaCO_3$ treatments, far fewer variables were significant predictors of function, though macrofauna community indices remained significant and individually explained more variation than organic matter treatment. This implies a strong facilitation of ecosystem function by the macrofauna community even when the geochemical complexities of sediments are enhanced. Organic matter treatment was the only predictor included in several full models (when fitted first), highlighting the dominant effect of this stressor on ecosystem function, however there was a substantial decrease in the variance it explained in $+CaCO_3$ treatments compared to $–CaCO_3$. This discrepancy may have been associated with the microbial component of the ecosystem, as it is the microbial community that converts and transforms solutes, though the macrofauna and sediment characteristics are key controllers of solute transport rates and microbial microhabitats (Kristensen & Kostka, 2013). Changes to porewater chemistry may also have driven the responses observed, therefore including a greater number of carbonate chemistry parameters and microbial community indicators in models may result in greater explanation of the functional response.

In this short-term, single-application experiment, $CaCO_3$ effectively reduced the negative effects of organic matter enrichment on ecosystem function. However, over longer timeframes and repeated $CaCO_3$ applications there may be potential effects associated with its addition. The extent of these effects will likely be dependent upon the difference in grain size between the ambient sediment and the added $CaCO_3$, and whether this difference substantially alters grain size distribution. If so, benthic ecosystem functions could be affected as diffusive solute exchange across the sediment–seawater interface is highly dependent upon sediment permeability (Huettel & Gust, 1992), and the effects of macrofaunal bioturbation on function change along gradients of sediment grain size (Needham *et al.*, 2011). To avoid such impacts $CaCO_3$ should be crushed or ground to match the sediment characteristics of the receiving site prior to addition, whilst keeping in mind that the dissolution rates of $CaCO_3$ will vary as a function of grain size (Pickett & Andersson, 2015).

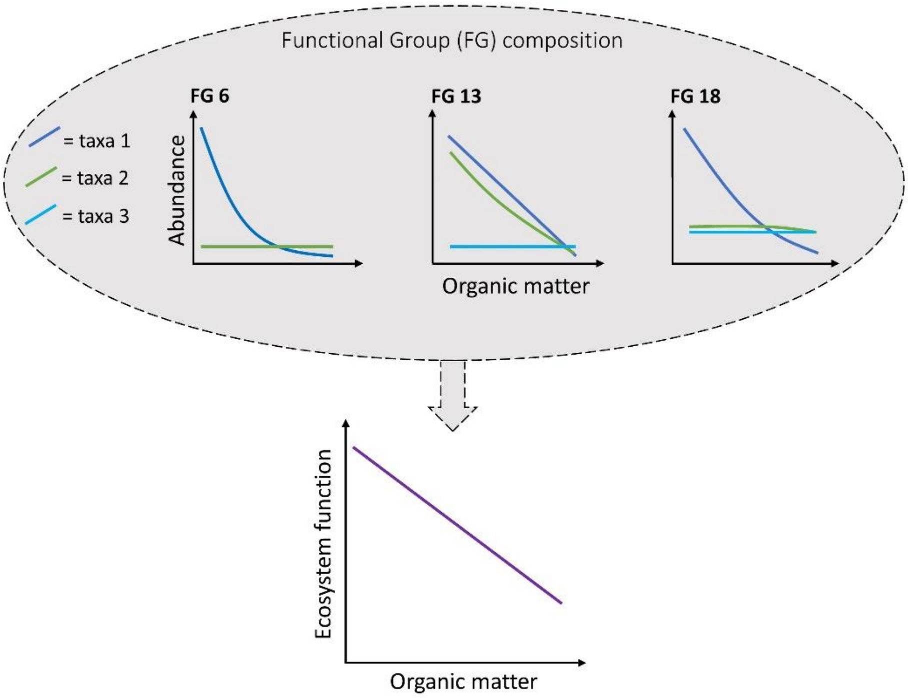
3.6 Conclusions

Benthic ecosystem functions respond demonstrably to experimentally elevated organic matter and $CaCO_3$ content in a field setting, despite complex interactions occurring between levels of biological organisation and environmental characteristics that influence function. Porewater acidification resulting from organic matter enrichment has negative effects on primary production and nutrient cycling. These negative effects are dampened by the addition of biogenic $CaCO_3$, thus increasing the resilience of the ecosystem to this acidification stressor. The lack of a beneficial effect of $CaCO_3$ on macrofauna biodiversity suggests other stressors associated with eutrophication (hypoxia, H_2S toxicity) were stronger determinants of community structure than pH. The positive effects of $CaCO_3$ addition may therefore be microbially mediated, and future research should focus on the combined response of macro- and microbiological communities to coastal eutrophication *in situ*. Developing a mechanistic understanding of how $CaCO_3$ provides resilience to ecosystem function will be crucial, as losses of key $CaCO_3$-producing species are likely under future coastal acidification stressors.

CHAPTER 4:

Resilience of benthic macrofauna to experimental organic enrichment: Consequences for ecosystem function

4.1 Graphical abstract



4.2 Introduction

Natural and anthropogenic disturbances are frequent in marine benthic environments, such that macrobenthic communities may be considered a mosaic of patches each at differing stages of recovery (Probert, 1984; Ellis *et al.*, 2000a). Organic matter enrichment is one of the most common disturbances to seafloor environments and has been a focus of benthic ecology research for decades (Pearson & Rosenberg, 1978; Brown *et al.*, 1987; Fernández-Rodríguez *et al.*, 2019). In urbanised coastal environments, anthropogenic activities can enhance organic matter delivery to the benthos directly, via excess feed and fecal matter from aquaculture operations (Weston, 1990; Cranford *et al.*, 2007), sewage treatment effluent (Grizzle & Penniman, 1991) and run-off from agricultural land (Dalzell *et al.*, 2005), or indirectly through nutrient enrichment-induced algal blooms (eutrophication) (Teichberg *et al.*, 2010). Although organic matter is critical for supporting benthic food webs (Deegan & Garritt, 1997), excess quantities stimulate biotic activity during decomposition resulting in sedimentary hypoxia, acidification and increased concentrations of toxic reduced solutes (Cai *et al.*, 2011; Howarth *et al.*, 2011; Wallace *et al.*, 2014). Such unfavourable conditions can cause shifts in the composition of benthic macrofaunal communities and drive alterations in benthic ecosystem function, although these linkages have rarely been documented *in situ* (see Gammal *et al.* (2017) and Norkko *et al.* (2019) for examples).

Shifts in the composition of benthic macrofaunal communities alter the representation of taxa-specific traits that influence processes like bioturbation, bioirrigation and shell formation; these processes underpin nutrient recycling, primary production and carbon sequestration functions, among others (Naeem *et al.*, 1994; Hooper *et al.*, 2005; Snelgrove *et al.*, 2014)., Burrowing organisms stimulate microbial activity by aerating sediments and translocating organic material. This significantly increases rates of organic matter decomposition, which is pertinent to the resilience of benthic ecosystems against organic matter enrichment (Heilskov & Holmer, 2001; Josefson *et al.*, 2012). Decreases in benthic biodiversity may therefore lead to harmful positive feedbacks that result in increasingly enriched sediments. To investigate the relationship between benthic biodiversity and ecosystem function (B–EF), macrofaunal taxa have frequently been combined into functional groups (FG) based on the combinations of traits they possess and how these are expected to influence ecosystem processes and functions (Bremner *et al.*, 2006; Queirós *et al.*, 2013; Greenfield *et al.*, 2016). This way, functional groups can act as proxies of ecosystem function and be used to explore relationships with taxonomic and functional diversity (see Danovaro *et al.* (2008) and Douglas *et al.* (2017) for examples in marine ecosystems). In some instances, changes in the abundance and richness of functional groups may have a stronger ability to explain ecosystem functions than traditional taxonomic classifications (McGill *et al.*, 2006; Cadotte *et al.*, 2011; Gagic *et al.*, 2015).

A central tenet of B–EF research is that the stability of ecosystem functions increases alongside the biodiversity that underpins them (i.e. the biological insurance hypothesis (Yachi & Loreau, 1999)). The underlying theory is that when a larger number of taxa support a function, there is a greater guarantee that some will remain to provide said function when environmental perturbations may exclude others (i.e. there is redundancy), thus reducing fluctuations in function over space and time (Naeem, 1998). Empirical evidence identifying the mechanism by which biodiversity provides resilience is a coveted goal of current B–EF research, with proposed mechanisms including density compensation, where the abundance of one taxa contributing to the function increases as a result of a decrease in the abundance of another (Gonzalez & Loreau, 2009), and response diversity, where populations of taxa providing the function respond differently to the same environmental change (Elmqvist *et al.*, 2003). Experimental manipulations have provided some support that these stabilising mechanisms exist in terrestrial ecosystems (see review by Mori *et al.* (2013) and also Stavert *et al.* (2017)), although there has likewise been evidence to the contrary (Valone & Barber, 2008). The existence of these mechanisms in marine ecosystems is yet to be confirmed, which has profound implications for how effectively ecosystems can be managed for the preservation of valuable ecosystem functions and services (Costanza *et al.*, 2014).

In their review, Hooper *et al.* (2005) concluded that B–EF field research lagged considerably behind theoretical advancements, and that this was particularly so in marine ecosystems. Consequently, empirical field-based evidence that stress-induced changes to benthic community composition alter ecosystem function has grown in the last decade (e.g. in response to metal contamination (Lohrer *et al.*, 2010), hypoxia (Villnäs *et al.*, 2012; Gammal *et al.*, 2017; Norkko *et al.*, 2019) and mud deposition (Rodil *et al.*, 2011; Pratt *et al.*, 2014a)). This relationship has also been explored for organic matter enrichment originating from aquaculture operations, either by measuring functions directly (e.g. Christensen *et al.* (2003) and Giles *et al.* (2006)), or by demonstrating shifts in functional traits (e.g. Papageorgiou *et al.* (2009) , Villnäs *et al.* (2011) and Sanz-Lázaro and Marín (2011)). However, these studies are conducted exclusively in subtidal environments and evidence demonstrating the functional response of intertidal ecosystems to organic matter enrichment are lacking. Van Colen *et al.* (2012) examined the recovery of mudflat functions impacted by eutrophication according to changes in the functional composition of the macrofaunal community, finding the replacement of small invertebrates by large bioturbators was crucial for restoring function. Indeed, substantial differences in the functional role of juvenile (< 10 mm) and adult (> 10 mm) bivalves *Austrovenus stutchburyi* and *Macomona liliana* have previously been demonstrated in New Zealand (Norkko *et al.*, 2013). However, there has been no complementary investigation of how community shifts drive the initial breakdown.

The similar responses of benthic macrofaunal communities to organic matter enrichment across broad geographical areas enabled the development of conceptual successional models (Pearson & Rosenberg, 1978; Gray & Mirza, 1979). Whilst the classic Pearson–Rosenberg model has received worldwide support (e.g. Weston (1990) and Magni *et al.* (2009)), challenges to the generality of this model have also been made (e.g. Maurer *et al.* (1993) and Gray *et al.* (2002)). Furthermore, the context-specificity of B–EF relationships has been shown by numerous *in situ* studies (including Geert *et al.* (2009), Gammal *et al.* (2017) and Crespo *et al.* (2018)), highlighting the need to document the response of estuarine communities to organic enrichment locally to understand the potential consequences for ecosystem function. There has been no comprehensive examination of the response of intertidal communities in New Zealand to organic matter over-enrichment, although the effect of multiple stressors that include organic matter has been assessed (Ellis *et al.*, 2000b; Thrush *et al.*, 2008), and Gladstone-Gallagher *et al.* (2016) experimented with small quantities (220 g dw m^{-2}) of organic detrital additions. The effects of this detritus on benthic ecosystem functions were minor, supposedly due to the low palatability of the macrodetritus to temperate benthic communities (Gladstone-Gallagher *et al.*, 2014; Gladstone-Gallagher *et al.*, 2016); however, the fresh depositions from algal blooms are expected to be highly labile (Karlson *et al.*, 2008) and thus more likely to elicit a eutrophication response.

Eutrophication-induced organic matter enrichment is a significant stressor worldwide requiring management interventions. In the previous chapter of this thesis, I demonstrated a decrease in the total abundance and richness of an intertidal benthic macrofauna community following organic matter enrichment, and a simultaneous impairment of ecosystem function (net primary production (NPP) and nutrient cycling (increased ammonium (NH_4^+) efflux)). In the present chapter, I examined how taxa responded to organic matter enrichment and the consequences for functional group diversity, and whether this was connected to the shifts in ecosystem function. Functional groups were predicted to have reduced richness (i.e. lower redundancy) and abundance with increasing organic matter enrichment, ultimately reducing the resilience of benthic ecosystem functions to further perturbations. I expected that shifts in community composition would have some ability to explain those in ecosystem functions and that this would be greater for functional classifications of the assemblage than taxonomic, assuming the functional roles played by taxa are more important than their identity (following (Cadotte *et al.*, 2011) and Gagic *et al.* (2015)).

4.3 Methods

Data collection took place as part of another experiment (see Chapter 3/Drylie *et al.* (2019)) in which $CaCO_3$ was added to treatment plots alongside varying concentrations of organic matter (field and laboratory analysis methods are repeated here for completeness). This was to investigate whether $CaCO_3$ would alleviate the negative effects of eutrophication-induced acidification on benthic ecosystem function and macrofaunal diversity. No response of the macrofauna community to $CaCO_3$ treatment was detected (results presented in Appendix 4); therefore, treatments were pooled to focus on organic matter enrichment. Ecosystem functions did respond to $CaCO_3$ treatment, but treatments are pooled for analyses correlating biodiversity and ecosystem function to see if there were any general effects of the macrofauna.

4.3.1 Study site and experimental setup

The study took place in the mid-intertidal zone of Tuapiro estuary (37°29.406', 175°57.074'), a sub-estuary of Tauranga Harbour, New Zealand. The estuary has a diurnal tidal cycle and the site experiences inundation periods of ~5 h. Experimental plots were established within a 12 m x 30 m (360 m^2) area of homogeneous fine sand (median grain size 191 µm), where surface sediments were characterised by low ambient organic content (< 2 %) typical of many New Zealand sandflats (Table 4.1) (Robertson *et al.*, 2015).

On 31st January 2017 (austral summer), forty 1.44 m^2 plots were established (2 m apart) in a 10 column by 4 row array. Ambient (no treatment), procedural controls (PC; sediment disturbed to mimic treatment setup) and $CaCO_3$ controls (plots that received $CaCO_3$ (crushed oyster shell fragments < 5 mm) but no organic matter) were randomly allocated (all n = 4). The remaining plots were randomly assigned one of nine organic matter enrichment treatments ranging from 250–2250 g dw m^{-2} of organic plant-derived slow-release garden fertiliser, increasing in 250 g increments in a gradient design. Replication was carried out for the lowest (250 g m^{-2} (n = 3)), middle (1250 g m^{-2} (n = 2)) and highest (2000 (n = 2) and 2250 g m^{-2} (n = 3)) organic matter treatments. Plots were excavated to 5 cm depth with spades before evenly distributing organic matter across plots and replacing sediment. The organic matter treatment range was chosen to create a gradient in sediment organic content (OC) that would elicit a response typical of eutrophically stressed systems (i.e. acidified porewaters, reduced oxygen availability), and treatment materials were chosen to mimic natural deposits of organic matter (from algal blooms (Teichberg *et al.*, 2010)) in estuarine systems. Slow-release fertiliser ensured sustained organic matter decomposition led to changes in the biogeochemical properties of the sediment. Sediments were not sieved to remove infauna prior to treatment setup. This is because I was interested in the disturbance to the community caused by organic matter enrichment, rather than the recovery of the community following enrichment. The consequence of this is that the composition of the starting assemblage may have differed between plots.

4.3.2 Field sampling

Sediment properties were sampled *in situ* after 62 d (3rd–4th April 2017, early austral autumn) and macrofauna communities after 70 d (11th April 2017), having allowed time for sediment properties to respond to experimental treatments (as determined from preliminary investigations). Six small cores (2.6 cm diameter x 2 cm depth) were collected and pooled from within each plot for analysis of sediment grain size and proxies of microphytobenthos (MPB) abundance (Chl *a* and phaeopigments (phaeo) concentration). These samples were stored frozen in the dark. Four medium cores (5 cm diameter x 7 cm depth) were collected and pooled per plot for analysis of OC at 0–2 cm and 2–5 cm depth sections. Two large cores (13 cm diameter x 15 cm depth) were collected from each plot and sieved over a 500 µm mesh *in situ* before preserving in 70 % isopropyl alcohol for characterisation of the macrofauna community. Porewater pH was measured in each plot using a digital field probe (Eutech pHTestr1, 2; Singapore) at ~1 cm depth.

4.3.3 Laboratory analyses

Sediment samples were thawed, homogenised and divided for analysis of sediment properties. Grain size samples (~10 g) were digested in 10 % hydrogen peroxide to remove organic matter before measuring using a Malvern Mastersizer 2000 (particle size range 0.05 – 2000 µm; Malvern, UK). Sediment pigment samples (Chl *a* and phaeo) were processed using standard procedures (Arar & Collins, 1997) and analysed on a Turner 10-AU fluorometer using an acidification step. Organic and $CaCO_3$ contents were determined from 5 g dried sediment samples (100 ^{o}C until constant weight) according to standard loss on ignition (LOI) procedures for OC (Heiri *et al.*, 2001) and $CaCO_3$ (Wang *et al.*, 2011). Macrofauna samples were stained with Rose of Bengal and all individuals retained on a 500 µm mesh were identified to lowest possible taxonomic level (species 89 % of the time). The term "taxa" will be used throughout the rest of this chapter when referring to either species or organisms identified to a higher taxonomic level to avoid confusion.

4.3.4 Data analysis

All statistical analyses were performed in PRIMER v7 with the PERMANOVA+ package (following protocols outlined by Anderson *et al.* (2008)) unless stated otherwise, and macrofauna abundance data were averaged from two cores per plot. Abundance data were untransformed for all analyses to avoid down-weighting the influence of abundant taxa, as these often have the largest effect on ecosystem function (Smith & Knapp, 2003; Solan *et al.*, 2004). Furthermore, when analyses were run with (square root) transformed data the outcomes were unchanged.

Two preliminary analyses were conducted to inform the approach for the main analyses. In the first preliminary analysis, organic matter treatment (0–2250 g dw m^{-2}) was distinguished from other environmental variables (OC 2–5 cm (2– 4 %) and porewater pH (7.4–6.3)) as the best explanatory variable describing macrofaunal community composition, using the BIOENV procedure within the BEST routine of PRIMER. Organic matter treatment alone accounted for most of the variation in community composition (Spearman coefficient = 0.8), and the addition of other environmental variables did not substantially improve this. Organic content (2–5 cm) and porewater pH were significantly correlated with organic matter treatment (Pearson coefficient = 0.7 and -0.7, respectively), highlighting collinearity between these environmental gradients. Organic matter treatment was therefore used as the explanatory variable against which taxa- and community-specific responses were analysed.

In the second preliminary analysis, the response of the entire macrofauna community to organic matter treatment was first considered using cluster analysis, which identified three broad groupings of treatments according to their effect on taxonomic community composition (see Appendix 5). These groups can be identified as representing low (0–250 g dw m^{-2}), medium (500–1250 g dw m^{-2}) and high (1500–2250 g dw m^{-2}) levels of organic matter enrichment. All further analyses (except probability of occurrence models) utilised these broader groups to identify general trends and drivers of change between levels of enrichment. Species accumulation curves (see Appendix 6) indicated that most taxa present in the experimental area had been sampled after the collection of 10 samples; each broad group of organic matter treatments contained at least 10 samples; therefore, it was unnecessary to remove the effect of unequal sample sizes for analyses (Melo *et al.*, 2003).

For the main analyses, all taxa identified in the study area were allocated to functional groups as defined by Greenfield *et al.* (2016), which characterise taxa according to their influence on sediment biogeochemistry and stability via functional attributes such as feeding behaviour, living position and adult body size. A suite of univariate descriptors of the taxonomic and functional community (total abundance, richness (number of taxa or functional groups), Shannon–Wiener (S–W) diversity (*H′*) and Pielou's evenness (*J′*)) were then determined and summarised according to the level of organic matter enrichment. Pielou's evenness represents the degree of dominance within a community, with a value of 0 indicating total numeric dominance by a single taxon and 1 indicating equal abundances of multiple taxa (Pielou, 1966). The S–W diversity index is a measure of biodiversity that accounts for the abundance and evenness of the taxa present and is therefore comparable across treatments (Heip & Engels, 2009). Exploring these indices for both the taxonomic and functional classifications of the community allowed any disparity between them to be identified, and one-way PERMANOVA tested for statistically significant differences between organic matter enrichment levels (Clarke & Gorley, 2006).

Examination of univariate indices indicated changes in the macrofauna community between broad levels of organic matter enrichment. Therefore, analyses of similarity (ANOSIM) were performed, paired with non-metric multi-dimensional scaling (nMDS) ordination (Bray & Curtis, 1957), to see whether this translated into shifts in (multivariate) community composition. The stress value associated with an nMDS ordination reflects how well the 2-dimensional ordination summarises the observed distances between samples in 3-dimensional space, i.e. how accurate it is. Generally, stress < 0.05 is excellent, < 0.1 is good, < 0.2 is OK, and < 0.3 is poor (Clarke & Ainsworth, 1993). Similarity percentages (SIMPER) analyses identified taxa and functional groups that were key contributors to the dissimilarity between enrichment levels, and the percent dissimilarity to standard deviation ratio (Diss/SD) determined whether these taxa could effectively discriminate between levels (a threshold of Diss/SD >1.3 indicated a good discriminating taxa) (Clarke *et al.*, 2014).

The sensitivity of individual taxa to organic matter enrichment was explored in two ways. Occurrence was first illustrated as a function of organic matter treatment by tabulating presence/absence data for all taxa, followed secondly by construction of probability of occurrence models for common taxa to highlight the nature of the relationship between occurrence and organic matter treatment. Taxa were modelled if they were common (occurring in ≥ 50 % of plots) and abundant (> 2 ind. core^{-1}) in control plots, to ensure model robustness. This resulted in the selection of 16 taxa, including bivalves *A. stutchburyi* and *M. liliana*. for which separate models were constructed for juvenile and adult size classes. Occurrence was modelled using logistic regression, utilising binomial error distributions and a logit link function (as in Thrush *et al.* (2003), and following Zuur *et al.* (2009)) and were constructed with the GLM package in R (version 3.5.1). Models were validated via visual inspection and consideration of concordance statistics to determine predictive accuracy (as in Thrush *et al.* (2003) and Ysebaert *et al.* (2002)). The concordance statistic is the rank correlation between predicted probabilities of the outcome occurring and the observed response (Austin & Steyerberg, 2012).

A subset of five functional groups were used to investigate the resilience of ecosystem function to organic matter enrichment. Groups were chosen that were potentially sensitive to enrichment, indicated by at least one taxon within the group having a low tolerance according to SIMPER analyses and/or probability of occurrence models, as these were expected to represent the greatest risk to ecosystem function in the absence of stabilising mechanisms. Changes in the richness and abundance of these functional groups were expected to be most important for ecosystem function (Cadotte *et al.*, 2011; Gagic *et al.*, 2015); therefore, only these indices were explored. Response diversity was detected statistically using two-way PERMANOVA following the approach of Winfree and Kremen (2009); changes in the abundance of the functional group were analysed (Bray–Curtis similarity index) with the level of organic enrichment and the contributing taxa as fixed factors. A significant (p(perm) < 0.05) interaction between organic enrichment and taxa indicated the effect of enrichment on the functional group varied depending on taxon identity, i.e. response diversity. Separate analyses were conducted for each functional group. Density compensation was investigated (as well as the nature of any significant PERMANOVA interactions) by plotting the changes in abundance of taxa within each functional group according to organic enrichment level. It should be noted that in these analyses, taxa abundances are not strictly independent of one another as taxa occur in the same core. Typically, non-independence of samples increases the chance of making a Type 1 error (i.e. rejecting the null hypothesis when it is in fact true) (Underwood & Underwood, 1997). However, I did not want to remove the influence of taxa interactions from my analyses as a statistically significant interaction between OM enrichment and taxa would show, importantly, the effect of OM given this context.

Finally, the relationship between macrofaunal community composition and ecosystem function was investigated as a function of organic matter enrichment. Ecosystem function data originated from Chapter 3 (Drylie *et al.*, 2019) where methods of collection and analysis are given in detail. Only NPP and NH_4^+ flux were significantly affected by organic matter enrichment; therefore, only these functions were considered. The relationship was first identified by overlaying vectors representing individual functions on macrofaunal community nMDS plots. A bivariate measure of ecosystem function (combining both functions) was presented via nMDS ordination (based on Euclidean distance matrices), and vectors representing the abundance of taxa and functional groups that had a Pearson correlation > 0.5 with either axis were overlaid to identify those that were associated with functional change. Lastly, RELATE analyses quantified and tested the statistical significance of the (Pearson) correlation between similarity matrices representing ecosystem functions (Euclidean) and macrofaunal community composition (Bray–Curtis) (Clarke & Gorley, 2006).

4.4 Results

4.4.1 Sediment characteristics

Surface (0–2 cm) sediment characteristics across different organic matter enrichment levels were similar 62 d after treatment setup, composed of fine sand (median grain size ranged from 183–193 µm) with low mud (< 5 %) and organic (< 3 %) content (Table 4.1). Organic content (OC) at 2–5 cm sediment depth (organic matter was added to plots at 5 cm depth) increased from 2 % in control plots to 4 % in high enrichment plots, resulting in a decrease in porewater pH from 7.3 to 6.6.

Table 4.1. Sediment characteristics in low (n = 15; 0–250 g dw m^{-2}), medium (n = 10; 500–1250 g dw m^{-2}) and high (n = 14; 1500–2250 g dw m^{-2}) organic matter enrichment plots 62 d after treatment setup. Porewater pH was measured at approximately 2 cm depth, all other characteristics were measured at 0–2 cm depth. Means (and standard deviation) are shown.

	Low		Medium		High	
Median grain size (µm)	192	(4)	188	(7)	191	(3)
Mud content (%)	3.1	(1.4)	3.6	(1.4)	2.3	(0.7)
Organic content (%)						
0–2 cm	1.9	(0.2)	2.1	(0.3)	1.9	(0.2)
2–5 cm	2.1	(0.3)	2.5	(0.5)	3.7	(1.4)
Porewater pH	7.3	(0.2)	6.8	(0.3)	6.6	(0.4)
Chlorophyll *a* (µg g^{-1} dw sediment)	11.3	(1.7)	11.4	(1.7)	12.1	(3.5)
Phaeopigment (µg g^{-1} dw sediment)	5.4	(4.9)	7.1	(3.2)	9.4	(7.9)

4.4.2 Univariate community response

Across the 39 plots and 78 cores sampled, 5062 invertebrates representing 37 taxa were identified. Fifteen functional groups which encompassed traits spanning multiple feeding modes, body types and depths of habitation were identified (Table 4.2). Polychaetes were numerically dominant with 13 taxa comprising 64 % of the total individuals collected. Bivalves (5 taxa, 21 % of total individuals) and gastropods (7 taxa, 7 % of total individuals) were the next most important groups. Decapods, amphipods, cumaceans, anthozoans, nemerteans and oligochaetes all contributed comparatively small proportions of total abundance (9 % combined) and diversity. Eight taxa occurred in all organic matter treatments, whilst seven occurred in only one and another four occurred in only two; rare taxa were found across the whole range of organic matter treatments (see Appendix 7).

Table 4.2. Functional groups represented in experimental plots and example species from each (as defined by Greenfield *et al.* 2015). Abbreviations: Hex = Hexanauplia, Biv = Bivalvia, Gas = Gastropoda, Ant = Anthozoa, Pol = Polychaeta, Amp = Amphipoda, Dec = Decapoda.

Group	Description	Example species	
1	Calcified, suspension-feeding, attached	Hex	*Austrominius modestus*
2	Calcified, suspension-feeding, top 2 cm, freely mobile	Biv	*Austrovenus stutchburyi*
3	Calcified, suspension-feeding, top 2 cm, limited mobility	Biv	*Arthritica bifurca*
5	Calcified, deposit/predator/scavenger/grazer, above surface, freely mobile	Gas	*Zeacumantus lutulentus*
6	Calcified, deposit-feeding, top 2 cm, limited mobility	Biv	*Lasaea parengaensis*
8	Calcified, deposit-feeding, deep, limited mobility, no habitat structure, large	Biv	*Macomona liliana*
9	Soft-bodied, suspension feeding, attached	Ant	*Anthopleura hermaphroditica*
10	Soft-bodied, suspension feeding, tube structure	Pol	*Boccardia syrtis*
12	Soft-bodied, deposit feeding, below surface, freely mobile	Pol	*Scoloplos cylindrifer*
13	Soft-bodied, deposit feeding, below surface, limited mobility	Pol	*Aonides trifida, Prionospio aucklandica*
18	Soft-bodied, predator/scavenger, below surface, deep, freely mobile, no habitat structure	Pol	*Ceratonereis* sp., *Perinereis vallata*
19	Soft-bodied, predator/scavenger, below surface, deep, limited mobility		Oligochaeta
22	Rigid, deposit-feeding, predator/scavenger, top 2 cm, freely mobile, no habitat structure	Amp	*Paracalliope novizealandiae*
23	Rigid, above surface, freely mobile	Dec	*Halicarcinus whitei, Colurostylis lemurum*
26	Rigid, predator/scavenger, below surface, freely mobile, large burrow former	Dec	*Austrohelice crassa, Hemiplax hirtipes*

Plotting univariate descriptors of the macrofauna community according to broader groupings of organic matter enrichment enabled detection of general trends. There were significant step-wise decreases in total abundance (median of ~100 ind. core^{-1} in low versus 25 ind. core^{-1} in high) and richness (median of 20 taxa core^{-1} in low versus 14 taxa core^{-1} in high) with increasing levels of organic enrichment (Figures 4.1a & b). Taxonomic S–W diversity remained stable across levels of organic matter enrichment whilst the evenness of the community increased significantly (Figures 4.1c & d), suggesting a decrease in dominance and a homogenisation of the community. Univariate indices of functional group composition showed similar responses as taxonomic composition (Figure 4.2); there was a significant decrease in the total number of functional groups present (from a median of 12 core^{-1} in low to 9 core^{-1} in high) as organic enrichment increased (Figure 4.2a), but no significant effect on functional group S–W diversity (Figure 4.2b). Functional group evenness increased significantly with increasing organic enrichment (Figure 4.2c), again suggesting reduced dominance.

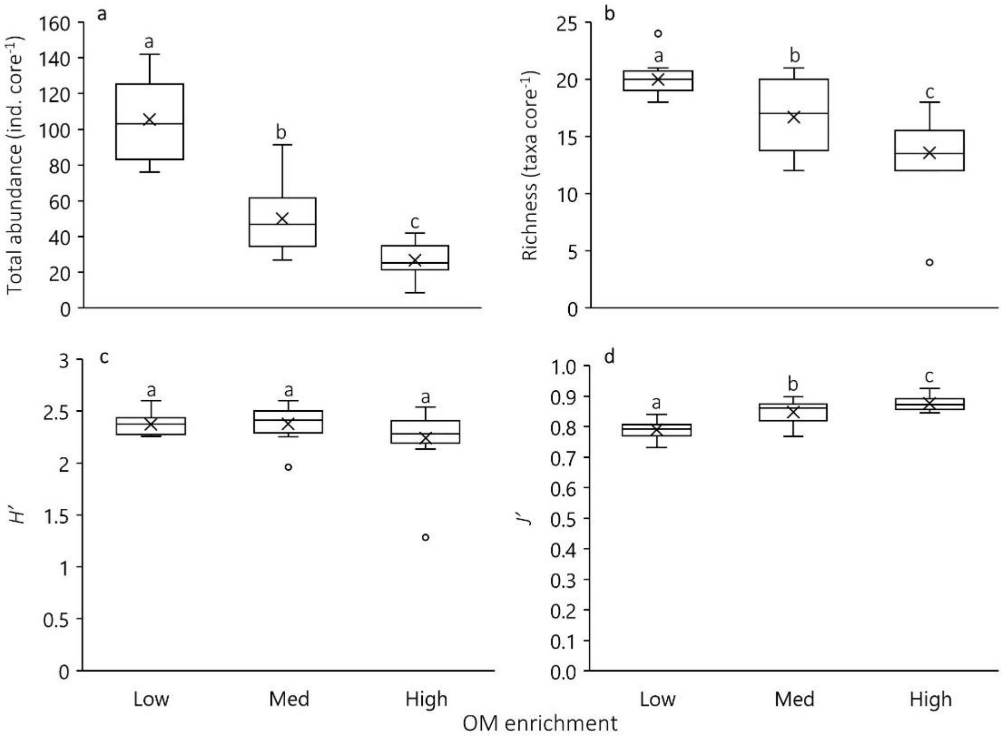


Figure 4.1. Univariate response of taxonomic macrofauna community to low (0–250 g dw m^{-2}, n = 12), medium (500–1250 g dw m^{-2}, n = 10) and high (1500–2250 g dw m^{-2}, n = 14) levels of organic matter enrichment: a) total abundance, b) richness, c) Shannon-Wiener diversity (*H′*) and d) Pielou's evenness (*J′*). Boxplot whiskers show the non-outlier range, with circles marking outliers. Box limits represent 25th and 75th percentiles, lines intersecting boxes are median values and crosses within boxes are means. Boxes not sharing the same letter are significantly different from each other (one-way PERMANOVA, followed by pairwise tests; p(perm) < 0.05; see Appendix 8).

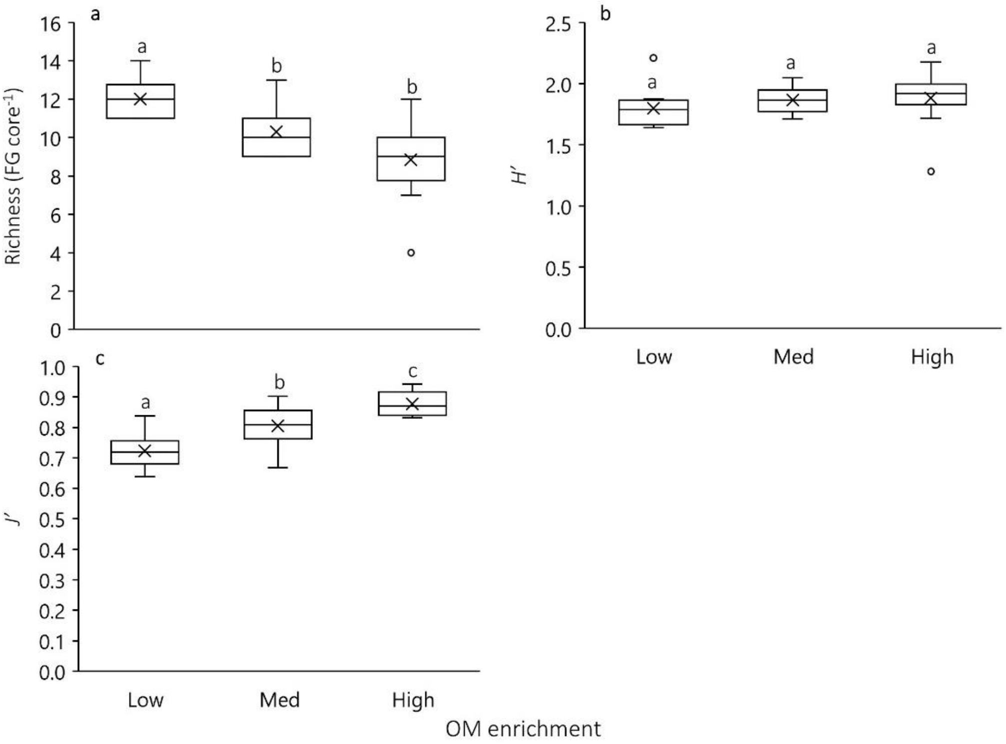


Figure 4.2. Univariate response of functional macrofauna community to low (0–250 g dw m^{-2}, n = 12), medium (500–1250 g dw m^{-2}, n = 10) and high (1500–2250 g dw m^{-2}, n = 14) levels of organic matter enrichment: a) total abundance, b) richness, c) Shannon-Wiener diversity (*H′*) and d) Pielou's evenness (*J′*). Boxplot whiskers show the non-outlier range, with circles marking outliers. Box limits represent 25th and 75th percentiles, lines intersecting boxes are median values and crosses within boxes are means. Boxes not sharing the same letter are significantly different from each other (one-way PERMANOVA, followed by pairwise tests; p(perm) < 0.05; see Appendix 8).

4.4.3 Multivariate community response

When the macrofaunal assemblage was considered as a whole, nMDS ordination displayed clear separation between low, medium and high levels of organic matter enrichment in terms of taxonomic and functional composition (Figure 4.3). Differences between taxonomic versus functional assemblage ordinations were minor, reflecting the tendency for functional groups to be represented by only a few taxa. Significant differences in community composition between all levels of enrichment were confirmed by ANOSIM (Table 4.3), with the greatest dissimilarity (65–70 %) occurring between low and high levels (Table 4.4).

The polychaetes *Prionopsio aucklandica* and *Aonides trifida* were revealed by SIMPER analysis as the greatest contributors to dissimilarity in taxonomic community composition between levels of organic enrichment (19–26 % and 14–18 %, respectively) (Table 4.4). Consequently FG 13, to which *P. aucklandica* and *A. trifida* both belong, accounted for the majority (41–49 %) of the dissimilarity in functional composition between levels of enrichment. All of the taxa contributing to the dissimilarity between low and high or low and medium enrichment communities were good discriminating taxa (Diss/SD > 1.3). Only two of the five (*P. aucklandica* and *A. stutchburyi*) were when considering medium versus high treatments, suggesting it is more difficult to detect shifts in community composition between these levels.

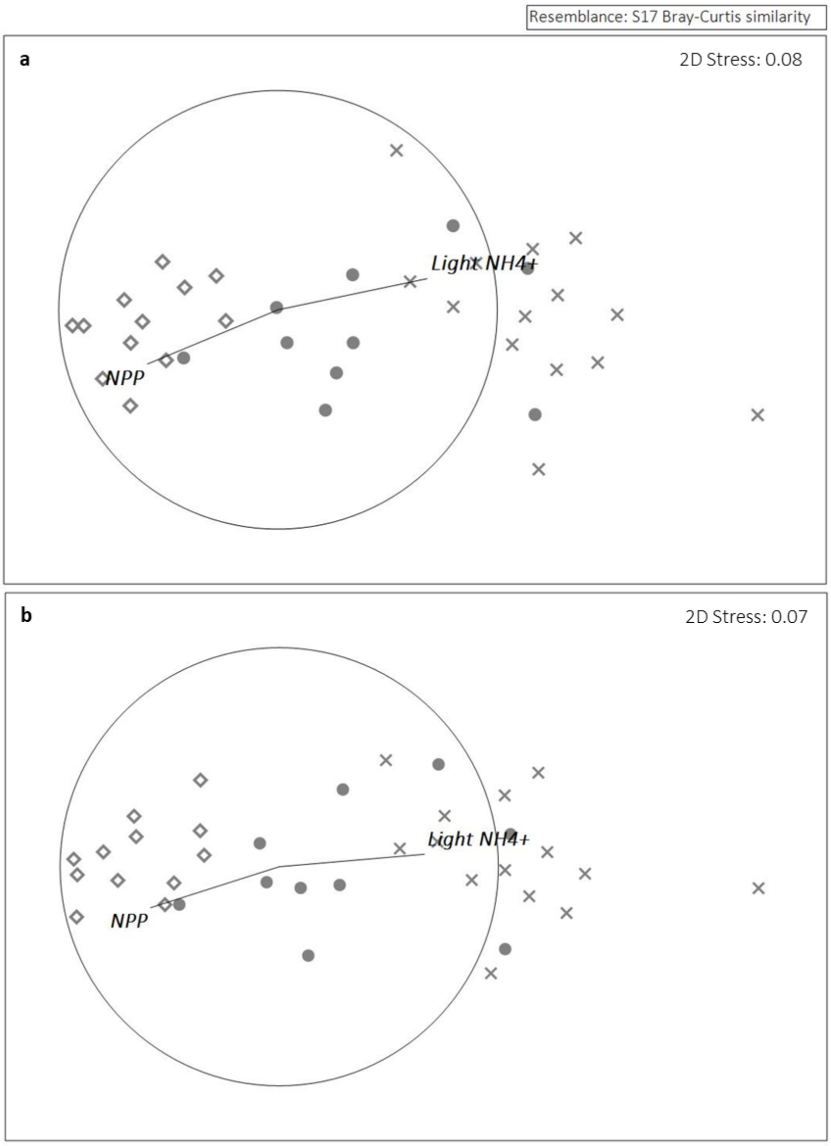


Figure 4.3. Non-metric MDS ordination of a) taxonomic and b) functional macrofauna community composition at different levels of organic matter enrichment: diamonds = low (0–250 g dw m^{-2}), filled circles = medium (500–1250 g dw m^{-2}), crosses = high (1750–2250 g dw m^{-2}). Vector overlays show the relationship with ecosystem functions that had a Pearson correlation > 0.5 with either axis (circle limits represent a correlation of 1). NPP = net primary production; Light NH_4^+ = light ammonium flux.

Table 4.3. Results of global and pairwise analyses of similarity (ANOSIM) comparing taxonomic and functional community composition between low (0–250 g dw m^{-2}, n = 12), medium (500–1250 g dw m^{-2}, n = 10) and high (1500–2250 g dw m^{-2}, n = 14) levels of organic matter enrichment. Significant results ($p < 0.05$) are indicated in bold.

	Global		Pairwise tests		
	R statistic	Sig.	Groups	R statistic	Sig.
Taxonomic	0.69	**<0.01**	Low, Med	0.59	**<0.01**
			Med, High	0.37	**<0.01**
			Low, High	0.96	**<0.01**
Functional	0.69	**<0.01**	Low, Med	0.58	**<0.01**
			Med, High	0.38	**<0.01**
			Low, High	0.96	**<0.01**

Table 4.4. Results of SIMPER analysis comparing community composition (taxonomic and functional) between low (0–250 g dw m^{-2}, n = 12), medium (500–1250 g dw m^{-2}, n = 10) and high (1500–2250 g dw m^{-2}, n = 14) levels of organic matter enrichment. Percent dissimilarity between treatments, key taxa and functional groups (FG) that cumulatively contributed (cont.) > 50 % to the dissimilarity, and the percent dissimilarity to standard deviation ratio (Diss/SD) is shown. See table 4.2 for FG description.

Taxonomic				Functional			
Taxa	Diss.	Cont.	Diss/SD	FG	Diss.	Cont.	Diss/SD
Low vs. Med	**49.8**				**44.8**		
Prionospio aucklandica		23.4	1.72	13		45.5	1.85
Aonides trifida		16.3	1.69	12		11.3	1.50
Lasaea parengaensis		8.50	1.86				
Ceratonereis sp.		8.42	1.74				
Low vs. High	**69.9**				**65.2**		
Prionospio aucklandica		26.0	3.09	13		49.1	3.83
Aonides trifida		18.1	3.05	18		10.8	1.86
Scoloplos cylindrifer		7.90	1.47				
Med vs. High	**48.6**				**42.8**		
Prionospio aucklandica		18.9	1.60	13		40.5	1.47
Aonides trifida		14.1	1.19	12		10.8	0.96
Scoloplos cylindrifer		9.31	0.96				
Austrovenus stutchburyi		7.21	1.51				
Zeacumantus lutulentus		5.48	1.25				

4.4.4 Individual taxon/ functional group response

Only one modelled taxon, the gastropod *Zeacumantus lutulentus* (FG 5), responded positively to increasing organic matter enrichment and had an increased probability of occurring; all others responded negatively and had taxa-specific relationships with enrichment (see Appendix 9). Both SIMPER analyses and probability of occurrence plots identified functional groups 6 and 13 as potentially sensitive to organic enrichment, as *L. parengaensis* (FG 6) and *A. trifida* (FG 13) were both good discriminating taxa and had occurrences that exhibited sigmoidal–decreasing relationships with increasing organic matter (Appendix 9, Figure A9.1b). Interestingly, *P. aucklandica* (FG 13) was not highlighted as being sensitive by probability of occurrence models as substantial decreases were not apparent until ~1250 g dw m^{-2} (boundary between medium and high levels of enrichment; Appendix 9, Figure A9.1a) but was the main contributor to the dissimilarity between levels of organic enrichment in SIMPER analyses. Taxa belonging to functional groups 2, 18 and 22 were also highlighted as sensitive to enrichment in terms of their abundance or occurrence, and alongside functional groups 6 and 13 represented different combinations of traits, richness and abundance; therefore, these functional groups were examined further. Functional group 13 (deposit-feeding, limited mobility polychaetes) had the highest richness, represented by 6 taxa across the study area, and was the most abundant (989 individuals overall). Contrastingly, functional groups 2 (suspension-feeding, mobile bivalves) and 6 (deposit-feeding, limited-mobility bivalves) were the least diverse containing only 2 taxa each and had intermediate and low abundances (248 and 143 individuals overall, respectively). Functional group 22 (deposit-feeding/predatory, mobile crustaceans) contained only 45 individuals across the whole study area but had an intermediate richness, consisting of 4 taxa.

Changes in the richness and abundance of these functional groups were proposed to influence ecosystem function, and both indices were affected by organic matter enrichment: the abundance of all five groups significantly decreased as enrichment increased, whereas the richness of only groups 13 and 22 did (Figure 4.4; Table 4.5). Functional group 13 dominated numerically at low levels of organic matter enrichment (Figure 4.4a). However, significant step-wise decreases in abundance with increasing enrichment resulted in 91 % of the individuals in the group being lost between low and high levels. This resulted in similar mean abundances of functional groups 13, 18 (both 4 ind. $core^{-1}$) and 2 (3 ind. $core^{-1}$) at high enrichment levels, explaining the increase in evenness previously demonstrated. FG 18 also exhibited step-wise decreases in abundance, losing 69 % of individuals between low and high levels of enrichment, and an 88 % decrease in the abundance of FG 6 occurred between low and medium levels of enrichment (this group was not found in high enrichment plots on average). There was no consistent trend in the abundance of FG 22 with enrichment. Richness was highest in functional groups 13 and 18 at all enrichment levels, despite the significant decreases in the richness of FG 13 (4 taxa core^{-1} at low levels and 3 taxa core^{-1} at high levels of enrichment) (Figure 4.4b).

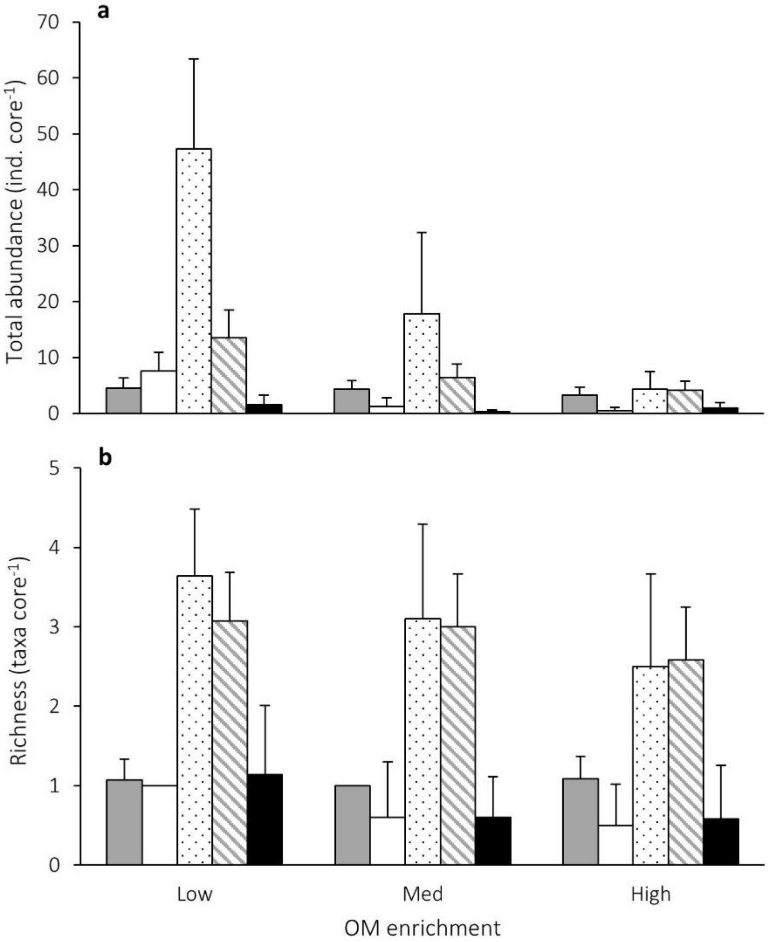


Figure 4.4. a) Abundance and b) taxonomic richness of potentially sensitive functional groups (FG) at low (0–250 g dw m^{-2}, n = 12), medium (500–1250 g dw m^{-2}, n = 10) and high (1500–2250 g dw m^{-2}, n = 14) levels of organic matter enrichment. FGs: 2 (grey bars), 6 (white bars), 13 (dotted bars), 18 (striped bars) and 22 (black bars), see Table 4.2 for descriptions. Bars represent means and standard deviation bars are shown. See table 4.2 for FG descriptions.

Table 4.5. Results of one-way PERMANOVA comparing functional group (FG) abundance and taxa richness between low (0–250 g dw m^{-2}, n = 12), medium (500–1250 g dw m^{-2}, n = 10) and high (1500–2250 g dw m^{-2}, n = 14) levels of organic matter enrichment. Significant (p(perm) < 0.05) results are highlighted in bold.

	FG	df	Pseudo-F	p(perm)	Pairwise tests	t	p(perm)
Abundance							
	2	2	8.13	**<0.01**	Low, Med	0.62	0.57
					Low, High	3.49	**<0.01**
					Med, High	3.38	**<0.01**
	6	2	32.9	**<0.01**	Low, Med	5.27	**<0.01**
					Low, High	8.92	**<0.01**
					Med, High	1.45	0.14
	13	2	14.1	**<0.01**	Low, Med	3.08	**<0.01**
					Low, High	5.63	**<0.01**
					Med, High	2.13	**0.01**
	18	2	19.2	**<0.01**	Low, Med	3.80	**<0.01**
					Low, High	6.12	**<0.01**
					Med, High	2.21	**0.03**
	22	2	3.41	**0.03**	Low, Med	2.77	**<0.01**
					Low, High	0.92	0.38
					Med, High	1.87	**<0.01**
Richness							
	2	2	0.39	1			
	6	2	2.94	0.08			
	13	2	4.23	**0.02**	Low, Med	0.94	0.48
					Low, High	2.74	**0.01**
					Med, High	1.73	0.11
	18	2	2.12	0.13			
	22	2	6.19	**<0.01**	Low, Med	3.24	**<0.01**
					Low, High	2.49	**0.04**
					Med, High	1.00	0.49

Significant interactions between organic matter enrichment and the abundance of contributing taxa were found for all functional groups, indicating response diversity (see Appendix 10); plotting the abundance of the individual taxa revealed the nature of this response diversity (Figure 4.5). At low levels of enrichment, functional groups were dominated by one or two taxa regardless of their total richness. In the low diversity bivalve groups 2 and 6, virtually all of the abundance was contributed by a single taxon (*A. stutchburyi* and *L. parengaensis*, respectively) as the other contributing taxon was rare, occurring in only one organic matter treatment (see Appendix 7), and thus had no relationship with enrichment (Figures 4.5a & b). However, *A. stutchburyi* was apparently resistant to organic enrichment, being present in similar numbers at each level (low and medium = 4 ind. core^{-1}, high = 3 ind. core^{-1}; Figure 4.5a), which supports the probability of occurrence model for this taxon and explains the minor overall decreases in the abundance of FG 2. Contrastingly, *L. parengaensis* was highly sensitive to organic enrichment with 95 % of individuals being lost between low and high enrichment levels (Figure 4.5b), resulting in the near extinction of FG 6.

The high diversity deposit-feeding polychaete group, FG 13, was dominated by *P. aucklandica* and *A. trifida* at low levels of enrichment, whilst the four remaining taxa contributed little (< 10 %) to functional group abundance (Figure 4.5c). Both of these taxa were highly sensitive to organic enrichment (*P. aucklandica* abundance decreased by 89 % and *A. trifida* by 94 % between low and high enrichment plots), whereas the less abundant taxa showed little response; the sensitivity of the dominant taxa to organic enrichment therefore caused the dramatic decreases in overall functional group abundance. The predatory deep-dwelling polychaetes, FG 18, were dominated by *Ceratonereis* sp. at low levels of enrichment; however, this taxon was also sensitive to organic matter enrichment (decreasing in abundance by 83 % between low and high levels) such that it occurred in similar numbers to *P. vallata* at high levels (both 2 ind. core^{-1}). *P. vallata,* and to a lesser extent *N. aestuariensis*, were tolerant to organic enrichment and therefore provided a contrasting response and maintained a low abundance of the functional group. There was evidence of density compensation in the low abundance group of mobile crustaceans, FG 22, as a decrease in the abundance of amphipods of the family Lysianassidae with increasing organic enrichment was counteracted by an increase in the abundance of the amphipod *Paracalliope novizealandiae* (Figure 4.5e); this explains the apparent resilience of this functional group to organic enrichment despite significant losses in richness and low overall abundances.

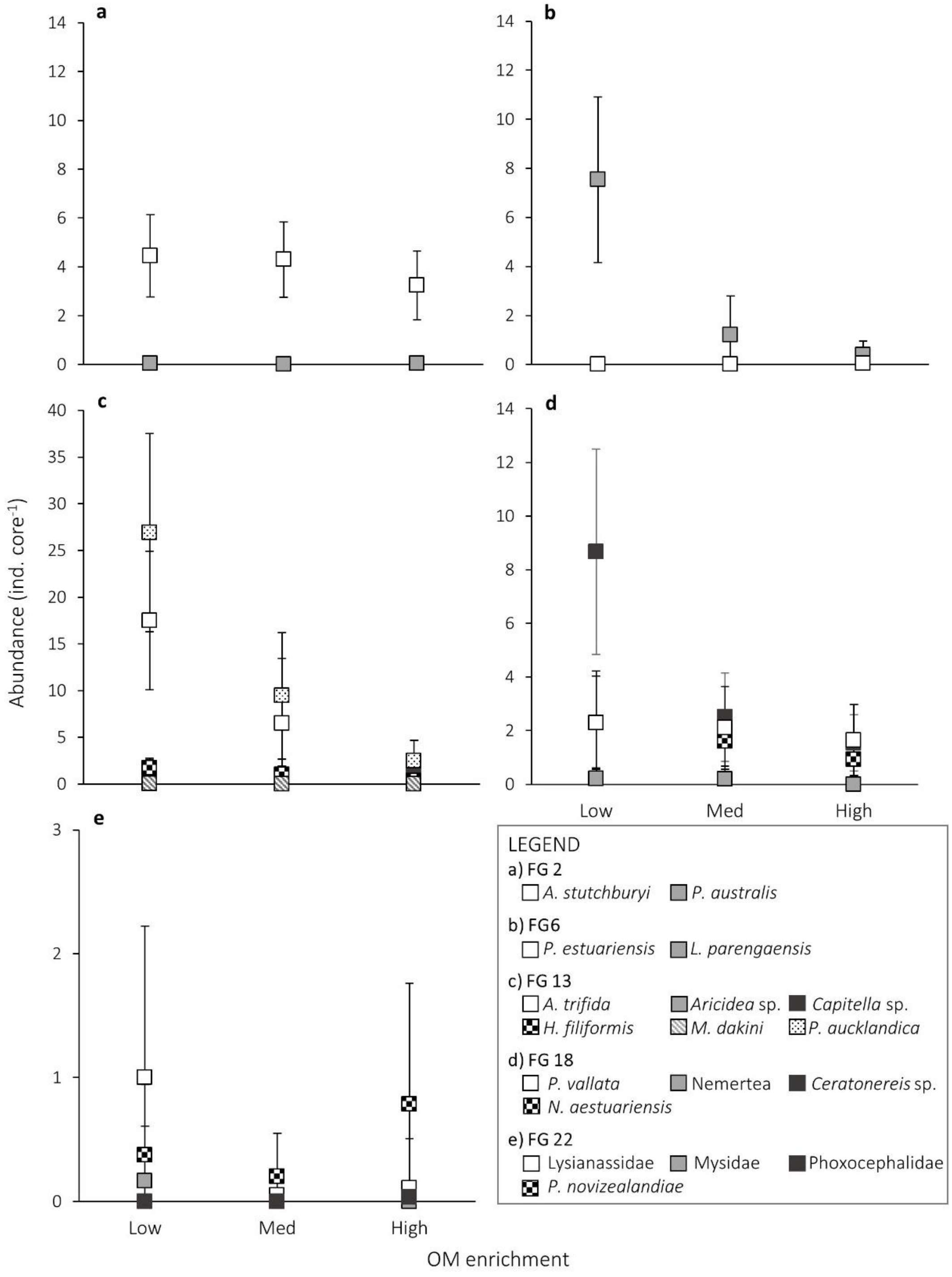


Figure 4.5. Mean abundance of taxa contributing to potentially sensitive functional groups (FG) at low (0–250 g dw m^{-2}, n = 12), medium (500–1250 g dw m^{-2}, n = 10) and high (1500–2250 g dw m^{-2}, n = 14) levels of organic matter enrichment. See Table 4.2 for description of functional groups. Standard deviation bars are shown. Note change in y-axis scale between figures.

4.4.5 Relationship with ecosystem function

A relationship between shifts in benthic macrofauna community composition and ecosystem function (NPP and $NH_4^+{}_{light}$ flux) was highlighted by the correlation of vectors representing these functions with nMDS axis 1 in Figure 4.3, the axis of greatest taxonomic and functional community change (NPP: Pearson's r = -0.6, $NH_4^+{}_{light}$ flux: Pearson's r = 0.7). When both ecosystem functions were combined into a single metric, the functioning of low organic matter enrichment plots was distinct from that in medium (PERMANOVA post-hoc: t = 4.28, p(perm) = <0.01), and high enrichment plots (PERMANOVA post-hoc: t = 5.99, p(perm) = <0.01), but there was no separation between medium and high (PERMANOVA post-hoc: t = 1.68, p(perm) = 0.1) (Figure 4.6). Of the functional groups examined in detail, decreases in the abundance of FGs 6, 13 and 18 were well-correlated with the directional shifts in ecosystem function (nMDS axis 1, Pearson's r = -6–-7; Figure 4.6a). Based on the dominance of functional groups by one or two taxa (Figure 4.5), it was predicted these relationships would reflect changes in the abundance of a few key taxa. Accordingly, taxonomic overlays identified 6 taxa that were well-correlated with nMDS axis 1 and belonged predominantly to these functional groups (Figure 4.6b); *P. aucklandica* and *A. trifida* (both FG 13) and *L. parengaensis* (FG 6) showed the strongest correlation (Pearson's r = -0.7), and the correlation of *Ceratonereis* sp. (FG 18), *S. benhami* (FG 12) and *H. filiformis* (FG 13) was slightly weaker (Pearson's r = 0.6). Additionally, taxonomic classifications of the macrofaunal community were associated with a greater amount of the variance in ecosystem functions than functional classifications (Table 4.6).

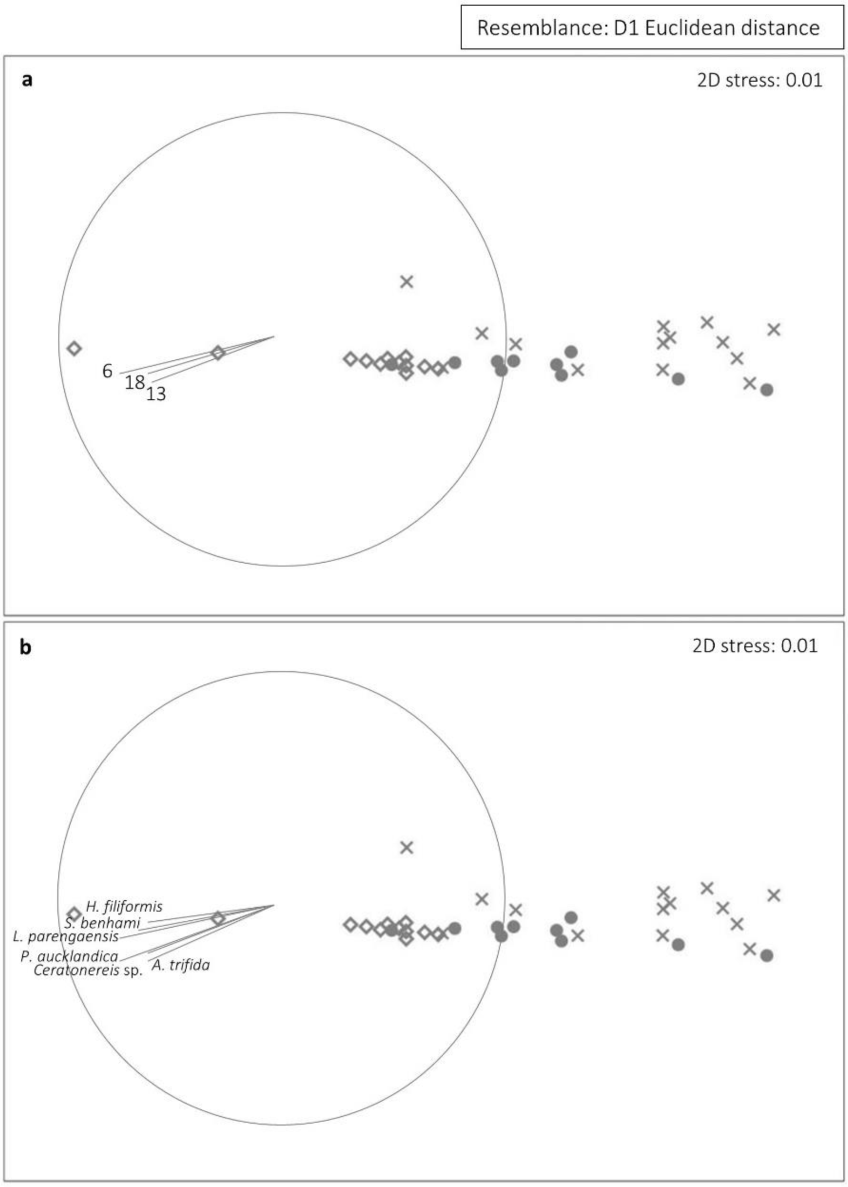


Figure 4.6. Non-metric MDS ordinations showing organic matter enrichment (diamonds = low (0–250 g dw m^{-2}), filled circles = medium (500–1250 g dw m^{-2}), crosses = high (1750–2250 g dw m^{-2})) effects on ecosystem function (net primary production and $NH_4^+{}_{light}$ flux combined) with vector overlays showing a) functional groups (FG) and b) taxa driving separation between samples (only those with a Pearson correlation > 0.5 with either axis are presented, circle limits represent a correlation of 1). See Table 4.2 for FG descriptions.

Table 4.6. Results of RELATE analyses testing correlations between macrofaunal community composition (taxonomic and functional) and ecosystem function. NPP = net primary production; $NH_4^+{}_{light}$ = light ammonium flux. Significant ($p < 0.05$) results are indicated in bold.

	Ecosystem function	Rho statistic	Sig.
Taxonomic	NPP	0.28	**<0.01**
	$NH_4^+{}_{light}$	0.42	**<0.01**
Functional	NPP	0.27	**<0.01**
	$NH_4^+{}_{light}$	0.38	**<0.01**

4.5 Discussion

The delivery of excess organic matter to benthic habitats is increasingly common as coastlines become urbanised by growing human populations. Resultant alterations to the rate of microbial decomposition processes can result in hypoxia, acidification and increased concentrations of toxic solutes in sediments (Cai *et al.*, 2011; Howarth *et al.*, 2011). The *in situ* taxonomic and functional response of a macrofaunal sandflat community to increasing levels of organic matter enrichment was investigated and linked to ecosystem function. Increasing organic enrichment caused significant losses in abundance and richness and increased both taxonomic and functional community evenness, resulting in distinct assemblages at low, medium and high levels of enrichment. There was evidence of response diversity and density compensation within functional groups which offered them resilience; however, declines in sensitive taxa that dominated functional groups were important drivers of ecosystem function nonetheless.

Taxa sensitivities to organic matter enrichment could not be summarised according to functional groups, as those within the same group often exhibited differing relationships with enrichment; this resulted in the detection of significant response diversity. To my knowledge, this is the first documentation of response diversity in a marine ecosystem. This stabilising mechanism is thought to provide resilience to ecosystem functions as tolerant taxa within a functional group persist when environmental perturbations mean sensitive taxa are lost (Elmqvist *et al.*, 2003). Consequently, the long-term resilience provided by response diversity is also dependent on the redundancy of the underpinning functional group, as the pool of taxa available to provide resilience against further perturbations will be reduced following an initial disturbance (Mori *et al.*, 2013; Oliver *et al.*, 2015). This means response diversity is likely to have a stronger influence on the overall resilience of FGs 13, 18 and 22, which each contained a minimum of four taxa, than FGs 2 and 6, which contained only two. As such, FGs 2 and 6 may be more vulnerable to multiple disturbances (whether successional or co-occurring), although this will also depend on the individual resistance of contributing taxa to the specific disturbances (Gladstone-Gallagher *et al.*, 2019).

Furthermore, response diversity only stabilised total functional group abundance, to some extent, in FG 22; in all other groups, taxa that exhibited resistance to organic matter enrichment were often much less abundant than those that were sensitive, supporting the early "drivers and passengers" concept of Walker *et al.* (1999) that minor taxa (passengers) may be analogues of dominant taxa (drivers) in terms of function, and that they play a crucial role in maintaining ecosystem function when required to 'substitute' during periods of stress. The implication is that despite preserving the functional group, response diversity may not necessarily protect against a loss in its functional viability if it underpins a density-dependent function (Mori *et al.*, 2013). For example, despite having the highest richness (and thus redundancy) and exhibiting response diversity, all taxa in FG 13 were either rare or sensitive to organic enrichment, resulting in substantial losses in abundance which could partially explain the breakdown of ecosystem functions. This illustrates that redundancy and response diversity alone cannot be used to infer resilience, and changes in total abundance must also be considered.

It is important to note that my enrichment study took place at one reasonably small spatial scale where the broad-scale occupancy of functional groups across the wider estuary was not considered. This characteristic will be vitally important for the resilience of functional groups in cases where small-scale disturbances (such as algal mat depositions) reduce functional group richness and thus the effectiveness of response diversity (Oliver *et al.*, 2015), as was seen with FGs 6 and 22. If these functional groups have a high occupancy across the wider estuary and a high potential for dispersal (for example FG 22 is freely mobile) then they may still be considered resilient to local organic enrichment, as the potential for re-establishment following disturbance (i.e. recovery) would be high (Lundquist *et al.*, 2010). If the broad-scale occupancy or dispersal potential of these groups is low, however (FG 6 has limited mobility), then such disturbances may result in a lasting exclusion of the functional group (Thrush *et al.*, 1996; Greenfield *et al.*, 2016). These considerations further highlight the complexity of defining functional group resilience, which requires the assessment of multiple processes and characteristics across different spatial scales (Gladstone-Gallagher *et al.*, 2019).

Evidence of density compensation was also found in response to organic matter enrichment: the abundance of an amphipod in the family Lysianassidae decreased with increasing enrichment, whilst the abundance of *P. novizealandiae* increased, such that there was only a minor overall change in the abundance of FG 22. Gonzalez & Loreau (2009) reviewed the dynamics that may enable this stabilising mechanism to arise and highlighted response diversity as a key precursor. However, it is difficult to distinguish whether the trend observed in amphipod abundance arose due to the removal of a competitive interaction between the two, which allowed *P. novizealandiae* to flourish where it was previously out-competed, or whether the Lysianassid amphipod was indeed negatively affected by the excess organic matter whilst *P. novizealandiae* was positively affected. The exploitation of the same fundamental niche by these taxa, and previous documentation of interspecific competition between amphipod species (Dennert, 1974; Grant, 1981; Shucksmith *et al.*, 2009), perhaps indicates the former is most likely. The identification of interactions such as these are important for managers attempting to conserve ecosystem function, as they can help determine whether the loss of a taxa can be tolerated or whether it is likely to influence the overall performance of the functional group. This is not to say there is not inherent value in conserving biodiversity, however, which has itself been recognised as an ecosystem service (Mace *et al.*, 2012), and is crucial for ensuring long-term resilience (Oliver *et al.*, 2015).

Models revealed taxon-specific sensitivities to organic matter enrichment; however, decreases in the probability of occurrence of most common and abundant taxa were not apparent until loading reached ~1000–1250 g dw m^{-2}. Additionally, no plots were azoic and rare taxa were not at greater risk of exclusion than common taxa, indicating that the assemblage as a whole was either highly resistant to large organic matter inputs, or that recruitment from the wider landscape had already re-populated plots. On a dynamic sandflat such as the study site rates of taxonomic turnover (beta-diversity) may have been high, which itself represents a form of resilience as recovery can occur quickly following a disturbance (Gladstone-Gallagher *et al.*, 2019). The stability of the S–W diversity index would however suggest there was little effect of enrichment on benthic biodiversity, but the significant losses in abundance and shifts in community structure that were identified by multivariate analyses were masked. Decreases in the biodiversity of a benthic community exposed to toxic metal contaminants also went undetected by this index (Hewitt *et al.*, 2010), highlighting the importance of considering multiple indices simultaneously to avoid dependence on a metric that may lack sensitivity to the stressor of interest (Borja & Dauer, 2008).

The homogenisation of the macrofaunal assemblage indicated by the increase in evenness with organic enrichment contradicts the expectation that a small number of opportunistic taxa would dominate at high levels (Pearson & Rosenberg, 1978). Additionally, large adult *M. liliana* were present in the highest organic matter enrichment plots, although the feeding (sediment surface) and living position of this bivalve (below the depth of organic matter burial) may have granted it protection from the enrichment disturbance (Hewitt *et al.*, 1996). Likewise, adult *A. stutchburyi* appeared resistant to organic enrichment in this study. If eutrophication were persistent, bottom-water hypoxia would likely exclude large bivalves (Levin *et al.*, 2009); however, the one-off organic matter deposition and hydrologically dynamic nature of the study site means hypoxia was unlikely to develop during my experiment. An increase in the evenness of benthic communities with eutrophication was also demonstrated by Dolbeth *et al.* (2019), which resulted in an increase in bioturbation and a consequent increase in nutrient efflux from sediments. Although I also observed an increase in nutrient efflux, the dramatic declines in abundance with increasing organic enrichment would have precluded such an increase in bioturbation activity.

The response of benthic ecosystem functions to organic matter enrichment could be partially explained by shifts in macrofaunal community composition, evidencing the link between biodiversity and ecosystem function (Loreau *et al.*, 2001; Ieno *et al.*, 2006). Specifically, the sensitivity of FGs 6, 13 and 18 to organic enrichment was important in driving NPP and $NH_4^+{}_{light}$ fluxes, and the greater effect of enrichment on the abundance than the richness of functional groups points to a density-dependent relationship. This is in line with previous *in situ* studies conducted in New Zealand estuaries that have demonstrated decreases in ecosystem functions according to losses in the density of key taxa (see Pratt *et al.* (2014a), Needham *et al.* (2011) and Sandwell *et al.* (2009)). Each of the functional groups important for ecosystem function represent different modes and depths of bioturbation, which has been shown to influence MPB primary productivity (Lohrer *et al.*, 2004), coupled nitrification–denitrification (Laverock *et al.*, 2011) and organic matter decomposition (Heilskov & Holmer, 2001; Josefson *et al.*, 2012) by transporting solutes across oxic boundaries and increasing the sediment's effective exchange surface area (Berg *et al.*, 2001). A decrease in the stimulation of these processes owing to a decrease in bioturbation is predicted to explain the impairment of ecosystem function (Biles *et al.*, 2002).

The correlation between FG 6 and ecosystem function is particularly troublesome, as this group was increasingly absent at high levels of enrichment and had characteristics (limited mobility) that may hinder its recovery. Further investigation of the broad-scale occupancy of the group would help inform whether its small-scale sensitivity warrants concern; however, a strongly supported ecological theory is that abundance and occupancy are positively correlated (Brown, 1984; Gaston, 1999; Gaston *et al.*, 2000; Steenweg *et al.*, 2018). Recent evidence of this in estuarine macrofaunal communities (Barnes, 2019) suggests FG 6 should be prioritised for conservation efforts to preserve ecosystem function. Taxonomic classifications of the macrofaunal community were however more effective in explaining the response of ecosystem function to organic enrichment than functional, suggesting taxa-specific traits were more important than functional biodiversity (as was also reported by Norling *et al.* (2007) in a benthic ecosystem). Therefore attention may be best focussed on the key taxon within vulnerable functional groups (*L. parengaensis* in FG 6). A functional composition approach may seemingly dilute the effects of key individual taxa when considering local scales, but this approach may have greater merit when considering large geographical areas that encompass benthic environments composed of varying taxa (e.g. Dissanayake *et al.* (2018) and Villnas *et al.* (2018)).

4.6 Conclusions

The findings of this study advance benthic ecologists' understanding of how an increasingly pervasive stressor alters intertidal benthic ecosystem structure and function, and contributes a first description of functional response diversity in a marine ecosystem to the field of biodiversity-ecosystem function. The macrofaunal community studied is typical of New Zealand sandflats, i.e. dominated by deposit-feeding polychaetes and suspension- and deposit-feeding bivalves (Hayward *et al.*, 1981; Pridmore *et al.*, 1990); therefore, I expect the trends identified here to be applicable to similar habitat types across the country. Functional groups were dominated by one or two taxa regardless of their overall richness, and despite exhibiting stabilising mechanisms (response diversity and density compensation) that provided resilience against organic matter enrichment, became functionally extinct due to significant decreases in the abundance of key sensitive taxa: a decline in the rate of various modes of bioturbation are hypothesised to explain the significant relationship between the macrofaunal shifts and impaired ecosystem functions. The identification of the interactions between organic matter enrichment, macrofaunal community shifts and resilience and ecosystem function can help guide the management of this stressor to maintain ecosystem functions and services, and ultimately reveal that preserving macrofaunal biodiversity is crucial to do so.

CHAPTER 5: General Discussion

Coastal marine ecosystems are being altered by human activities at a greater rate than ever before. Over the course of this thesis I have investigated how two of the most significant consequences of such alterations, elevated water column turbidity and organic matter over-enrichment, affect the structure and functioning of estuarine benthic ecosystems. Through observational and experimental field studies my three research chapters have highlighted that naturally occurring processes and mechanisms can increase the resilience of intertidal soft-sediments to these stressors, though declines in ecosystem function still occur.

5.1 Summary

Large-scale deforestation and land-use changes in New Zealand have dramatically increased the natural rate of delivery of terrigenous sediments to estuaries (Thrush *et al.*, 2004; Seers & Shears, 2015). The subsequent increase in suspended sediment concentrations (SSC) elevates water column turbidity, which reduces the rate and efficiency of benthic primary production (PP) during submergence due to reduced light availability (Pratt *et al.*, 2014b). In **Chapter 2** I characterised this phenomenon as a temporally displaced stressor and investigated whether PP during periods of emergence could compensate for the losses experienced during submergence in turbid locations. Initially this required the development of a method and experimental guidelines that enabled the measurement of PP and benthic metabolism in emerged intertidal habitats *in situ* (see Appendix 1), as protocols for doing so in vegetated habitats were lacking from the literature. I demonstrated that in microphytobenthos- (MPB) and seagrass-dominated habitats, rates of net (NPP) and gross (GPP) PP were always greater during periods of emergence than submergence, and that this difference increased in magnitude with increases in site turbidity. Also, the seagrass habitat at the most turbid location exhibited the greatest photosynthetic efficiency during both tidal states. Assuming allochthonous inputs to estuarine systems are of minor importance in New Zealand (Jones *et al.*, 2017), the dominant contribution of emerged PP to total benthic PP in turbid locations represents a critical pathway providing resilience to benthic food webs against increasing SSC.

The delivery of excess organic matter to benthic ecosystems is exacerbated by human activities that overload estuaries with nutrients, namely nitrogen, due to the associated stimulation of water column algal production (eutrophication) (Nixon, 1995; Howarth & Marino, 2006). The biogeochemical changes associated with the degradation of organic matter can create undesirable conditions in estuarine sediments, including acidification, hypoxia and the accumulation of toxic solutes (Middelburg *et al.*, 1993; Cai *et al.*, 2011), which can cause shifts in benthic macrofaunal community composition and ecosystem function (Gray *et al.*, 2002; Norkko *et al.*, 2019). The aim of **Chapter 3** was to determine whether the addition of biogenic calcium carbonate ($CaCO_3$) could reduce the negative effects of eutrophication-induced acidification on benthic ecosystem function by increasing the capacity of sediments to buffer fluctuations in pH. To this end, I conducted a manipulative field experiment utilising a gradient of organic matter enrichment that was duplicated with and without the addition of $CaCO_3$. The addition of $CaCO_3$ did not improve sedimentary conditions sufficiently to preserve macrofaunal biodiversity, likely due to the greater importance of co-occurring stressors in structuring the benthic community. Nevertheless, NPP and the cycling of NH_4^+ (ecosystem functions) were positively influenced by the presence of $CaCO_3$, evidenced by the maintenance of these functions to a higher level of organic enrichment in sediments supplied with excess $CaCO_3$ than those without. The microbial communities ultimately responsible for the transformation of solutes are postulated to have benefitted from undetected improvements in sediment porewater chemistry due to the presence of $CaCO_3$, though this is an area requiring further research.

In New Zealand estuaries, sensitivities of macrofaunal taxa to sediment contaminants such as mud and metals have been assessed with an aim to develop benthic health models that can detect the presence of these stressors (e.g. Ellis *et al.* (2015), Hewitt and Ellis (2010) and Thrush *et al.* (2003)). Excess organic matter is also considered a contaminant of growing concern but has not received the same level of scrutiny, thus far only being included in studies of multiple-stressor effects on benthic macrofauna (Ellis *et al.*, 2015). To fill this gap, in **Chapter 4** I described the taxonomic and functional response of an intertidal macrofaunal community typical of sandflats in North Island, New Zealand to a gradient of organic enrichment and investigated the effect that individual taxa sensitivities had on functional group composition. Taxa-specific sensitivities to organic enrichment were observed that could not be summarised according to functional group, and all common taxa except the deposit-feeding gastropod *Zeacumantus lutulentus* were negatively affected by enrichment. Despite functional groups exhibiting response diversity, which afforded them resilience to organic enrichment, groups were dominated by only one or two key taxa that were sensitive to organic enrichment, thus resulting in drastic decreases in functional group abundance. Decreases in the abundance of these key taxa, and consequently their functional group, were significantly related to the declines in ecosystem function measured in Chapter 3. This suggested a density-dependent relationship, which is in accordance with the findings of previous local *in situ* studies of biodiversity–ecosystem functioning (see Pratt *et al.* (2014a) and Sandwell *et al.* (2009)).

Collectively, the research chapters of my thesis demonstrate how benthic ecosystems are likely to respond to local anthropogenically-induced stressors, and how natural mechanisms may provide resilience to benthic ecosystem structure and function. However, there will be additional factors that determine how effective these mechanisms are in providing resilience, namely, whether multiple co-occurring stressors and stressor thresholds exist.

5.2 Multiple stressors

Estuarine ecosystems are subject to multiple anthropogenic stressors as their position between terrestrial and marine environments means the negative effects of human activities in both realms are experienced here (Stoms *et al.*, 2005; Halpern *et al.*, 2009). As such, stressors rarely occur in isolation but rather overlap in space and time. Researchers investigating the effects of co-occurring anthropogenic stressors have mostly concentrated on those that occur at local to regional scales (including elevated turbidity, eutrophication, toxic contaminant pollution and over-fishing; see review by Crain *et al.* (2008)), however in the near future, human-induced climatic stressors that occur across global scales (including global warming, sea-level rise and ocean acidification) will also be of increasing concern, and recent literature has started to reflect this (Cheng *et al.*, 2015; Mitchell *et al.*, 2015; Hewitt *et al.*, 2016). Theoretically, the interactive effects of two stressors (A and B) can manifest through additive (A + B), multiplicative (A x B) or comparative (the effects of A override those of B) pathways, which may be synergistic (observed effects exceed those predicted from individual effects) or antagonistic (observed effects are less than predicted from individual effects) (Bruland *et al.*, 1991; Soluk, 1993; Hay, 1996; Folt *et al.*, 1999). I suspect that the efficacy of the resilience mechanisms highlighted in this thesis will vary depending on the specific identity of co-occurring stressors and how they interact with one another.

5.2.1 Local scales

Increases in turbidity and organic matter enrichment are likely to co-occur in estuarine systems because terrestrial runoff, especially if originating from agricultural catchments, is often laden with nutrient-rich soils that both elevate SSC and stimulate water-column algal production (Davies-Colley *et al.*, 2004). These stressors may interact additively to drastically reduce O_2 availability in the benthos, potentially driving hypoxia, as decreases in benthic PP due to a light-limitation of photosynthesis (Pratt *et al.*, 2014b) will produce less O_2, whilst the accumulation of organic matter in the sediments and its subsequent remineralisation increasingly consumes O_2 (Middelburg *et al.*, 1993). The occurrence of such interactions have achieved some notoriety, for example, in Chesapeake Bay (Kemp *et al.*, 2005) and the Baltic Sea (Cederwall & Elmgren, 1990). In such cases, the resilience afforded by $CaCO_3$ to eutrophication-induced acidification may become unimportant as hypoxic stress overrides any negative effects associated with acidification.

The potential for detrimental feedback loops to occur were additionally highlighted in a companion study to Chapter 3: Bulmer *et al.* (2018) showed that increases in turbidity reduced the capacity for intertidal seagrass habitats to remove excess nutrients from the water column, a trend that was also reported for MPB by Pratt *et al.* (2014b), which may increase the likelihood of algal blooms. Such blooms would further reduce light penetration to the benthos allowing positive feedbacks to ensue (Vahtera *et al.*, 2007; Lapointe *et al.*, 2019), ultimately resulting in the organic enrichment of sediments as blooms collapse. In this instance, emerged PP would initially be an important mechanism of resilience sustaining overall benthic PP as light availability diminishes, but as organic enrichment progresses the degradative interactions described above may promote hypoxia (and acidification) as an overriding stressor. Furthermore, excess $CaCO_3$ maintained the processing of NH_4^+ to a higher degree of organic enrichment in eutrophically-acidified sediments (Chapter 3). This suggests that any losses of sedimentary $CaCO_3$ (see section 5.2.2) may further reduce the capacity of benthic ecosystems to remove excess nitrogen, further enabling water column production (Kristensen *et al.*, 2014).

5.2.2 Global context

Between 1993 and 2014 the warming of Earth's atmosphere and oceans has driven sea level rise (SLR) of 3.3 mm yr^{-1} through the thermal expansion of seawater and freshwater input from land ice melt(Chen *et al.,* 2017) ; these rates are predicted to accelerate over the 21st Century (Nerem *et al.*, 2018). The direct physical and social effects on coastal human populations are clear (Graham *et al.*, 2013; IPCC, 2014a), however the indirect effects that may materialise due to impaired intertidal ecosystem functions are less so. For example, where SLR co-occurs spatially with high water-column turbidity, the capacity of benthic ecosystems to remain productive will be reduced. This will be particularly so if coastal developments (such as sea defences) prevent the natural retreat of the coastline. In such cases, the spatial extent of intertidal habitats will be reduced, termed 'coastal squeeze' (Pontee, 2013), resulting in a smaller contribution of emerged PP to total PP. I demonstrated that intertidal sandflat and seagrass habitats in moderately to highly turbid environments were only autotrophic during emergence (Chapter 2), therefore coastal squeeze (and in extreme cases the total eradication of the intertidal zone) may result in entirely heterotrophic ecosystems and declines in seagrass biomass (Park, 1999; Turner & Schwarz, 2006b). Importantly, the complexity of benthic food webs may be reduced if PP is insufficient to support higher trophic levels, although this will also be dependent on the availability and lability of allochthonous resources (Doi, 2009). In a future world, then, the ability of emerged PP to provide resilience against elevated water-column turbidity will be dependent on the specific vulnerability of the ecosystem to SLR, and the potential for intertidal habitats to retreat sufficiently quickly.

An additional consequence of anthropogenic modifications to global biogeochemical cycles is the absorption of greater concentrations of atmospheric CO_2 by global surface waters, which is driving worldwide declines in seawater pH and changes in carbonate chemistry (ocean acidification; OA) (Doney *et al.*, 2009). Where local eutrophication-induced acidification occurs against this backdrop the dissolution of $CaCO_3$ deposits in coastal sediments may occur more rapidly and completely, as empirical research suggests these pathways will act synergistically to dramatically increase the acidification of coastal waters beyond what is expected from atmospheric CO_2 input alone (Cai *et al.*, 2011). Persistent acidification under these scenarios is likely to also trigger the loss of living $CaCO_3$ from benthic communities (Fabry *et al.*, 2008; Green *et al.*, 2013), the consequences of which would be two-fold, as the resilience to eutrophication-induced acidification afforded by $CaCO_3$ would be lost as well as the dominating influence of large bivalves on ecosystem processes (e.g. sediment stability (Lelieveld *et al.*, 2003), PP (Lohrer *et al.*, 2016), nutrient cycling and community structure (Thrush *et al.*, 2006)). The key species in New Zealand, *Austrovenus stutchburyi* and *Macomona liliana*, are apparently very tolerant to organic matter enrichment (Chapter 4), however ecosystem functions responded negatively to enrichment prior to their loss (Chapter 3). If OA excludes *A. stutchburyi* and *M. liliana* entirely and diminishes $CaCO_3$ deposits, even greater shifts in ecosystem function than were observed in my study are expected.

Alternatively, eutrophication may counteract the effects of OA on carbonate chemistry by increasing the assimilation of CO_2, an interaction that was modelled by Borges and Gypens (2010). A shift from net heterotrophy to net autotrophy is likely responsible for the opposing effects, owing to a net removal of CO_2 due to uptake by photosynthesis. Such antagonistic interactions may provide a temporary relief from OA, though as discussed, the promotion of eutrophication has numerous negative consequences that culminate with benthic acidification. Regardless, despite evidence that some marine autotrophs indeed respond to elevated CO_2 by increasing PP (Koch *et al.*, 2013; Cartaxana *et al.*, 2015; Vopel *et al.*, 2018), the effect on MPB communities was shown by Johnson *et al.* (2013) and Dutkiewicz *et al.* (2015) to be species-specific, resulting in shifts in community composition. This suggests that real-world autotrophic responses to OA may not be easily predicted, and that alterations to the PP of estuarine systems are likely (Flynn *et al.*, 2015). Furthermore, expectations that CO_2 removal by increased rates of photosynthesis will create refuges for associated communities by maintaining an area of high pH (Krause-Jensen *et al.*, 2015) require further validation.

The co-occurrence of multiple stressors undoubtedly presents a challenge in understanding benthic ecosystem response and resilience to current and future world scenarios. This was adeptly demonstrated by Crain *et al.* (2008) who showed in their meta-analysis that the observed interactions between two marine stressors were context-dependent and usually varied according to the level of a third environmental factor or stressor. Nevertheless, understanding the mechanism by which each stressor individually drives ecosystem response may help interpret or predict how and when cumulative stressors interact. For example, stress-induced taxa tolerances (as explored in Chapter 4) may lead to antagonistic interactions since tolerance to one stressor (organic enrichment) can improve tolerance to a second stressor that acts through similar mechanisms (e.g. OA) (Blanck, 2002). However the reverse may also be true, as Hewitt *et al.* (2016) recently showed that taxa sensitive to sediment mud content exhibited stronger reactions to climate-related environmental change at sites also affected by increases in mud. Ecological compensatory mechanisms (as demonstrated in Chapter 2) might alternatively lead to synergistic interactions as exposure to one stressor (elevated turbidity) will select for mechanisms (emerged PP) robust to that stressor but susceptible to a second (e.g. SLR). Such *a priori* predictions can help direct future research on multiple stressors towards those stressor combinations that might be expected to have the biggest negative impacts.

5.3 Stressor thresholds

It is likely that thresholds in the quantity of SSC and organic matter enrichment exist beyond which the mechanisms of resilience I have identified (emerged PP and $CaCO_3$ buffering) can no longer afford protection to benthic ecosystem structure and function. This is not to be confused with a stressor-associated tipping point, a limit beyond which a nonlinear change in some ecosystem property occurs and favours an alternative ecosystem state (Holling, 1973; Folke *et al.*, 2004), but is rather a precursor that represents a level of stress where resilience mechanisms become impaired or are overridden and declines in biodiversity or function begin.

For instance, benthic ecosystems may be considered less robust once submerged PP is lost due to elevated turbidity, as the entire ecosystem becomes dependent on emerged PP rather than this representing an additional source. Quantifying the threshold in turbidity where submerged PP is lost is therefore crucial for understanding when the resilience of benthic PP might be compromised. My study sites in Chapter 2 did not encompass this threshold, as PP was detected during submergence in both seagrass and sandflat habitats even at the most turbid location (although only 4 % of total GPP was contributed by submerged PP in seagrass habitats). I can therefore postulate that the threshold exceeds 68 NTU in the Kaipara Harbour (the turbidity measured at the most turbid site), however site-specific factors including the degree of acclimation that photosynthesising organisms exhibit to ambient light conditions (see Kohlmeier *et al.* (2014a)) are likely to affect these thresholds and preclude broad geographical generalisations. It is important to also note that despite emerged PP maintaining benthic PP, increases in water-column turbidity likely result in decreases in numerous other functions such as nutrient retention (Pratt *et al.*, 2014b; Bulmer *et al.*, 2018), sediment oxygenation during submergence (Baillie, 1986) and subsequently coupled nitrification–denitrification (An & Joye, 2001), highlighting the need to consider all co-occurring pressures to comprehensively determine the state of ecosystem function.

Contrastingly, in Chapter 3 I demonstrated that $CaCO_3$ dampened the negative effects of organic matter enrichment on benthic NPP and NH_4^+ cycling and despite this, declines in NPP were still apparent in +$CaCO_3$ treatments (evidenced by the linear decrease in Figure 3.3a). This suggests that the threshold in the quantity of organic enrichment where $CaCO_3$ can no longer prevent negative effects *was* encompassed by my treatments, and the immediate declines imply that this threshold was in fact below my lowest treatment (250 g dw m^{-2}). However, the additional biogeochemical changes associated with organic matter degradation (such as hypoxia) may also have driven the declines in NPP, such that the true capacity for $CaCO_3$ to provide resilience against acidification was masked by the effects of co-occurring stressors. Laboratory-based studies that isolate acidification as a stressor could shed more light on the mechanism underpinning $CaCO_3$-based resilience, which may then enable the identification of resilience thresholds (see Section 5.4).

Nevertheless, resilience thresholds will likely change with future climatic change (Scheffer *et al.*, 2015), making the regular reassessment of thresholds and tipping points necessary. For example, at sites with high turbidity in the Kaipara Harbour emerged PP is able to sustain benthic PP. This means a relatively high level of water column SSC can be tolerated, however if in the future a substantial portion of the intertidal area is lost to SLR, the contribution of emerged PP will be vastly reduced such that benthic PP may be insufficient to support local food webs. In this instance, water column SSC will need to be maintained at a much lower level to allow submerged PP to contribute (see Figure 5.1a). Similarly, the level of organic enrichment that the Tuapiro sandflat can tolerate before declines in function and biodiversity occur (Chapters 3 and 4) is expected to decrease when global OA is a more prominent stressor, as the capacity for $CaCO_3$ to provide resilience will be exhausted sooner by the multiple acidification pathways and previously tolerant taxa may be excluded (Figure 5.1b). A sound understanding of local stressor thresholds and tipping points may thus be used to minimise the impacts of global stressors at the estuary-wide scale, as acceptable limits in rates of sediment and nutrient loading could be set and adjusted according to the predicted interactive effects (Brown *et al.*, 2013).

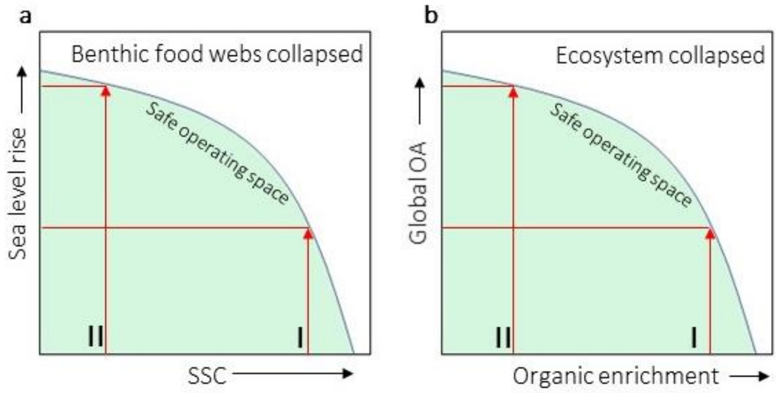


Figure 5.1. Conceptual diagram illustrating how global climatic stressors may interact with a) suspended sediment concentrations (SSC) (causing turbidity) and b) organic enrichment (causing localised acidification) to affect benthic ecosystem function (PP = primary production). When global stressors are at low levels, ecosystem function can withstand high local stress (I), whereas local stressors have to be managed at lower levels to preserve ecosystem function when global stressors increase (II). Adapted from Scheffer *et al.* (2015).

Evidently, the quantification of stressor thresholds beyond which benthic ecosystem resilience is lost would be incredibly valuable for local organisations tasked with managing estuarine ecosystems. The management of stressors such as eutrophication are commonly retrospective, i.e. action is taken after there is evidence that a regime shift has occurred. Whilst Kelly *et al.* (2015) noted that in marine ecosystems tipping point-based management rooted in science is an effective strategy, the use of more subtle thresholds of resilience-loss may help switch to a more proactive approach as a loss of resilience usually paves the way for a switch to an alternative state (Scheffer *et al.*, 2001; Lindegren *et al.*, 2012). As it is challenging, costly and sometimes impossible to reverse the regime shift associated with crossing a tipping point (Selkoe *et al.*, 2015), this tactic would be more desirable. Conducting further gradient-based experimental designs based on the boundaries I have identified will help elucidate where these thresholds lie, and doing so *in situ* at multiple locations encompassing a wide range of environmental conditions will help identify any context-dependency of these thresholds.

5.4 Future directions & concluding remarks

In this thesis I have conducted three studies that investigate the response of benthic ecosystems to two contemporary global stressors, and in doing so, I have advanced current understanding of the resilience of benthic ecosystem functions against them. Key questions that should be prioritised in future research to continue developing this knowledge are:

- How generalisable are the trends I have identified?
- How will the interaction of multiple stressors alter the response and resilience of benthic ecosystems?
- How does $CaCO_3$ increase benthic resilience to eutrophication-induced acidification?
- Where are the thresholds in turbidity and organic matter enrichment beyond which mechanisms of resilience fail or become less robust?

To increase the ability to generalise my results it will be necessary to conduct further field studies following similar protocols that are placed within larger spatial and temporal frameworks. A limitation of the small-scale field studies I have conducted is their inability to encompass the broad environmental variation that occurs over seasons and geographical areas (Thrush *et al.*, 1997). Therefore I cannot say, for example, whether the importance of emerged PP to overall benthic PP is consistent throughout the year (as factors such as photosynthesising biomass, sediment temperature and light intensity vary), or whether the role of $CaCO_3$ in buffering the effects of eutrophication-induced acidification is the same in different sediments types. It would be valuable to answer these questions in future research so the true value of these mechanisms of resilience can be understood. To achieve this, my experiments could be repeated at multiple locations throughout New Zealand that are characterised by different sediment types (ranging from sand to mud) and macrofaunal community compositions, and a smaller number of contrasting locations could be chosen to repeat these experiments seasonally.

Multiple stressor effects must be the focus of future benthic ecology research, despite the difficulties associated with implementing such studies and interpreting the potentially complex results. In addition to recording the response of benthic ecosystem structure and function to multiple sources of stress, future endeavours should investigate how the mechanisms of resilience I have identified (emerged PP, $CaCO_3$ buffering and response diversity) are affected by different combinations of stressors. This could be achieved by first identifying pairs of stressors about which informed *a priori* predictions can be made on the nature of their interaction (as outlined in section 5.2.2) and testing whether the mechanisms of resilience become stronger, weaker or disappear entirely when these stressors are combined. Considering acidification as an example, evidence that $CaCO_3$ buffering becomes stronger with exposure to two sources of acidification stress might be that ecosystem function is maintained more effectively during exposure to both rather than one of the stressors. A reduction in the ability of $CaCO_3$ to maintain function during exposure to both stressors would indicate this mechanism became weaker, and no difference between $CaCO_3$-enriched and unenriched sediments would suggest the resilience mechanism had failed. Such approaches could vastly increase our understanding of the sensitivity of benthic ecosystems to cumulative stressors.

Vital to understanding when and if $CaCO_3$ provides resilience will be to know *how* it provides resilience. I demonstrated in Chapter 3 that even in the midst of the complex interactions and ecological 'noise' of the field, $CaCO_3$ dampened negative effects of eutrophication-induced acidification on ecosystem functions, however the coarse sampling techniques used to measure changes in sediment biogeochemistry could not elucidate the mechanism by which it did so. Future laboratory-based studies that control environmental conditions and conduct precise, small-scale measurements of changes in porewater pH, total alkalinity, dissolved inorganic carbon and calcium ion concentrations in $CaCO_3$-enriched versus unenriched sediments will be necessary to identify this. Such measurements would also help to determine whether my hypothesis that $CaCO_3$ addition improved sediment conditions for the microbial community is plausible.

Finally, studies conducted across gradients of turbidity and organic enrichment with higher resolutions (i.e. smaller increments between treatments) than utilised in my experiments will be key in identifying where critical thresholds lie for ecosystem resilience. My findings may indicate appropriate levels of each stressor to target in such studies. For example, Chapter 2 indicated that critical thresholds of turbidity beyond which submerged PP would be lost in the Kaipara Harbour exceeded 68 NTU, and Chapter 3 suggested that the resilience afforded to ecosystem function by $CaCO_3$ buffering was overridden prior to organic matter enrichment quantities of 250 g dw m^{-2}. Gradient-based experimental designs that utilise small increments in turbidity and organic enrichment centred on these values would allow the detection of more precise resilience thresholds, which could feed directly into the management of these stressors.

5.4.1 Final remarks

The outcomes of my research chapters and the extrapolation of their findings to consider the combined effects of increasing SSC and eutrophication, among other stressors, paints an overwhelmingly bleak picture for the future functioning of estuarine benthic habitats and the goods and services they provide. As there are many unknowns around the mechanisms providing resilience and the cumulative effects of multiple coastal stressors, precautionary management at the local scale that integrates land-use, watershed processes and coastal biodiversity (using modelling approaches like those suggested by Stoms *et al.* (2005)) with an aim to drastically reduce or prevent terrestrial run-off to estuaries is essential. Global climate change pressures that will be incomprehensibly more difficult to manage may soon override local stressors (as evidenced by Ballard *et al.* (2019), who demonstrate that decadal trends in precipitation and temperature have already affected nitrogen loading), and reactive management may have little capacity to lessen the effects on estuarine ecosystems. However, as articulated by Adger (2010), "societies have inherent capacities to adapt to climate change… and these capacities are bound up in their ability to act collectively"; targeted *in situ* studies that increase our understanding of benthic ecosystem response to a changing world, as presented in this thesis, are invaluable in contributing to this adaptability.

References

Adger, W. N. (2010). Social Capital, Collective Action, and Adaptation to Climate Change. In M. Voss (Ed.), *Der Klimawandel: Sozialwissenschaftliche Perspektiven* (pp. 327-345). Wiesbaden: VS Verlag für Sozialwissenschaften.

An, S., & Joye, S. B. (2001). Enhancement of coupled nitrification-denitrification by benthic photosynthesis in shallow estuarine sediments. *Limnology and Oceanography, 46*(1), 62-74. https://doi.org/10.4319/lo.2001.46.1.0062

Anderson, M., Gorley, R. N., & Clarke, R. K. (2008). *PERMANOVA+ for PRIMER: Guide to software and statistical methods*: Primer-E Limited.

Anderson, M. J. (2008). Animal-sediment relationships re-visited: Characterising species' distributions along an environmental gradient using canonical analysis and quantile regression splines. *Journal of Experimental Marine Biology and Ecology, 366*(1–2), 16-27. http://doi.org/10.1016/j.jembe.2008.07.006

Anthony, K. R. N., Ridd, P. V., Orpin, A. R., Larcombe, P., & Lough, J. (2004). Temporal variation of light availability in coastal benthic habitats: Effects of clouds, turbidity, and tides. *Limnology and Oceanography, 49*(6), 2201-2211. http://doi.org/10.4319/lo.2004.49.6.2201

Arar, E. J., & Collins, G. B. (1997). *Method 445.0: In vitro determination of chlorophyll a and pheophytin a in marine and freshwater algae by fluorescence*: United States Environmental Protection Agency, Office of Research and Development, National Exposure Research Laboratory.

Austin, P. C., & Steyerberg, E. W. (2012). Interpreting the concordance statistic of a logistic regression model: relation to the variance and odds ratio of a continuous explanatory variable. *BMC Medical Research Methodology, 12*(1), 82. https://doi.org/10.1186/1471-2288-12-82

Baggini, C., Issaris, Y., Salomidi, M., & Hall-Spencer, J. (2015). Herbivore diversity improves benthic community resilience to ocean acidification. *Journal of Experimental Marine Biology and Ecology, 469*, 98-104. https://doi.org/10.1016/j.jembe.2015.04.019

Baillie, P. W. (1986). Oxygenation of intertidal estuarine sediments by benthic microalgal photosynthesis. *Estuarine, Coastal and Shelf Science, 22*(2), 143-159. https://doi.org/10.1016/0272-7714(86)90110-1

Ballard, T., Sinha, E., & Michalak, A. M. (2019). Long-term changes in precipitation and temperature have already impacted nitrogen loading. *Environmental Science & Technology*. https://doi.org/10.1021/acs.est.8b06898

Bannister, R. J., Valdemarsen, T., Hansen, P. K., Holmer, M., & Ervik, A. (2014). Changes in benthic sediment conditions under an Atlantic salmon farm at a deep, well-flushed coastal site. *Aquaculture Environment Interactions, 5*(1), 29-47. http://doi.org/10.3354/aei00092

Barnes, R. S. K. (2019). Abundance/occupancy/patchiness relations in estuarine seagrass macrobenthos. *Estuarine, Coastal and Shelf Science*, 106360. https://doi.org/10.1016/j.ecss.2019.106360

Barranguet, C., Kromkamp, J., & Peene, J. (1998). Factors controlling primary production and photosynthetic characteristics of intertidal microphytobenthos. *Marine Ecology Progress Series, 173*, 117-126. https://doi.org/10.3354/meps173117

Bartels, P., Ask, J., Andersson, A., Karlsson, J., & Giesler, R. (2018). Allochthonous organic matter supports benthic but not pelagic food webs in shallow coastal ecosystems. *Ecosystems, 21*(7), 1459-1470. https://doi.org/10.1007/s10021-018-0233-5

Baumann, H., Wallace, R. B., Tagliaferri, T., & Gobler, C. J. (2015). Large natural pH, CO_2 and O_2 fluctuations in a temperate tidal salt marsh on diel, seasonal, and interannual time scales. *Estuaries and Coasts, 38*(1), 220-231. http://doi.org/10.1007/s12237-014-9800-y

Bautista, M. F., & Paerl, H. W. (1985). Diel N_2 fixation in an intertidal marine cyanobacterial mat community. *Marine Chemistry, 16*(4), 369-377. https://doi.org/10.1016/0304-4203(85)90057-X

Beman, J. M., Chow, C.-E., King, A. L., Feng, Y., Fuhrman, J. A., Andersson, A., . . . Hutchins, D. A. (2011). Global declines in oceanic nitrification rates as a consequence of ocean acidification. *Proceedings of the National Academy of Sciences, 108*(1), 208-213. http://doi.org/10.1073/pnas.1011053108

Berelson, W. M., Heggie, D., Longmore, A., Kilgore, T., Nicholson, G., & Skyring, G. (1998). Benthic nutrient recycling in Port Phillip Bay, Australia. *Estuarine, Coastal and Shelf Science, 46*(6), 917-934. https://doi.org/10.1006/ecss.1998.0328

Berg, P., Rysgaard, S., Funch, P., & Sejr, M. K. (2001). Effects of bioturbation on solutes and solids in marine sediments. *Aquatic Microbial Ecology, 26*(1), 81-94.

Beukema, J. J. (1976). Biomass and species richness of the macro-benthic animals living on the tidal flats of the Dutch Wadden Sea. *Netherlands Journal of Sea Research, 10*(2), 236-261. http://dx.doi.org/10.1016/0077-7579(76)90017-X

Bhadury, P. (2015). Effects of ocean acidification on marine invertebrates - a review. *Indian Journal of Geo-Marine Sciences, 44*(4), 1-11.

Biles, C., Paterson, D., Ford, R., Solan, M., & Raffaelli, D. (2002). Bioturbation, ecosystem functioning and community structure. *Hydrology and Earth System Sciences Discussions, 6*(6), 999-1005.

Binnerup, S. J., Jensen, K., Revsbech, N. P., Jensen, M. H., & Sørensen, J. (1992). Denitrification, dissimilatory reduction of nitrate to ammonium, and nitrification in a bioturbated estuarine sediment as measured with ^{15}N and microsensor techniques. *Applied and Environmental Microbiology, 58*(1), 303-313.

Birchenough, S. N. R., Reiss, H., Degraer, S., Mieszkowska, N., Borja, Á., Buhl-Mortensen, L., . . . Wätjen, K. (2015). Climate change and marine benthos: A review of existing research and future directions in the North Atlantic. *Wiley Interdisciplinary Reviews: Climate Change, 6*(2), 203-223. http://doi.org/10.1002/wcc.330

Blanchard, D., & Bourget, E. (1999). Scales of coastal heterogeneity: influence on intertidal community structure. *Marine Ecology Progress Series, 179*, 163-173. https://doi.org/10.3354/meps179163

Blanchard, G., & Guarini, J.-M. (1996). Studying the role of mud temperature on the hourly variation of the photosynthetic capacity of microphytobenthos in intertidal areas. *Oceanographic Literature Review, 6*(44), 612.

Blanck, H. (2002). A critical review of procedures and approaches used for assessing pollution-induced community tolerance (PICT) in biotic communities. *Human and Ecological Risk Assessment: An International Journal, 8*(5), 1003-1034. https://doi.org/10.1080/1080-700291905792

Borges, A. V., & Gypens, N. (2010). Carbonate chemistry in the coastal zone responds more strongly to eutrophication than ocean acidification. *Limnology and Oceanography, 55*(1), 346-353. https://doi.org/10.4319/lo.2010.55.1.0346

Borja, A., & Dauer, D. M. (2008). Assessing the environmental quality status in estuarine and coastal systems: Comparing methodologies and indices. *Ecological Indicators, 8*(4), 331-337. https://doi.org/10.1016/j.ecolind.2007.05.004

Boucher, G., Clavier, J., & Garrigue, C. (1994). Oxygen and carbon dioxide fluxes at the watersediment interface of a tropical lagoon. *Marine Ecology Progress Series, 107*(1/2), 185-193. https://doi.org/10.3354/meps107185

Braeckman, U., Foshtomi, M. Y., Van Gansbeke, D., Meysman, F., Soetaert, K., Vincx, M., & Vanaverbeke, J. (2014). Variable importance of macrofaunal functional biodiversity for biogeochemical cycling in temperate coastal sediments. *Ecosystems, 17*(4), 720-737. https://doi.org/10.1007/s10021-014-9755-7

Braeckman, U., Provoost, P., Gribsholt, B., Van Gansbeke, D., Middelburg, J. J., Soetaert, K., . . . Vanaverbeke, J. (2010). Role of macrofauna functional traits and density in biogeochemical fluxes and bioturbation. *Marine Ecology Progress Series, 399*, 173-186. http://doi.org/10.3354/meps08336

Bray, J. R., & Curtis, J. T. (1957). An ordination of the upland forest communities of southern Wisconsin. *Ecological Monographs, 27*(4), 325-349. https://doi.org/10.2307/1942268

Bremner, J., Rogers, S. I., & Frid, C. L. J. (2006). Methods for describing ecological functioning of marine benthic assemblages using biological traits analysis (BTA). *Ecological Indicators, 6*(3), 609-622. http://dx.doi.org/10.1016/j.ecolind.2005.08.026

Brown, C. J., Saunders, M. I., Possingham, H. P., & Richardson, A. J. (2013). Managing for interactions between local and global stressors of ecosystems. *PLoS ONE, 8*(6), e65765. https://doi.org/10.1371/journal.pone.0065765

Brown, J. H. (1984). On the relationship between abundance and distribution of species. *The American Naturalist, 124*(2), 255-279. https://doi.org/10.1086/284267

Brown, J. R., Gowen, R. J., & McLusky, D. S. (1987). The effect of salmon farming on the benthos of a Scottish sea loch. *Journal of Experimental Marine Biology and Ecology, 109*(1), 39-51. https://doi.org/10.1016/0022-0981(87)90184-5

Bruland, K. W., Donat, J. R., & Hutchins, D. A. (1991). Interactive influences of bioactive trace metals on biological production in oceanic waters. *Limnology and Oceanography, 36*(8), 1555-1577. https://doi.org/10.4319/lo.1991.36.8.1555

Bulmer, R. H., Kelly, S., & Jeffs, A. G. (2015a). Light reqirements of the seagrass, *Zostera muelleri*, determined by observations at the maximum depth limit in a temperate estuary, New Zealand. *New Zealand Journal of Marine and Freshwater Research*. https://doi.org/10.1080/00288330.2015.1120759

Bulmer, R. H., Lundquist, C. J., & Schwendenmann, L. (2015b). Sediment properties and CO_2 efflux from intact and cleared temperate mangrove forests. *Biogeosciences, 12*(20), 6169-6180. https://doi.org/10.5194/bg-12-6169-2015

Bulmer, R. H., Townsend, M., Drylie, T., & Lohrer, A. M. (2018). Elevated turbidity and the nutrient removal capacity of seagrass. *Frontiers in Marine Science, 5*(462). https://doi.org/10.3389/fmars.2018.00462

Cadotte, M. W., Carscadden, K., & Mirotchnick, N. (2011). Beyond species: functional diversity and the maintenance of ecological processes and services. *Journal of Applied Ecology, 48*(5), 1079-1087. https::/doi.org/10.1111/j.1365-2664.2011.02048.x

Cahoon, L. B., Nearhoof, J. E., & Tilton, C. L. (1999). Sediment grain size effect on benthic microalgal biomass in shallow aquatic ecosystems. *Estuaries, 22*(3), 735-741. https://doi.org/10.2307/1353106

Cahoon, L. B., & Safi, K. A. (2002). Distribution and biomass of benthic microalgae in Manukau Harbour, New Zealand. *New Zealand Journal of Marine and Freshwater Research, 36*(2), 257-266. 10.1080/00288330.2002.9517084

Cai, W.-J., Hu, X., Huang, W.-J., Murrell, M. C., Lehrter, J. C., Lohrenz, S. E., . . . Gong, G.-C. (2011). Acidification of subsurface coastal waters enhanced by eutrophication. *Nature Geoscience, 4*(11), 766-770. http://doi.org/10.1038/ngeo1297

Cartaxana, P., Vieira, S., Ribeiro, L., Rocha, R. J. M., Cruz, S., Calado, R., & da Silva, J. M. (2015). Effects of elevated temperature and CO2 on intertidal microphytobenthos. *BMC Ecology, 15*(1), 10. 10.1186/s12898-015-0043-y

Cederwall, H., & Elmgren, R. (1990). Biological effects of eutrophication in the Baltic Sea, particularly the coastal zone. *Ambio. Stockholm, 19*(3), 109-112.

Celis-Plá, P. S. M., Hall-Spencer, J. M., Horta, P., Milazzo, M., Korbee, N., Cornwall, C. E., & Figueroa, F. L. (2015). Macroalgal responses to ocean acidification depend on nutrient and light levels. *Frontiers in Marine Science, 2*. http://doi.org/10.3389/fmars.2015.00026

Chen, X., Zhang, X., Church, J. A., Watson, C. S., King, M. A., Monselesan, D., . . . Harig, C. (2017). The increasing rate of global mean sea-level rise during 1993–2014. *Nature Climate Change*, 7(7), 492-495. https://doi.org/10.1038/nclimate3325

Cheng, B. S., Bible, J. M., Chang, A. L., Ferner, M. C., Wasson, K., Zabin, C. J., . . . Grosholz, E. D. (2015). Testing local and global stressor impacts on a coastal foundation species using an ecologically realistic framework. *Global Change Biology, 21*(7), 2488-2499. https://doi.org/10.1111/gcb.12895

Chiba, S., Saito, H., Fletcher, R., Yogi, T., Kayo, M., Miyagi, S., . . . Fujikura, K. (2018). Human footprint in the abyss: 30 year records of deep-sea plastic debris. *Marine Policy, 96*, 204-212. https://doi.org/10.1016/j.marpol.2018.03.022

Christensen, P. B., Glud, R. N., Dalsgaard, T., & Gillespie, P. (2003). Impacts of longline mussel farming on oxygen and nitrogen dynamics and biological communities of coastal sediments. *Aquaculture, 218*(1-4), 567-588. https://doi.org/10.1016/S0044-8486(02)00587-2

Clarke, K., & Ainsworth, M. J. (1993). A method of linking multivariate community structure to environmental variables. *Marine Ecology Progress Series*, 92, 205-205.

Clarke, K., & Gorley, R. N. (2006). *Primer v6: User Manual/Tutorial*.

Clarke, K. R., Gorley, R., Somerfield, P., & Warwick, R. (2014). *Change in marine communities: an approach to statistical analysis and interpretation*: Primer-E Ltd.

Clavier, J., Chauvaud, L., Carlier, A., Amice, E., Van der Geest, M., Labrosse, P., . . . Hily, C. (2011). Aerial and underwater carbon metabolism of a Zostera noltii seagrass bed in the Banc d'Arguin, Mauritania. *Aquatic Botany, 95*(1), 24-30. https://doi.org/10.1016/j.aquabot.2011.03.005

Clements, J. C., & Chopin, T. (2017). Ocean acidification and marine aquaculture in North America: potential impacts and mitigation strategies. *Reviews in Aquaculture, 9*(4), 326-341. http://doi.org/10.1111/raq.12140

Coelho, H., Vieira, S., & Serôdio, J. (2009). Effects of desiccation on the photosynthetic activity of intertidal microphytobenthos biofilms as studied by optical methods. *Journal of Experimental Marine Biology and Ecology, 381*(2), 98-104. 10.1016/j.jembe.2009.09.013

Colijn, F., Admiraal, W., Baretta, J. W., & Ruardij, P. (1987). Primary production in a turbid estuary, the Ems-Dollard: Field and model studies. *Continental Shelf Research, 7*(11), 1405-1409. https://doi.org/10.1016/0278-4343(87)90045-8

Cosby, B. J., Cowling, E. B., Galloway, J. N., Erisman, J. W., Aber, J. D., Howarth, R. W., & Seitzinger, S. P. (2003). The Nitrogen Cascade. *Bioscience, 53*(4), 341-356. https://doi.org/10.1641/0006-3568(2003)053[0341:TNC]2.0.CO;2

Costanza, R., de Groot, R., Sutton, P., van der Ploeg, S., Anderson, S. J., Kubiszewski, I., . . . Turner, R. K. (2014). Changes in the global value of ecosystem services. *Global Environmental Change, 26*, 152-158. http://dx.doi.org/10.1016/j.gloenvcha.2014.04.002

Crain, C. M., Kroeker, K., & Halpern, B. S. (2008). Interactive and cumulative effects of multiple human stressors in marine systems. *Ecology Letters, 11*(12), 1304-1315. https://doi.org/10.1111/j.1461-0248.2008.01253.x

Cranford, P. J., Strain, P. M., Dowd, M., Hargrave, B. T., Grant, J., & Archambault, M. (2007). Influence of mussel aquaculture on nitrogen dynamics in a nutrient enriched coastal embayment. *Marine Ecology Progress Series, 347*, 61-78.

Crespo, D., Solan, M., Leston, S., Pardal, M. A., & Dolbeth, M. (2018). Ecological consequences of invasion across the freshwater–marine transition in a warming world. *Ecology and Evolution, 8*(3), 1807-1817. https://doi.org/10.1002/ece3.3652

Cumming, H. (2016). *Effects of turbidity on the aerobic physiology and feeding behaviour of juvenile snapper (Pagrus auratus).* University of Auckland, Research Space Auckland.

Dahms, H.-U., Schizas, N. V., James, R. A., Wang, L., & Hwang, J.-S. (2018). Marine hydrothermal vents as templates for global change scenarios. *Hydrobiologia, 818*(1), 1-10. https://doi.org/10.1007/s10750-018-3598-8

Dalsgaard, T. (2003). Benthic primary production and nutrient cycling in sediments with benthic microalgae and transient accumulation of macroalgae. *Limnology and Oceanography, 48*(6), 2138-2150. https://doi.org/10.4319/lo.2003.48.6.2138

Dalzell, B. J., Filley, T. R., & Harbor, J. M. (2005). Flood pulse influences on terrestrial organic matter export from an agricultural watershed. *Journal of Geophysical Research: Biogeosciences, 110*(G2).

Danovaro, R., Gambi, C., Dell'Anno, A., Corinaldesi, C., Fraschetti, S., Vanreusel, A., . . . Gooday, A. J. (2008). Exponential decline of deep-sea ecosystem functioning linked to benthic biodiversity loss. *Current Biology, 18*(1), 1-8. https://doi.org/10.1016/j.cub.2007.11.056

Davies-Colley, R. J., Nagels, J. W., Smith, R. A., Young, R. G., & Phillips, C. J. (2004). Water quality impact of a dairy cow herd crossing a stream. *New Zealand Journal of Marine and Freshwater Research, 38*(4), 569-576. https://doi.org/10.1080/00288330.2004.9517262

de Winton, M. D., Hawes, I., Clayton, J. S., Champion, P. D., & Smith, R. K. (1998). Sea lettuce dynamics and ecophysiology in Tauranga Harbour, Bay of Plenty. NIWA Client Report BPR802: Environment B.O.P., Tauranga District Council, Western Bay of Plenty District Council.

Deegan, L. A., & Garritt, R. H. (1997). Evidence for spatial variability in estuarine food webs. *Marine Ecology Progress Series, 147*, 31-47. https://doi.org/10.3354/meps147031

Dennert, H. G. (1974). Tolerance differences and interspecific competition in three members of the amphipod genus *Gammarus*. *Bijdragen tot de Dierkunde, 44*(1), 83. https://doi.org/10.1163/26660644-04401004

Diaz, R. J., & Rosenberg, R. (2008). Spreading dead zones and consequences for marine ecosystems. *Science, 321*(5891), 926-929. http://doi.org/10.1126/science.1156401

Dissanayake, N. G., Frid, C. L. J., Drylie, T. P., & Caswell, B. A. (2018). Ecological functioning of mudflats: global analysis reveals both regional differences and widespread conservation of functioning. *Marine Ecology Progress Series, 604*, 1-20. https://doi.org/10.3354/meps12728

Doi, H. (2009). Spatial patterns of autochthonous and allochthonous resources in aquatic food webs. *Population Ecology, 51*(1), 57-64. https://doi.org/10.1007/s10144-008-0127-z

Dolbeth, M., Cardoso, P. G., Ferreira, S. M., Verdelhos, T., Raffaelli, D., & Pardal, M. A. (2007). Anthropogenic and natural disturbance effects on a macrobenthic estuarine community over a 10-year period. *Marine Pollution Bulletin*, 54(5), 576-585. https://doi.org/10.1016/j.marpolbul.2006.12.005

Dolbeth, M., Crespo, D., Leston, S., & Solan, M. (2019). Realistic scenarios of environmental disturbance lead to functionally important changes in benthic species-environment interactions. *Marine Environmental Research, 150*, 104770. https://doi.org/10.1016/j.marenvres.2019.104770

Doney, S. C., Fabry, V. J., Feely, R. A., & Kleypas, J. A. (2009). Ocean acidification: The other CO_2 problem. *Annual Review of Marine Science, 1*, 169-192. https://doi.org/10.1146/annurev.marine.010908.163834

Doney, S. C., Ruckelshaus, M., Duffy, J. E., Barry, J. P., Chan, F., English, C. A., . . . Talley, L. D. (2012). Climate change impacts on marine ecosystems. *Annual Review of Marine Science, 4*(1), 11-37. https://doi.org/10.1146/annurev-marine-041911-111611

Donohue, P. J. C., Calosi, P., Bates, A. H., Laverock, B., Rastrick, S., Mark, F. C., . . . Widdicombe, S. (2012). Impact of exposure to elevated pCO($_2$) on the physiology and behaviour of an important ecosystem engineer, the burrowing shrimp *Upogebia deltaura*. *Aquatic Biology, 15*(1), 73-86. http://doi.org/10.3354/ab00408

Dormann, C. F., Elith, J., Bacher, S., Buchmann, C., Carl, G., Carré, G., . . . Lautenbach, S. (2013). Collinearity: a review of methods to deal with it and a simulation study evaluating their performance. *Ecography, 36*(1), 27-46. http://doi.org/10.1111/j.1600-0587.2012.07348.x

Douglas, E. J., Pilditch, C. A., Kraan, C., Schipper, L. A., Lohrer, A. M., & Thrush, S. F. (2017). Macrofaunal functional diversity provides resilience to nutrient enrichment in coastal sediments. *Ecosystems*, 1-13. https://doi.org/10.1007/s10021-017-0113-4

Drylie, T. P., Lohrer, A. M., Needham, H. R., Bulmer, R. H., & Pilditch, C. A. (2018). Benthic primary production in emerged intertidal habitats provides resilience to high water column turbidity. *Journal of Sea Research, 142*, 101-112. http://doi.org/10.1016/j.seares.2018.09.015

Drylie, T. P., Needham, H. R., Lohrer, A. M., Hartland, A., & Pilditch, C. A. (2019). Calcium carbonate alters the functional response of coastal sediments to eutrophication-induced acidification. *Scientific Reports, 9*(1), 12012. https://doi.org/10.1038/s41598-019-48549-8

Du, G. Y., Li, W. T., Li, H., & Chung, I. K. (2012). Migratory responses of benthic diatoms to light and temperature monitored by chlorophyll fluorescence. *Journal of Plant Biology, 55*(2), 159-164. https://doi.org/10.1007/s12374-011-9200-9

Duarte, C., Hendriks, I., Moore, T., Olsen, Y., Steckbauer, A., Ramajo, L., . . . McCulloch, M. (2013). Is ocean acidification an open-ocean syndrome? Understanding anthropogenic impacts on seawater ph. *Estuaries and Coasts, 36*(2), 221-236. http://doi.org/10.1007/s12237-013-9594-3

Duarte, C. M., & Cebrián, J. (1996). The fate of marine autotrophic production. *Limnology and Oceanography, 41*(8), 1758-1766.

Dutkiewicz, S., Morris, J. J., Follows, M. J., Scott, J., Levitan, O., Dyhrman, S. T., & Berman-Frank, I. (2015). Impact of ocean acidification on the structure of future phytoplankton communities. *Nature Climate Change, advance online publication*. https://doi.org/10.1038/nclimate2722

Eilers, P. H. C., & Peeters, J. C. H. (1988). A model for the relationship between light intensity and the rate of photosynthesis in phytoplankton. *Ecological Modelling, 42*(3), 199-215. http://doi.org/10.1016/0304-3800(88)90057-9

Eisler, R. (2012). *Oceanic Acidification: A Comprehensive Overview*. Boca Raton: CRC Press.

Eklund, B. (1992). Practical guidance for flux chamber measurements of fugitive volatile organic emission rates. *Journal of the Air & Waste Management Association, 42*(12), 1583-1591. https://doi.org/10.1080/10473289.1992.10467102

Ellis, J., Cummings, V., Hewitt, J., Thrush, S., & Norkko, A. (2002). Determining effects of suspended sediment on condition of a suspension feeding bivalve (Atrina zelandica): results of a survey, a laboratory experiment and a field transplant experiment. *Journal of Experimental Marine Biology and Ecology, 267*(2), 147-174.

Ellis, J. I., Hewitt, J. E., Clark, D., Taiapa, C., Patterson, M., Sinner, J., . . . Thrush, S. F. (2015). Assessing ecological community health in coastal estuarine systems impacted by multiple stressors. *Journal of Experimental Marine Biology and Ecology, 473*, 176-187. https://doi.org/10.1016/j.jembe.2015.09.003

Ellis, J. I., Norkko, A., & Thrush, S. F. (2000a). Broad-scale disturbance of intertidal and shallow sublittoral soft-sediment habitats; effects on the benthic macrofauna. *Journal of Aquatic Ecosystem Stress and Recovery, 7*(1), 57-74. https://doi.org/10.1023/A:1009923530894

Ellis, J. I., Schneider, D. C., & Thrush, S. F. (2000b). Detecting anthropogenic disturbance in an environment with multiple gradients of physical disturbance, Manukau Harbour, New Zealand. *Hydrobiologia, 440*, 379-391. https://doi.org/10.1023/A:1004172622751

Elmqvist, T., Folke, C., Nyström, M., Peterson, G., Bengtsson, J., Walker, B., & Norberg, J. (2003). Response diversity, ecosystem change, and resilience. *Frontiers in Ecology and the Environment, 1*(9), 488-494. https://doi.org/10.1890/1540-9295(2003)001[0488:rdecar]2.0.co;2

Evrard, V., Huettel, M., Cook, P. L. M., Soetaert, K., Heip, C. H. R., & Middelburg, J. J. (2012). Importance of phytodetritus and microphytobenthos for heterotrophs in a shallow subtidal sandy sediment. *Marine Ecology Progress Series, 455*, 13-31. https://doi.org/10.3354/meps09676

Fabry, V. J., Seibel, B. A., Feely, R. A., & Orr, J. C. (2008). Impacts of ocean acidification on marine fauna and ecosystem processes. *Ices Journal of Marine Science, 65*(3), 414-432. 10.1093/icesjms/fsn048

Fang, C., & Moncrieff, J. B. (2001). The dependence of soil CO_2 efflux on temperature. *Soil Biology and Biochemistry, 33*(2), 155-165. https://doi.org/10.1016/S0038-0717(00)00125-5

Fernández-Rodríguez, V., Santos, C. S. G., & Pires, A. P. F. (2019). Meta-analysis of the effects of organic matter on polychaetes of the east coast of South America. *Marine Environmental Research*. https://doi.org/10.1016/j.marenvres.2019.06.001

Figueroa, P. (2013). Vanity, Pollution and Death on Mt. Everest. *Development & society : Biodiversity, ecosystems, pollution, culture, environment.* Retrieved August 31st, 2019, from https://ourworld.unu.edu/en/vanity-pollution-and-death-on-mt-everest

Findlay, R. H., & Watling, L. (1997). Prediction of benthic impact for salmon net-pens based on the balance of benthic oxygen supply and demand. *Marine Ecology Progress Series, 155*, 147-157. https://doi.org/10.3354/meps155147

Fisher, T. R., Harding, L. W., Stanley, D. W., & Ward, L. G. (1988). Phytoplankton, nutrients, and turbidity in the Chesapeake, Delaware, and Hudson estuaries. *Estuarine, Coastal and Shelf Science, 27*(1), 61-93. https://doi.org/10.1016/0272-7714(88)90032-7

Fitch, J. E., & Crowe, T. P. (2012). Combined effects of inorganic nutrients and organic enrichment on intertidal benthic macrofauna: an experimental approach. *Marine Ecology Progress Series, 461*, 59-70. http://doi.org/10.3354/meps09819

Flynn, K. J., Clark, D. R., Mitra, A., Fabian, H., Hansen, P. J., Glibert, P. M., . . . Brownlee, C. (2015). Ocean acidification with (de)eutrophication will alter future phytoplankton growth and succession. *Proceedings of the Royal Society B-Biological Sciences, 282*(1804), 42604-42604.

Folke, C., Carpenter, S., Walker, B., Scheffer, M., Elmqvist, T., Gunderson, L., & Holling, C. S. (2004). Regime shifts, resilience, and biodiversity in ecosystem management. *Annual Review of Ecology, Evolution, and Systematics, 35*, 557-581. https://doi.org/10.1146/annurev.ecolsys.35.021103.105711

Folt, C., Chen, C., Moore, M., & Burnaford, J. (1999). Synergism and antagonism among multiple stressors. *Limnology and Oceanography, 44*(3part2), 864-877. https://doi.org/10.4319/lo.1999.44.3_part_2.0864

Fourqurean, J. W., Duarte, C. M., Kennedy, H., Marbà, N., Holmer, M., Mateo, M. A., . . . McGlathery, K. J. (2012). Seagrass ecosystems as a globally significant carbon stock. *Nature Geoscience, 5*(7), 505-509. https://doi.org/10.1038/ngeo1477

Franklin, L. A., Osmond, C. B., & Larkum, A. W. D. (2003). Photoinhibition, UV-B and algal photosynthesis. In A. W. D. Larkum, S. E. Douglas & J. A. Raven (Eds.), *Photosynthesis in Algae* (pp. 351-384). Dordrecht: Springer Netherlands.

Gagic, V., Bartomeus, I., Jonsson, T., Taylor, A., Winqvist, C., Fischer, C., . . . Bommarco, R. (2015). Functional identity and diversity of animals predict ecosystem functioning better than species-based indices. *Proceedings of the Royal Society B: Biological Sciences, 282*(1801), 20142620. https://doi.org/10.1098/rspb.2014.2620

Gameiro, C., Zwolinski, J., & Brotas, V. (2011). Light control on phytoplankton production in a shallow and turbid estuarine system. *Hydrobiologia, 669*(1), 249. 10.1007/s10750-011-0695-3

Gammal, J., Norkko, J., Pilditch, C. A., & Norkko, A. (2017). Coastal hypoxia and the importance of benthic macrofauna communities for ecosystem functioning. *Estuaries and Coasts, 40*(2), 457-468. https://doi.org/10.1007/s12237-016-0152-7

Gaston, K. J. (1999). Implications of interspecific and intraspecific abundance-occupancy relationships. *Oikos, 86*(2), 195-207. https://doi.org/10.2307/3546438

Gaston, K. J., Blackburn, T. M., Greenwood, J. J. D., Gregory, R. D., Quinn, R. M., & Lawton, J. H. (2000). Abundance–occupancy relationships. *Journal of Applied Ecology, 37*(s1), 39-59. https://doi.org/10.1046/j.1365-2664.2000.00485.x

Gattuso, J.-P., Gentili, B., Duarte, C. M., Kleypas, J. A., Middelburg, J. J., & Antoine, D. (2006). Light availability in the coastal ocean: impact on the distribution of benthic photosynthetic organisms and contribution to primary production. *Biogeosciences Discussions, 3*(4), 895-959. https://doi.org/10.5194/bg-3-489-2006

Gattuso, J. P., Frankignoulle, M., & Wollast, R. (1998). Carbon and carbonate metabolism in coastal aquatic ecosystems. *Annual Review of Ecology and Systematics, 29*, 405-434. https://doi.org/10.2307/221714

Gazeau, F., Parker, L. M., Comeau, S., Gattuso, J.-P., O'Connor, W. A., Martin, S., . . . Ross, P. M. (2013). Impacts of ocean acidification on marine shelled molluscs. *Marine Biology, 160*(8), 2207-2245. http://doi.org/10.1007/s00227-013-2219-3

Geankoplis, C. J. (2003). *Transport Processes and Separation Process Principles*: Prentice Hall Professional Technical Reference.

Geert, H. J., Wynter, D. T., Perkins, M., Machairopoulou, M., & Neill, S. P. (2009). Context dependency of relationships between biodiversity and ecosystem functioning is different for multiple ecosystem functions. *Oikos, 118*(12), 1892-1900. https://doi.org/10.1111/j.1600-0706.2009.17556.x

Giles, H., Pilditch, C. A., & Bell, D. G. (2006). Sedimentation from mussel (Perna canaliculus) culture in the Firth of Thames, New Zealand: Impacts on sediment oxygen and nutrient fluxes. *Aquaculture, 261*(1), 125-140. https://doi.org/10.1016/j.aquaculture.2006.06.048

Gladstone-Gallagher, R. V., Lohrer, A. M., Lundquist, C. J., & Pilditch, C. A. (2016). Effects of detrital subsidies on soft-sediment ecosystem function are transient and source-dependent. *PLoS ONE, 11*(5), e0154790. https://doi.org/10.1371/journal.pone.0154790

Gladstone-Gallagher, R. V., Lundquist, C. J., & Pilditch, C. A. (2014). Response of temperate intertidal benthic assemblages to mangrove detrital inputs. *Journal of Experimental Marine Biology and Ecology, 460*, 80-88. https://doi.org/10.1016/j.jembe.2014.06.006

Gladstone-Gallagher, R. V., Pilditch, C. A., Stephenson, F., & Thrush, S. F. (2019). Linking traits across ecological scales determines functional resilience. *Trends in Ecology & Evolution*. https://doi.org/10.1016/j.tree.2019.07.010

González-Delgado, S., & Hernández, J. C. (2018). The importance of natural acidified systems in the study of ocean acidification: What have we learned? In C. Sheppard (Ed.), *Advances in Marine Biology* (Vol. 80, pp. 57-99): Academic Press.

Gonzalez, A., & Loreau, M. (2009). The causes and consequences of compensatory dynamics in ecological communities. *Annual Review of Ecology, Evolution, and Systematics, 40*(1), 393-414. https://doi.org/10.1146/annurev.ecolsys.39.110707.173349

Graham, S., Barnett, J., Fincher, R., Hurlimann, A., Mortreux, C., & Waters, E. (2013). The social values at risk from sea-level rise. *Environmental Impact Assessment Review, 41*, 45-52. https://doi.org/10.1016/j.eiar.2013.02.002

Grant, J. (1981). Dynamics of competition among estuarine sand-burrowing amphipods. *Journal of Experimental Marine Biology and Ecology, 49*(2), 255-265. https://doi.org/10.1016/0022-0981(81)90075-7

Gray, J. S., & Mirza, F. B. (1979). A possible method for the detection of pollution-induced disturbance on marine benthic communities. *Marine Pollution Bulletin, 10*(5), 142-146. https://doi.org/10.1016/0025-326X(79)90082-1

Gray, J. S., Wu, R. S.-s., & Or, Y. Y. (2002). Effects of hypoxia and organic enrichment on the coastal marine environment. *Marine Ecology Progress Series, 238*, 249-279. https://doi.org/10.3354/meps238249

Green, M. A., Waldbusser, G. G., Hubazc, L., Cathcart, E., & Hall, J. (2013). Carbonate mineral saturation state as the recruitment cue for settling bivalves in marine muds. *Estuaries and Coasts, 36*(1), 18-27. http://doi.org/10.1007/s12237-012-9549-0

Green, M. A., Waldbusser, G. G., Reilly, S. L., Emerson, K., & O'Donnell, S. (2009). Death by dissolution: Sediment saturation state as a mortality factor for juvenile bivalves. *Limnology and Oceanography, 54*(4), 1037-1047. http://doi.org/10.4319/lo.2009.54.4.1037

Green, M. O., & Coco, G. (2007). Sediment transport on an estuarine intertidal flat: Measurements and conceptual model of waves, rainfall and exchanges with a tidal creek. *Estuarine, Coastal and Shelf Science, 72*(4), 553-569. https://doi.org/10.1016/j.ecss.2006.11.006

Greenfield, B. L., Kraan, C., Pilditch, C. A., & Thrush, S. F. (2016). Mapping functional groups can provide insight into ecosystem functioning and potential resilience of intertidal sandflats. *Marine Ecology Progress Series, 548*, 1-10. http://doi.org/10.3354/meps11692

Greiner, C. M., Klinger, T., Ruesink, J. L., Barber, J. S., & Horwith, M. (2018). Habitat effects of macrophytes and shell on carbonate chemistry and juvenile clam recruitment, survival, and growth. *Journal of Experimental Marine Biology and Ecology, 509*, 8-15. http://doi.org/10.1016/j.jembe.2018.08.006

Grizzle, R. E., & Penniman, C. A. (1991). Effects of organic enrichment on estuarine macrofaunal benthos: a comparison of sediment profile imaging and traditional methods. *Marine Ecology Progress Series, 74*(2/3), 249-262.

Günther, C.-P. (1992). Dispersal of intertidal invertebrates: A strategy to react to disturbance of different scales? *Netherlands Journal of Sea Research, 30*, 45-56. http://doi.org/10.1016/0077-7579(92)90044-F

Hailes, S. F., & Carter, K. R. (2016). Kaipara Harbour ecological monitoring programme: Report on data collected between October 2009 and January 2016 *Auckland Council technical report*: Prepared by NIWA for Auckland Council.

Hales, B., Emerson, S., & Archer, D. (1994). Respiration and dissolution in the sediments of the western North Atlantic: estimates from models of in situ microelectrode measurements of porewater oxygen and pH. *Deep Sea Research Part I: Oceanographic Research Papers, 41*(4), 695-719. http://dx.doi.org/10.1016/0967-0637(94)90050-7

Hall-Spencer, J. M., Rodolfo-Metalpa, R., Martin, S., Ransome, E., Fine, M., Turner, S. M., . . . Buia, M.-C. (2008). Volcanic carbon dioxide vents show ecosystem effects of ocean acidification. *Nature, 454*, 96. https://doi.org/10.1038/nature07051

Hall, S. J. (1994). Physical disturbance and marine benthic communities: life in unconsolidated sediments. *Oceanography and Marine Biology: an annual review*.

Halpern, B. S., Ebert, C. M., Kappel, C. V., Madin, E. M. P., Micheli, F., Perry, M., . . . Walbridge, S. (2009). Global priority areas for incorporating land–sea connections in marine conservation. *Conservation Letters, 2*(4), 189-196. https://doi.org/10.1111/j.1755-263X.2009.00060.x

Halpern, B. S., Walbridge, S., Selkoe, K. A., Kappel, C. V., Micheli, F., D'Agrosa, C., . . . Watson, R. (2008). A global map of human impact on marine ecosystems. *Science, 319*(5865), 948-952. https://doi.org/10.1126/science.1149345

Hargrave, B. T. (1969). Similarity of oxygen uptake by benthic communities. *Limnology and Oceanography, 14*(5), 801-805. http://doi.org/10.4319/lo.1969.14.5.0801

Hargrave, B. T. (1973). Coupling carbon flow through some pelagic and benthic communities. *Journal of the Fisheries Research Board of Canada, 30*(9), 1317-1326. https://doi.org/10.1139/f73-212

Hargrave, B. T., Phillips, G. A., Doucette, L. I., White, M. J., Milligan, T. G., Wildish, D. J., & Cranston, R. E. (1997). Assessing benthic impacts of organic enrichment from marine aquaculture *The Interactions Between Sediments and Water* (pp. 641-650): Springer.

Hargrave, B. T., Prouse, N. J., Phillips, G. A., & Neame, P. A. (1983). Primary production and respiration in pelagic and benthic communities at two intertidal sites in the upper Bay of Fundy. *Canadian Journal of Fisheries and Aquatic Sciences, 40*(S1), s229-s243. 10.1139/f83-286

Hay, M. E. (1996). Defensive synergisms? Reply to Pennings. *Ecology, 77*(6), 1950-1952. https://doi.org/10.2307/2265799

Hayward, B. W., Grace, R. V., & Brook, F. J. (1981). Soft-bottom benthic macrofaunal communities of the eastern Bay of Islands, northern New Zealand. *Tane, 27*, 103-122.

Healy, R. W., Striegl, R. G., Russell, T. F., Hutchinson, G. L., & Livingston, G. P. (1996). Numerical evaluation of static-chamber measurements of soil—atmosphere gas exchange: identification of physical processes. *Soil Science Society of America Journal, 60*(3), 740-747. https://doi.org/10.2136/sssaj1996.03615995006000030009x

Heath, R. A. (1975). Stability of some New Zealand coastal inlets. *Marine and Freshwater Research, 9*(4), 449-457.

Heck, K. L., Hays, G., & Orth, R. J. (2003). Critical evaluation of the nursery role hypothesis for seagrass meadows. *Marine Ecology Progress Series, 253*, 123-136.

Heffner, R. A., Butler, M. J., & Reilly, C. K. (1996). Pseudoreplication Revisited. *Ecology, 77*(8), 2558-2562. https://doi.org/10.2307/2265754

Heggie, K., & Savage, C. (2009). Nitrogen yields from New Zealand coastal catchments to receiving estuaries. *New Zealand Journal of Marine and Freshwater Research, 43*(5), 1039-1052. https://doi.org/10.1080/00288330.2009.9626527

Heilskov, A. C., & Holmer, M. (2001). Effects of benthic fauna on organic matter mineralization in fish-farm sediments: importance of size and abundance. *Ices Journal of Marine Science, 58*(2), 427-434. https://doi.org/10.1006/jmsc.2000.1026

Heip, C., & Engels, P. (2009). Comparing species diversity and evenness indices. *Journal of the Marine Biological Association of the United Kingdom, 54*(3), 559-563. https://doi.org/10.1017/S0025315400022748

Heiri, O., Lotter, A. F., & Lemcke, G. (2001). Loss on ignition as a method for estimating organic and carbonate content in sediments: reproducibility and comparability of results. *Journal of Paleolimnology, 25*(1), 101-110. http://doi.org/10.1023/A:1008119611481

Henley, W. J. (1993). Measurement and interpretation of photosynthetic light-response curves in algae in the context of photoinhibition and diel changes. *Journal of Phycology, 29*(6), 729-739. 10.1111/j.0022-3646.1993.00729.x

Hewitt, J., & Ellis, J. (2010). Assessment of the benthic health model. Organisation: Auckland Regional Council TR2010/034.

Hewitt, J., Thrush, S., Lohrer, A., & Townsend, M. (2010). A latent threat to biodiversity: consequences of small-scale heterogeneity loss. *Biodiversity and Conservation, 19*(5), 1315-1323. https://doi.org/10.1007/s10531-009-9763-7

Hewitt, J. E., Ellis, J. I., & Thrush, S. F. (2016). Multiple stressors, nonlinear effects and the implications of climate change impacts on marine coastal ecosystems. *Global Change Biology, 22*(8), 2665-2675. https://doi.org/10.1111/gcb.13176

Hewitt, J. E., & Funnell, G. A. (2005). Benthic marine habitats and communities of the southern Kaipara: Prepared by NIWA for Auckland Regional Council.

Hewitt, J. E., Thrush, S. F., Cummings, V. J., & Pridmore, R. D. (1996). Matching patterns with processes: predicting the effect of size and mobility on the spatial distributions of the bivalves Macomona liliana and Austrovenus stutchburyi. *Marine Ecology Progress Series, 135*, 57-67. https://doi.org/10.3354/meps135057

Hewitt, J. E., Thrush, S. F., Dayton, P. K., & Bonsdorff, E. (2007). The effect of spatial and temporal heterogeneity on the design and analysis of empirical studies of scale-dependent systems. *The American Naturalist, 169*(3), 398-408. http://doi.org/10.1086/510925

Hillman, K., Walker, D., Larkum, A., & McComb, A. J. (1989). Productivity and Nutrient Limitation. In A. W. D. Larkum, A. J. McComb & S. A. Shephard (Eds.), *Biology of Seagrasses : A Treatise on the Biology of Seagrasses with Special Reference to the Australian Region*. Amsterdam, The Netherlands: Elsevier Science Pub.

Hohaia, A., Vopel, K., & Pilditch, C. A. (2014). Thin terrestrial sediment deposits on intertidal sandflats: effects on pore-water solutes and juvenile bivalve burial behaviour. *Biogeosciences, 11*(8), 2225-2235. https://doi.org/10.5194/bg-11-2225-2014

Holling, C. S. (1973). Resilience and stability of ecological systems. *Annual Review of Ecology and Systematics, 4*(1), 1-23.

Holmes, R. W., & Mahall, B. E. (1982). Preliminary observations on the effects of flooding and desiccation upon the net photosynthetic rates of high intertidal estuarine sediments. *Limnology and Oceanography, 27*(5), 954-958. https://doi.org/10.4319/lo.1982.27.5.0954

Hooper, D. U., Chapin, F. S., Ewel, J. J., Hector, A., Inchausti, P., Lavorel, S., . . . Wardle, D. A. (2005). Effects of biodiversity on ecosystem functioning: A consensus of current knowledge. *Ecological Monographs, 75*(1), 3-35. https://doi.org/10.1890/04-0922

Hopkins, W. G. (2006). *Photosynthesis and respiration*. New York: Chelsea House.

Hopkinson, C. S., & Wetzel, R. L. (1982). *In situ* measurements of nutrient and oxygen fluxes in a coastal marine benthic community. *Marine Ecology Progress Series, 10*, 29.

Howarth, R., Chan, F., Conley, D. J., Garnier, J., Doney, S. C., Marino, R., & Billen, G. (2011). Coupled biogeochemical cycles: eutrophication and hypoxia in temperate estuaries and coastal marine ecosystems. *Frontiers in Ecology and the Environment, 9*(1), 18-26. http://doi.org/10.1890/100008

Howarth, R. W., & Marino, R. (2006). Nitrogen as the limiting nutrient for eutrophication in coastal marine ecosystems: Evolving views over three decades. *Limnology and Oceanography, 51*(1part2), 364-376. https://doi.org/10.4319/lo.2006.51.1_part_2.0364

Huettel, M., & Gust, G. (1992). Solute release mechanisms from confined sediment cores in stirred benthic chambers and flume flows. *Marine Ecology Progress Series, 82*(2), 187-197.

Hurlbert, S. H. (1984). Pseudoreplication and the design of ecological field experiments. *Ecological Monographs, 54*(2), 187-211. https://doi.org/10.2307/1942661

Ieno, E. N., Solan, M., Batty, P., & Pierce, G. J. (2006). How biodiversity affects ecosystem functioning: roles of infaunal species richness, identity and density in the marine benthos. *Marine Ecology Progress Series, 311*, 263-271. https://doi.org/10.3354/meps311263

IPCC. (2014a). *Climate Change 2014: Impacts, adaptation and vulnerability. Contribution of Working Group II to the Fifth Assessment Report of the IPCC* (Vol. 4). Cambridge, UK and New York, NY, USA: Cambridge University Press.

IPCC. (2014b). Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change (pp. 151). Geneva, Switzerland: IPCC.

James, R. J., Lincoln-Smith, M. P., & Fairweather, P. G. (1995). Sieve mesh-size and taxonomic resolution needed to describe natural spatial variation of marine macrofauna. *Marine Ecology Progress Series, 118*, 187-198. https://doi.org/10.3354/meps118187

Jensen, L., Mueller, T., Tate, K., Ross, D., Magid, J., & Nielsen, N. (1996). Soil surface CO2 flux as an index of soil respiration in situ: a comparison of two chamber methods. *Soil Biology and Biochemistry, 28*(10-11), 1297-1306. https://doi.org/10.1016/S0038-0717(96)00136-8

Johnson, V. R., Brownlee, C., Rickaby, R. E. M., Graziano, M., Milazzo, M., & Hall-Spencer, J. M. (2013). Responses of marine benthic microalgae to elevated CO_2. *Marine Biology, 160*(8), 1813-1824. http://doi.org/10.1007/s00227-011-1840-2

Joint, I. R., & Pomroy, A. J. (1981). Primary production in a turbid estuary. *Estuarine, Coastal and Shelf Science, 13*(3), 303-316. http://dx.doi.org/10.1016/S0302-3524(81)80028-X

Jones, H. F. E., Pilditch, C. A., Hamilton, D. P., & Bryan, K. R. (2017). Impacts of a bivalve mass mortality event on an estuarine food web and bivalve grazing pressure. *New Zealand Journal of Marine and Freshwater Research, 51*(3), 370-392. https://doi.org/10.1080/00288330.2016.1245200

Josefson, A. B., Norkko, J., & Norkko, A. (2012). Burial and decomposition of plant pigments in surface sediments of the Baltic Sea: role of oxygen and benthic fauna. *Marine Ecology Progress Series, 455*, 33-49. https://doi.org/10.3354/meps09661

Kabwe, L. K., Hendry, M. J., Wilson, G. W., & Lawrence, J. R. (2002). Quantifying CO2 fluxes from soil surfaces to the atmosphere. *Journal of Hydrology, 260*(1), 1-14. https://doi.org/10.1016/S0022-1694(01)00601-1

Kanemasu, E. T., Powers, W. l., & Sij, J. W. (1974). Field chamber measurements of CO_2 flux from soil surface. *Soil Science, 118*(4), 233-237.

Karlson, A. M. L., Nascimento, F. J. A., & Elmgren, R. (2008). Incorporation and burial of carbon from settling cyanobacterial blooms by deposit-feeding macrofauna. *Limnology and Oceanography, 53*(6), 2754-2758. https://doi.org/10.4319/lo.2008.53.6.2754

Karlson, K., Bonsdorff, E., & Rosenberg, R. (2007). The impact of benthic macrofauna for nutrient fluxes from Baltic Sea sediments. *AMBIO: A Journal of the Human Environment, 36*(2), 161-167, 167. https://doi.org/10.1579/0044-7447(2007)36[161:TIOBMF]2.0.CO;2

Kelly, R. P., Erickson, A. L., Mease, L. A., Battista, W., Kittinger, J. N., & Fujita, R. (2015). Embracing thresholds for better environmental management. *Philosophical Transactions of the Royal Society B: Biological Sciences, 370*(1659), 20130276. https://doi.org/10.1098/rstb.2013.0276

Kemp, W. M., Boynton, W. R., Adolf, J. E., Boesch, D. F., Boicourt, W. C., Brush, G., . . . Stevenson, J. C. (2005). Eutrophication of Chesapeake Bay: historical trends and ecological interactions. *Marine Ecology Progress Series, 303*, 1-29. https://doi.org/10.3354/meps303001

Kennison, R. L., & Fong, P. (2014). Extreme eutrophication in shallow estuaries and lagoons of California is driven by a unique combination of local watershed modifications that trump variability associated with wet and dry seasons. *Estuaries and Coasts, 37*(1), 164-179. https://doi.org/10.1007/s12237-013-9687-z

Kessler, A. J., Roberts, K. L., Bissett, A., & Cook, P. L. M. (2018). Biogeochemical controls on the relative importance of denitrification and dissimilatory nitrate reduction to ammonium in estuaries. *Global Biogeochemical Cycles, 32*(7), 1045-1057. http://doi.org/10.1029/2018GB005908

Kitidis, V., Laverock, B., McNeill, L. C., Beesley, A., Cummings, D., Tait, K., . . . Widdicombe, S. (2011). Impact of ocean acidification on benthic and water column ammonia oxidation. *Geophysical Research Letters, 38*. http://doi.org/10.1029/2011gl049095

Koch, M., Bowes, G., Ross, C., & Zhang, X.-H. (2013). Climate change and ocean acidification effects on seagrasses and marine macroalgae. *Global Change Biology, 19*(1), 103-132. http://doi.org/10.1111/j.1365-2486.2012.02791.x

Kohler, K. E., & Gill, S. M. (2006). Coral Point Count with Excel extensions (CPCe): A Visual Basic program for the determination of coral and substrate coverage using random point count methodolgy. *Computer Geosciences, 32*, 1259-1269.

Kohlmeier, D., Pilditch, C. A., Bornman, J. F., & Bischof, K. (2014a). Site specific differences in morphometry and photophysiology in intertidal Zostera muelleri meadows. *Aquatic Botany, 116*, 104-109. https://doi.org/10.1016/j.aquabot.2014.02.011

Kohlmeier, D., Pilditch, C. A., Bornman, J. F., & Bischof, K. (2014b). Site specific differences in morphometry and photophysiology in intertidal Zostera muelleri meadows. *Aquatic Botany, 116*(Supplement C), 104-109. https://doi.org/10.1016/j.aquabot.2014.02.011

Kohlmeier, D., Pilditch, C. A., Bornman, J. F., & Bischof, K. (2016). Adjustment of photoprotection to tidal conditions in intertidal seagrasses. *Journal of the Marine Biological Association of the United Kingdom, 97*(3), 571-579.

Körtzinger, A., Hedges, J. I., & Quay, P. D. (2001). Redfield ratios revisited: Removing the biasing effect of anthropogenic CO_2. *Limnology and Oceanography, 46*(4), 964-970. http://doi.org/10.4319/lo.2001.46.4.0964

Krause-Jensen, D., Duarte, C. M., Hendriks, I. E., Meire, L., Blicher, M. E., Marbà, N., & Sejr, M. K. (2015). Macroalgae contribute to nested mosaics of pH variability in a subarctic fjord. *Biogeosciences, 12*(16), 4895-4911. https://doi.org/10.5194/bg-12-4895-2015

Kristensen, E., & Blackburn, T. (1987). The fate of organic carbon and nitrogen in experimental marine sediment systems: Influence of bioturbation and anoxia. *Journal of Marine Research, 45*(1), 231-257.

Kristensen, E., Delefosse, M., Quintana, C. O., Flindt, M. R., & Valdemarsen, T. (2014). Influence of benthic macrofauna community shifts on ecosystem functioning in shallow estuaries. *Frontiers in Marine Science, 1*(41). https://doi.org/10.3389/fmars.2014.00041

Kristensen, E., & Kostka, J. E. (2013). Macrofaunal burrows and irrigation in marine sediment: Microbiological and biogeochemical interactions *Interactions Between Macro- and Microorganisms in Marine Sediments* (pp. 125-157): American Geophysical Union.

Kromkamp, J., Barranguet, C., & Peene, J. (1998). Determination of microphytobenthos PSII quantum efficiency and photosynthetic activity by means of variable chlorophyll fluorescence. *Marine Ecology Progress Series, 162*, 45-55.

Kwon, B.-O., Koh, C.-H., Khim, J. S., Park, J., Kang, S.-G., & Hwang, J. H. (2014). The relationship between primary production of microphytobenthos and tidal cycle on the Hwaseong mudflat, west coast of Korea. *Journal of Coastal Research*, 1188-1196. doi:10.2112/JCOASTRES-D-11-00233.1

Lapointe, B. E., Herren, L. W., Brewton, R. A., & Alderman, P. (2019). Nutrient over-enrichment and light limitation of seagrass communities in the Indian River Lagoon, an urbanized subtropical estuary. *Science of The Total Environment*, 134068. https://doi.org/10.1016/j.scitotenv.2019.134068

Larson, F., & Sundback, K. (2008). Role of microphytobenthos in recovery of functions in a shallow-water sediment system after hypoxic events. *Marine Ecology Progress Series, 357*, 1-16. https://doi.org/10.3354/meps07426

Laverock, B., Gilbert, J. A., Tait, K., Osborn, A. M., & Widdicombe, S. (2011). Bioturbation: impact on the marine nitrogen cycle. *Biochemical Society Transactions, 39*, 315-320. https://doi.org/10.1042/bst0390315

Laverock, B., Kitidis, V., Tait, K., Gilbert, J., Osborn, A., & Widdicombe, S. (2013). Bioturbation determines the response of benthic ammonia-oxidizing microorganisms to ocean acidification. *Philosophical Transactions of the Royal Society of London B: Biological Sciences, 368*(1627), 20120441. http://doi.org/10.1098/rstb.2012.0441

Lee, K.-S., Park, S. R., & Kim, Y. K. (2007). Effects of irradiance, temperature, and nutrients on growth dynamics of seagrasses: A review. *Journal of Experimental Marine Biology and Ecology, 350*(1), 144-175. https://doi.org/10.1016/j.jembe.2007.06.016

Lelieveld, S. D., Pilditch, C. A., & Green, M. O. (2003). Variation in sediment stability and relation to indicators of microbial abundance in the Okura Estuary, New Zealand. *Estuarine, Coastal and Shelf Science, 57*(1), 123-136. https://doi.org/10.1016/S0272-7714(02)00336-0

Leopold, A., Marchand, C., Deborde, J., Chaduteau, C., & Allenbach, M. (2013). Influence of mangrove zonation on CO_2 fluxes at the sediment–air interface (New Caledonia). *Geoderma, 202-203*, 62-70. https://doi.org/10.1016/j.geoderma.2013.03.008

Leuschner, C., Landwehr, S., & Mehlig, U. (1998). Limitation of carbon assimilation of intertidal Zostera noltii and Z. marina by desiccation at low tide. *Aquatic Botany, 62*(3), 171-176. 10.1016/S0304-3770(98)00091-6

Levin, L. A., Ekau, W., Gooday, A. J., Jorissen, F., Middelburg, J. J., Naqvi, S. W. A., . . . Zhang, J. (2009). Effects of natural and human-induced hypoxia on coastal benthos. *Biogeosciences, 6*(10), 2063-2098. https://doi.org/10.5194/bg-6-2063-2009

Lindegren, M., Dakos, V., Gröger, J. P., Gårdmark, A., Kornilovs, G., Otto, S. A., & Möllmann, C. (2012). Early detection of ecosystem regime shifts: A multiple method evaluation for management application. *PLoS ONE, 7*(7), e38410. https://doi.org/10.1371/journal.pone.0038410

Lohrer, A. (2017). Kaipara Harbour sediment mitigation study: Harbour benthic ecology narrative: Prepared by NIWA for Streamlined Environmental Ltd.

Lohrer, A. M., Cummings, V. J., & Thrush, S. F. (2013). Altered sea ice thickness and permanence affects benthic ecosystem functioning in coastal antarctica. *Ecosystems, 16*(2), 224 - 236. http://doi.org/10.1007/s10021-012-9610-7

Lohrer, A. M., Halliday, N. J., Thrush, S. F., Hewitt, J. E., & Rodil, I. F. (2010). Ecosystem functioning in a disturbance-recovery context: Contribution of macrofauna to primary production and nutrient release on intertidal sandflats. *Journal of Experimental Marine Biology and Ecology, 390*(1), 6-13. http://dx.doi.org/10.1016/j.jembe.2010.04.035

Lohrer, A. M., Thrush, S. F., & Gibbs, M. M. (2004). Bioturbators enhance ecosystem function through complex biogeochemical interactions. *Nature, 431*(7012), 1092-1095. https://doi.org/10.1038/nature03042

Lohrer, A. M., Thrush, S. F., Hewitt, J. E., & Kraan, C. (2015). The up-scaling of ecosystem functions in a heterogeneous world. *Scientific Reports, 5*, 10349. http://doi.org/10.1038/srep10349

Lohrer, A. M., Townsend, M., Hailes, S. F., Rodil, I. F., Cartner, K., Pratt, D. R., & Hewitt, J. E. (2016). Influence of New Zealand cockles (Austrovenus stutchburyi) on primary productivity in sandflat-seagrass (Zostera muelleri) ecotones. *Estuarine, Coastal and Shelf Science*. http://dx.doi.org/10.1016/j.ecss.2016.08.045

Longstaff, B. J., & Dennison, W. C. (1999). Seagrass survival during pulsed turbidity events: the effects of light deprivation on the seagrasses *Halodule pinifolia* and *Halophila ovalis*. *Aquatic Botany, 65*(1), 105-121. https://doi.org/10.1016/S0304-3770(99)00035-2

Loreau, M., & de Mazancourt, C. (2013). Biodiversity and ecosystem stability: A synthesis of underlying mechanisms. *Ecology Letters, 16*(s1), 106-115. https://doi.org/10.1111/ele.12073

Loreau, M., Naeem, S., Inchausti, P., Bengtsson, J., Grime, J. P., Hector, A., . . . Wardle, D. A. (2001). Biodiversity and ecosystem functioning: Current knowledge and future challenges. *Science, 294*(5543), 804-808. https://doi.org/10.1126/science.1064088

Lotze, H. K., & Milewski, I. (2004). Two centuries of multiple human impacts and successive changes in a North Atlantic food web. *Ecological Applications, 14*(5), 1428-1447. https://doi.org/10.1890/03-5027

Lundquist, C. J., Thrush, S. F., Coco, G., & Hewitt, J. E. (2010). Interactions between disturbance and dispersal reduce persistence thresholds in a benthic community. *Marine Ecology Progress Series, 413*, 217-228. https://doi.org/10.3354/meps08578

Mace, G. M., Norris, K., & Fitter, A. H. (2012). Biodiversity and ecosystem services: a multilayered relationship. *Trends in Ecology & Evolution, 27*(1), 19-26. https://doi.org/10.1016/j.tree.2011.08.006

Magni, P., Tagliapietra, D., Lardicci, C., Balthis, L., Castelli, A., Como, S., . . . Viaroli, P. (2009). Animal-sediment relationships: Evaluating the 'Pearson–Rosenberg paradigm' in Mediterranean coastal lagoons. *Marine Pollution Bulletin, 58*(4), 478-486. https://doi.org/10.1016/j.marpolbul.2008.12.009

Manassa, R. P., Smith, T. M., Beardall, J., Keough, M. J., & Cook, P. L. M. (2017). Capacity of a temperate intertidal seagrass species to tolerate changing environmental conditions: Significance of light and tidal exposure. *Ecological Indicators*. https://doi.org/10.1016/j.ecolind.2017.04.056

Mason, G. E. (2010). *Poisoning and Acidification of the Earth's Oceans* (G. E. Mason Ed.). New York: Nova Science Publishers, Inc.

Maurer, D., Robertson, G., & Gerlinger, T. (1993). San Pedro Shelf California: testing the Pearson-Rosenberg model (PRM). *Marine Environmental Research, 35*(4), 303-321. https://doi.org/10.1016/0141-1136(93)90099-L

McGill, B. J., Enquist, B. J., Weiher, E., & Westoby, M. (2006). Rebuilding community ecology from functional traits. *Trends in Ecology & Evolution, 21*(4), 178-185. https://doi.org/10.1016/j.tree.2006.02.002

Melo, A. S., Pereira, R. A. S., Santos, A. J., Shepherd, G. J., Machado, G., Medeiros, H. F., & Sawaya, R. J. (2003). Comparing species richness among assemblages using sample units: Why not use extrapolation methods to standardize different sample sizes? *Oikos, 101*(2), 398-410. https://doi.org/10.1034/j.1600-0706.2003.11893.x

Melzner, F., Thomsen, J., Koeve, W., Oschlies, A., Gutowska, M., Bange, H., . . . Körtzinger, A. (2013). Future ocean acidification will be amplified by hypoxia in coastal habitats. *Marine Biology, 160*(8), 1875-1888. http://doi.org/10.1007/s00227-012-1954-1

Middelburg, J. J., Vlug, T., Jaco, F., & van der Nat, W. A. (1993). Organic matter mineralization in marine systems. *Global and Planetary Change, 8*(1), 47-58. https://doi.org/10.1016/0921-8181(93)90062-S

Mielnick, P. C., & Dugas, W. A. (2000). Soil CO_2 flux in a tallgrass prairie. *Soil Biology and Biochemistry, 32*(2), 221-228. https://doi.org/10.1016/S0038-0717(99)00150-9

Migné, A., Davoult, D., Spilmont, N., Menu, D., Boucher, G., Gattuso, J. P., & Rybarczyk, H. (2002). A closed-chamber CO2-flux method for estimating intertidal primary production and respiration under emersed conditions. *Marine Biology, 140*(4), 865-869. https://doi.org/10.1007/s00227-001-0741-1

Migné, A., Davoult, D., Spilmont, N., Ouisse, V., & Boucher, G. (2016). Spatial and temporal variability of CO2 fluxes at the sediment–air interface in a tidal flat of a temperate lagoon (Arcachon Bay, France). *Journal of Sea Research, 109*, 13-19. http://dx.doi.org/10.1016/j.seares.2016.01.003

Migné, A., Spilmont, N., Boucher, G., Denis, L., Hubas, C., Janquin, M. A., . . . Davoult, D. (2009). Annual budget of benthic production in Mont Saint-Michel Bay considering cloudiness, microphytobenthos migration, and variability of respiration rates with tidal conditions. *Continental Shelf Research, 29*(19), 2280-2285. http://dx.doi.org/10.1016/j.csr.2009.09.004

Migné, A., Spilmont, N., & Davoult, D. (2004). In situ measurements of benthic primary production during emersion: seasonal variations and annual production in the Bay of Somme (eastern English Channel, France). *Continental Shelf Research, 24*(13–14), 1437-1449. http://dx.doi.org/10.1016/j.csr.2004.06.002

Migné, A., Trigui, R. J., Davoult, D., & Desroy, N. (2018). Benthic metabolism over the emersion - Immersion alternation in sands colonized by the invasive Manila clam Ruditapes philippinarum. *Estuarine, Coastal and Shelf Science*. https://doi.org/10.1016/j.ecss.2017.11.030

Millennium Ecosystem Assessment. (2005). Current state and trends *Washington, DC*.

Miller, D. C., Geider, R. J., & MacIntyre, H. L. (1996). Microphytobenthos: The ecological role of the "secret garden" of unvegetated, shallow-water marine habitats. II. Role in sediment stability and shallow-water food webs. *Estuaries, 19*(2), 202-212. https://doi.org/10.2307/1352225

Mitbavkar, S., & Anil, A. C. (2004). Vertical migratory rhythms of benthic diatoms in a tropical intertidal sand flat: influence of irradiance and tides. *Marine Biology, 145*(1), 9-20. https://doi.org/10.1007/s00227-004-1300-3

Mitchell, S. B., Jennerjahn, T. C., Vizzini, S., & Zhang, W. (2015). Changes to processes in estuaries and coastal waters due to intense multiple pressures – An introduction and synthesis. *Estuarine, Coastal and Shelf Science, 156*, 1-6. https://doi.org/10.1016/j.ecss.2014.12.027

Moncreiff, C. A., Sullivan, M. J., & Daehnick, A. E. (1992). Primary production dynamics in seagrass beds of Mississippi Sounds: the contributions of seagrass, epiphytic algae, sand microflora, and phytoplankton. *Marine Ecology Progress Series, 87*, 161-171.

Moore, K. A., Neckles, H. A., & Orth, R. J. (1996). Zostera marina (eelgrass) growth and survival along a gradient of nutrients and turbidity in the lower Chesapeake Bay. *Marine Ecology Progress Series, 142*(1/3), 247-259.

Mori, A. S., Furukawa, T., & Sasaki, T. (2013). Response diversity determines the resilience of ecosystems to environmental change. *Biological Reviews, 88*(2), 349-364. https://doi.org/10.1111/brv.12004

Naeem, S. (1998). Species redundancy and ecosystem reliability. *Conservation Biology, 12*(1), 39-45. https://doi.org/10.1111/j.1523-1739.1998.96379.x

Naeem, S., Thompson, L., Lawler, S., Lawton, J., & Woodfin, R. (1994). Declining biodiversity can alter the performance of ecosystems. *Nature, 368*, 734-736. https://doi.org/10.1038/368734a0

Needham, H. R., Pilditch, C. A., Lohrer, A. M., & Thrush, S. F. (2011). Context-specific bioturbation mediates changes to ecosystem functioning.

Ecosystems, 14(7), 1096 - 1109. https://doi.org/10.1007/s10021-011-9468-0

Nerem, R. S., Beckley, B. D., Fasullo, J. T., Hamlington, B. D., Masters, D., & Mitchum, G. T. (2018). Climate-change–driven accelerated sea-level rise detected in the altimeter era. *Proceedings of the National Academy of Sciences, 115*(9), 2022-2025. https://doi.org/10.1073/pnas.1717312115

Newell, R. I. E., & Koch, E. W. (2004). Modeling seagrass density and distribution in response to changes in turbidity stemming from bivalve filtration and seagrass sediment stabilization. *Estuaries, 27*(5), 793-806. https://doi.org/10.1007/bf02912041

Nixon, S. W. (1995). Coastal marine eutrophication: A definition, social causes, and future concerns. *Ophelia, 41*(1), 199-219. https://doi.org/10.1080/00785236.1995.10422044

Norkko, A., Villnäs, A., Norkko, J., Valanko, S., & Pilditch, C. (2013). Size matters: Implications of the loss of large individuals for ecosystem function. *Scientific Reports, 3*, 2646. https://doi.org/10.1038/srep02646

Norkko, J., Pilditch, C. A., Gammal, J., Rosenberg, R., Enemar, A., Magnusson, M., . . . Norkko, A. (2019). Ecosystem functioning along gradients of increasing hypoxia and changing soft-sediment community types. *Journal of Sea Research*, 101781. https://doi.org/10.1016/j.seares.2019.101781

Norling, K., Rosenberg, R., Hulth, S., Grémare, A., & Bonsdorff, E. (2007). Importance of functional biodiversity and species-specific traits of benthic fauna for ecosystem functions in marine sediment. *Marine Ecology Progress Series, 332*, 11-23. http://doi.org/10.3354/meps332011

Norman, J. M., Garcia, R., & Verma, S. B. (1992). Soil surface CO_2 fluxes and the carbon budget of a grassland. *Journal of Geophysical Research: Atmospheres, 97*(D17), 18845-18853. https://doi.org/10.1029/92jd01348

Oliver, T. H., Heard, M. S., Isaac, N. J. B., Roy, D. B., Procter, D., Eigenbrod, F., . . . Bullock, J. M. (2015). Biodiversity and resilience of ecosystem functions. *Trends in Ecology & Evolution, 30*(11), 673-684. https://doi.org/10.1016/j.tree.2015.08.009

Orr, J. C., Fabry, V. J., Aumont, O., Bopp, L., Doney, S. C., Feely, R. A., . . . Yool, A. (2005). Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature, 437*(7059), 681-686. http://doi.org/10.1038/nature04095

Otani, S., Kozuki, Y., Yamanaka, R., Sasaoka, H., Ishiyama, T., Okitsu, Y., . . . Fujiki, Y. (2010). The role of crabs (*Macrophthalmus japonicus*) burrows on organic carbon cycle in estuarine tidal flat, Japan. *Estuarine, Coastal and Shelf Science, 86*(3), 434-440. https://doi.org/10.1016/j.ecss.2009.07.033

Ouisse, V., Migne, A., & Davoult, D. (2011). Community-level carbon flux variability over a tidal cycle in *Zostera marina* and *Z. noltii* beds. *Marine Ecology Progress Series, 437*, 79-87. https://doi.org/10.3354/meps09274

Page, M., Trustrum, N., & Gomez, B. (2000). Implications of a century of anthropogenic erosion for future land use in the Gisborne-East Coast region of New Zealand. *New Zealand Geographer, 56*(2), 13-24. https://doi.org/10.1111/j.1745-7939.2000.tb01571.x

Papageorgiou, N., Sigala, K., & Karakassis, I. (2009). Changes of macrofaunal functional composition at sedimentary habitats in the vicinity of fish farms. *Estuarine, Coastal and Shelf Science, 83*(4), 561-568. https://doi.org/10.1016/j.ecss.2009.05.002

Park, S. G. (1999). Changes in the abundance of seagrass (*Zostera* spp.) in Tauranga Harbour from 1959-96. *Environment B.O.P.*(Environmental Report 99/30).

Park, S. R., Kim, S., Kim, Y. K., Kang, C.-K., & Lee, K.-S. (2016). Photoacclimatory responses of *Zostera marina* in the intertidal and subtidal zones. *PLoS ONE, 11*(5), e0156214. 10.1371/journal.pone.0156214

Parsons, D. M., Morrison, M. A., Thrush, S. F., Middleton, C., Smith, M., Spong, K. T., & Buckthought, D. (2013). The influence of habitat structure on juvenile fish in a New Zealand estuary. *Marine Ecology, 34*(4), 492-500. https://doi.org/10.1111/maec.12050

Pearson, T. H., & Rosenberg, R. (1978). Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. *Oceanogr. Mar. Biol. Ann. Rev, 16*, 229-311.

Pickett, M., & Andersson, A. (2015). Dissolution rates of biogenic carbonates in natural seawater at different pCO_2 conditions: A laboratory study. *Aquatic Geochemistry, 21*(6), 459-485. http://doi.org/10.1007/s10498-015-9261-3

Pielou, E. C. (1966). The measurement of diversity in different types of biological collections. *Journal of theoretical biology, 13*, 131-144.

Pilditch, C., A., Valanko, S., Norkko, J., & Norkko, A. (2015). Post-settlement dispersal: The neglected link in maintenance of soft-sediment biodiversity. *Biology Letters, 11*(2), 20140795. http://doi.org/10.1098/rsbl.2014.0795

Pinckney, J., & Zingmark, R. G. (1991). Effects of tidal stage and sun angles on intertidal benthic microalgal productivity. *Marine Ecology Progress Series, 76*(1), 81-89.

Polis, G. (1998). Stability is woven by complex webs. *Nature, 395*, 744-745. https://doi.org/10.1038/27323

Pontee, N. (2013). Defining coastal squeeze: A discussion. *Ocean & Coastal Management, 84*, 204-207. https://doi.org/10.1016/j.ocecoaman.2013.07.010

Pratt, D. R., Lohrer, A. M., Pilditch, C. A., & Thrush, S. F. (2014a). Changes in ecosystem function across sedimentary gradients in estuaries. *Ecosystems, 17*(1), 182-194. https://doi.org/10.1007/s10021-013-9716-6

Pratt, D. R., Pilditch, C. A., Lohrer, A. M., & Thrush, S. F. (2014b). The effects of short-term increases in turbidity on sandflat microphytobenthic productivity and nutrient fluxes. *Journal of Sea Research, 92*, 170-177. https://doi.org/10.1016/j.seares.2013.07.009

Pridmore, R. D., Thrush, S. F., Hewitt, J. E., & Roper, D. S. (1990). Macrobenthic community composition of six intertidal sandflats in Manukau Harbour, New Zealand. *New Zealand Journal of Marine and Freshwater Research, 24*(1), 81-96. https://doi.org/10.1080/00288330.1990.9516404

Probert, P. K. (1984). Disturbance, sediment stability, and trophic structure of soft-bottom communities. *Journal of Marine Research, 42*(4), 893-921. https://doi.org/10.1357/002224084788520837

Pumpanen, J., Kolari, P., Ilvesniemi, H., Minkkinen, K., Vesala, T., Niinistö, S., . . . Hari, P. (2004). Comparison of different chamber techniques for measuring soil CO2 efflux. *Agricultural and Forest Meteorology, 123*(3), 159-176. https://doi.org/10.1016/j.agrformet.2003.12.001

Queirós, A. M., Birchenough, S. N. R., Bremner, J., Godbold, J. A., Parker, R. E., Romero-Ramirez, A., . . . Widdicombe, S. (2013). A bioturbation classification of European marine infaunal invertebrates. *Ecology and Evolution, 3*(11), 3958-3985. https://doi.org/10.1002/ece3.769

Quillien, N., Nordström, M. C., Le Bris, H., Bonsdorff, E., & Grall, J. (2017). Green tides on inter- and subtidal sandy shores: Differential impacts on infauna and flatfish. *Journal of the Marine Biological Association of the United Kingdom, 98*(4), 699-712. https://doi.org/10.1017/S0025315416002010

Rao, A. M. F., Malkin, S. Y., Montserrat, F., & Meysman, F. J. R. (2014). Alkalinity production in intertidal sands intensified by lugworm bioirrigation. *Estuarine Coastal and Shelf Science, 148*, 36-47. http://doi.org/10.1016/j.ecss.2014.06.006

Ray, A., & Svejcar, T. (1999). A chamber design for measuring net CO_2 exchange on rangeland. *Journal of Range Management, 52*(1), 27-31. 10.2307/4003488

Ricart, A. M., York, P. H., Rasheed, M. A., Pérez, M., Romero, J., Bryant, C. V., & Macreadie, P. I. (2015). Variability of sedimentary organic carbon in patchy seagrass landscapes. *Marine Pollution Bulletin, 100*(1), 476-482. https://doi.org/10.1016/j.marpolbul.2015.09.032

Riera, R., Sacramento, A., Perez, Ó., Monterroso, Ó., Ramos, E., Rodríguez, M., & Almansa, E. (2013). Effects of organic enrichment on macrofauna community structure: An experimental approach. *Brazilian Journal of Oceanography, 61*(4), 223-229. http://dx.doi.org/10.1590/S1679-87592013000400003

Ringwood, A. H., & Keppler, C. J. (2002). Water quality variation and clam growth: Is pH really a non-issue in estuaries? *Estuaries, 25*(5), 901-907. http://doi.org/10.1007/bf02691338

Rodil, I. F., Lohrer, A. M., Chiaroni, L. D., Hewitt, J. E., & Thrush, S. F. (2011). Disturbance of sandflats by thin terrigenous sediment deposits: consequences for primary production and nutrient cycling. *Ecological Applications, 21*(2), 416-426. https://doi.org/10.1890/09-1845.1

Rodil, I. F., Lohrer, A. M., & Thrush, S. F. (2013). Sensitivity of heterogeneous marine benthic habitats to subtle stressors. *PLoS ONE, 8*(11), 1-10. http://doi.org/10.1371/journal.pone.0081646

Rossi, F., Gribsholt, B., Middelburg, J. J., & Heip, C. (2008). Context-dependent effects of suspension feeding on intertidal ecosystem functioning. *Marine Ecology Progress Series, 354*, 47-57. https://doi.org/10.3354/meps07213

Ruiz, J. M., & Romero, J. (2001). Effects of in situ experimental shading on the Mediterranean seagrass Posidonia oceanica. *Marine Ecology Progress Series, 215*, 107-120.

Ruiz, J. M., & Romero, J. (2003). Effects of disturbances caused by coastal constructions on spatial structure, growth dynamics and photosynthesis of the seagrass *Posidonia oceanica*. *Marine Pollution Bulletin, 46*(12), 1523-1533. https://doi.org/10.1016/j.marpolbul.2003.08.021

Sanderson, E. W., Jaiteh, M., Levy, M. A., Redford, K. H., Wannebo, A. V., & Woolmer, G. (2002). The Human Footprint and the Last of the Wild: The human footprint is a global map of human influence on the land surface, which suggests that human beings are stewards of nature, whether we like it or not. *Bioscience, 52*(10), 891-904. https://doi.org/10.1641/0006-3568(2002)052[0891:thfatl]2.0.co;2

Sandwell, D. R., Pilditch, C. A., & Lohrer, A. M. (2009). Density dependent effects of an infaunal suspension-feeding bivalve (*Austrovenus stutchburyi*) on sandflat nutrient fluxes and microphytobenthic productivity. *Journal of Experimental Marine Biology and Ecology, 373*(1), 16-25. http://dx.doi.org/10.1016/j.jembe.2009.02.015

Sanz-Lázaro, C., & Marín, A. (2011). Diversity patterns of benthic macrofauna caused by marine fish farming. *Diversity, 3*(2). https://doi.org/10.3390/d3020176

Scheffer, M., Barrett, S., Carpenter, S. R., Folke, C., Green, A. J., Holmgren, M., . . . Walker, B. (2015). Creating a safe operating space for iconic ecosystems. *Science, 347*(6228), 1317-1319. https://doi.org/10.1126/science.aaa3769

Scheffer, M., Carpenter, S., Foley, J. A., Folke, C., & Walker, B. (2001). Catastrophic shifts in ecosystems. *Nature, 413*(6856), 591-596.

Scheffer, M., & Carpenter, S. R. (2003). Catastrophic regime shifts in ecosystems: linking theory to observation. *Trends in Ecology & Evolution, 18*(12), 648-656. https://doi.org/10.1016/j.tree.2003.09.002

Schlacher, T. A., & Wooldridge, T. H. (1996). How sieve mesh size affects sample estimates of estuarine benthic macrofauna. *Journal of Experimental Marine Biology and Ecology*, 201(1), 159-171. https://doi.org/10.1016/0022-0981(95)00198-0

Schwarz, A.-M. (2004). Contribution of photosynthetic gains during tidal emersion to production of Zostera capricorni in a North Island, New Zealand estuary. *New Zealand Journal of Marine and Freshwater Research, 38*(5), 809-818. 10.1080/00288330.2004.9517280

Seers, B. M., & Shears, N. T. (2015). Spatio-temporal patterns in coastal turbidity – Long-term trends and drivers of variation across an estuarine-open coast gradient. *Estuarine, Coastal and Shelf Science, 154*(Supplement C), 137-151. https://doi.org/10.1016/j.ecss.2014.12.018

Seitzinger, S. P. (1988). Denitrification in freshwater and coastal marine ecosystems: Ecological and geochemical significance. *Limnology and Oceanography, 33*(4part2), 702-724. 10.4319/lo.1988.33.4part2.0702

Seitzinger, S. P., & Nixon, S. W. (1985). Eutrophication and the rate of denitrification and N_2O production in coastal marine sediments. *Limnology and Oceanography, 30*(6), 1332-1339. http://doi.org/10.4319/lo.1985.30.6.1332

Selkoe, K. A., Blenckner, T., Caldwell, M. R., Crowder, L. B., Erickson, A. L., Essington, T. E., . . . Zedler, J. (2015). Principles for managing marine ecosystems prone to tipping points. *Ecosystem Health and Sustainability, 1*(5), 1-18. 10.1890/ehs14-0024.1

Shucksmith, R., Cook, E. J., Hughes, D. J., & Burrows, M. T. (2009). Competition between the non-native amphipod *Caprella mutica* and two native species of caprellids *Pseudoprotella phasma* and *Caprella linearis*. *Journal of the Marine Biological Association of the United Kingdom, 89*(6), 1125-1132. https://doi.org/10.1017/S0025315409000435

Silva, J., Santos, R., Calleja, M. L., & Duarte, C. M. (2005). Submerged versus air-exposed intertidal macrophyte productivity: From physiological to community-level assessments. *Journal of Experimental Marine Biology and Ecology, 317*(1), 87-95. 10.1016/j.jembe.2004.11.010

Smith, M. D., & Knapp, A. K. (2003). Dominant species maintain ecosystem function with non-random species loss. *Ecology Letters, 6*(6), 509-517. https://doi.org/10.1046/j.1461-0248.2003.00454.x

Snelgrove, P. V., Thrush , S. F., Wall, D. H., & Norkko, A. (2014). Real-world biodiversity-ecosystem functioning: A seafloor perspective. *Trends in Ecology & Evolution, 29*(7), 398 - 405. https://doi.org/10.1016/j.tree.2014.05.002

Solan, M., Cardinale, B. J., Downing, A. L., Engelhardt, K. A. M., Ruesink, J. L., & Srivastava, D. S. (2004). Extinction and ecosystem function in the marine benthos. *Science, 306*(5699), 1177-1180. http://doi.org/10.1126/science.1103960

Soluk, D. A. (1993). Multiple predator effects: Predicting combined functional response of stream fish and invertebrate predators. *Ecology, 74*(1), 219-225. https://doi.org/10.2307/1939516

Spilmont, N., Davoult, D., & Migné, A. (2006). Benthic primary production during emersion: In situ measurements and potential primary production in the Seine Estuary (English Channel, France). *Marine Pollution Bulletin, 53*(1–4), 49-55. http://dx.doi.org/10.1016/j.marpolbul.2005.09.016

Spilmont, N., Migné, A., Seuront, L., & Davoult, D. (2007). Short-term variability of intertidal benthic community production during emersion and the implication in annual budget calculation. *Marine Ecology Progress Series, 333*, 95-101.

Spivak, A. C., Sanderman, J., Bowen, J. L., Canuel, E. A., & Hopkinson, C. S. (2019). Global-change controls on soil-carbon accumulation and loss in coastal vegetated ecosystems. *Nature Geoscience, 12*(9), 685-692. https://doi.org/10.1038/s41561-019-0435-2

Stavert, J. R., Pattemore, D. E., Gaskett, A. C., Beggs, J. R., & Bartomeus, I. (2017). Exotic species enhance response diversity to land-use change but modify functional composition. *Proceedings of the Royal Society B: Biological Sciences, 284*(1860), 20170788. https://doi.org/10.1098/rspb.2017.0788

Steenweg, R., Hebblewhite, M., Whittington, J., Lukacs, P., & McKelvey, K. (2018). Sampling scales define occupancy and underlying occupancy–abundance relationships in animals. *Ecology, 99*(1), 172-183. https://doi.org/10.1002/ecy.2054

Stephenson, F., Bulmer, R. H., Meredyth-Young, M., Meysick, L., Hewitt, J. E., & Lundquist, C. J. (2019). Effects of benthic protection extent on recovery dynamics of a conceptual seafloor community. *Frontiers in Marine Science, 6*(607). https://doi.org/10.3389/fmars.2019.00607

Stoms, D. M., Davis, F. W., Andelman, S. J., Carr, M. H., Gaines, S. D., Halpern, B. S., . . . Warner, R. R. (2005). Integrated coastal reserve planning: Making the land–sea connection. *Frontiers in Ecology and the Environment, 3*(8), 429-436. https://doi.org/10.1890/1540-9295(2005)003[0429:icrpmt]2.0.co;2

Streever, W. J., Genders, A. J., & A. Cole, M. (1998). A closed chamber CO2 flux method for estimating marsh productivity. *Aquatic Botany, 62*(1), 33-44. https://doi.org/10.1016/S0304-3770(98)00080-1

Stumm, W., & Morgan, J. J. (2012). *Aquatic Chemistry: Chemical Equilibria and Rates in Natural Waters* (Vol. 126): John Wiley & Sons.

Sullivan, B. K., Doering, P. H., Oviatt, C. A., Keller, A. A., & Frithsen, J. B. (1991). Interactions with the benthos alter pelagic food web structure in coastal waters. *Canadian Journal of Fisheries and Aquatic Sciences, 48*(11), 2276-2284. https://doi.org/10.1139/f91-267

Sulpis, O., Boudreau, B. P., Mucci, A., Jenkins, C., Trossman, D. S., Arbic, B. K., & Key, R. M. (2018). Current $CaCO_3$ dissolution at the seafloor caused by anthropogenic CO_2. *Proceedings of the National Academy of Sciences, 115*(46), 11700-11705. http://doi.org/10.1073/pnas.1804250115

Sunda, W. G., & Cai, W.-J. (2012). Eutrophication induced CO_2-acidification of subsurface coastal waters: Interactive effects of temperature, salinity, and atmospheric pCO_2. *Environmental Science & Technology, 46*(19), 10651-10659. http://doi.org/10.1021/es300626f

Sundback, K., Miles, A., & Goransson, E. (2000). Nitrogen fluxes, denitrification and the role of microphytobenthos in microtidal shallow-water sediments: an annual study. *Marine Ecology Progress Series, 200*, 59-76. https://doi.org/10.3354/meps200059

Suzuki, I., Dular, U., & Kwok, S. C. (1974). Ammonia or ammonium ion as substrate for oxidation by *Nitrosomonas europaea* cells and extracts. *Journal of Bacteriology, 120*(1), 556-558.

Swales, A., Williamson, R. B., Van Dam, L. F., Stroud, M. J., & McGlone, M. S. (2002). Reconstruction of urban stormwater contamination of an estuary using catchment history and sediment profile dating. *Estuaries, 25*(1), 43-56. https://doi.org/10.1007/BF02696048

Tait, L. W., & Schiel, D. R. (2010). Primary productivity of intertidal macroalgal assemblages: comparison of laboratory and in situ photorespirometry. *Marine Ecology Progress Series, 416*, 115-125. https://doi.org/10.3354/meps08781

Talke, S. A., & Stacey, M. T. (2008). Suspended sediment fluxes at an intertidal flat: The shifting influence of wave, wind, tidal, and freshwater forcing. *Continental Shelf Research, 28*(6), 710-725. https://doi.org/10.1016/j.csr.2007.12.003

Teichberg, M., Fox, S. E., Olsen, Y. S., Valiela, I., Martinetto, P., Iribarne, O., . . . Tagliapietra, D. (2010). Eutrophication and macroalgal blooms in temperate and tropical coastal waters: nutrient enrichment experiments with Ulva spp. *Global Change Biology, 16*(9), 2624-2637. http://doi.org/10.1111/j.1365-2486.2009.02108.x

Thistle, D. (1981). Natural physical disturbances and communities of marine soft bottoms. *Marine Ecology Progress Series, 6*(2), 223-228.

Thomsen, J., Haynert, K., Wegner, K. M., & Melzner, F. (2015). Impact of seawater carbonate chemistry on the calcification of marine bivalves. *Biogeosciences, 12*(14), 4209-4220. http://doi.org/10.5194/bg-12-4209-2015

Thrush, S., Pridmore, R., Bell, R., Cummings, V., Dayton, P., Ford, R., . . . Hines, A. H. (1997). Sandflat habitat: Scaling from experiments to conclusions. *Journal of Experimental Marine Biology and Ecology*.

Thrush, S. F., Hewitt, J. E., Cummings, V. J., Ellis, J. I., Hatton, C., Lohrer, A., & Norkko, A. (2004). Muddy waters: elevating sediment input to coastal and estuarine habitats. *Frontiers in Ecology and the Environment, 2*(6), 299-306. https://doi.org/10.1890/1540-9295(2004)002[0299:MWESIT]2.0.CO;2

Thrush, S. F., Hewitt, J. E., Gibbs, M., Lundquist, C., & Norkko, A. (2006). Functional role of large organisms in intertidal communities: Community effects and ecosystem function. *Ecosystems, 9*(6), 1029-1040. http://doi.org/10.1007/s10021-005-0068-8

Thrush, S. F., Hewitt, J. E., Hickey, C. W., & Kelly, S. (2008). Multiple stressor effects identified from species abundance distributions: Interactions between urban contaminants and species habitat relationships. *Journal of Experimental Marine Biology and Ecology, 366*(1), 160-168. https://doi.org/10.1016/j.jembe.2008.07.020

Thrush, S. F., Hewitt, J. E., Kraan, C., Lohrer, A. M., Pilditch, C. A., & Douglas, E. (2017). Changes in the location of biodiversity–ecosystem function hot spots across the seafloor landscape with increasing sediment nutrient loading. *Proceedings of the Royal Society B: Biological Sciences, 284*(1852). http://doi.org/10.1098/rspb.2016.2861

Thrush, S. F., Hewitt, J. E., & Lohrer, A. M. (2012). Interaction networks in coastal soft-sediments highlight the potential for change in ecological resilience. *Ecological Applications, 22*(4), 1213-1223. 10.1890/11-1403.1

Thrush, S. F., Hewitt, J. E., Norkko, A., Nicholls, P. E., Funnell, G. A., & Ellis, J. I. (2003). Habitat change in estuaries: predicting broad-scale responses of intertidal macrofauna to sediment mud content. *Marine Ecology Progress Series, 263*, 101-112. https://doi.org/10.3354/meps263101

Thrush, S. F., Hewitt, J. E., Parkes, S., Lohrer, A. M., Pilditch, C., Woodin, S. A., . . . van Colen, C. (2014). Experimenting with ecosystem interaction networks in search of threshold potentials in real-world marine ecosystems. *Ecological Society of America, 95*(6), 1451-1457.

Thrush , S. F., Whitlatch, R. B., Pridmore, R. D., Hewitt, J. E., Cummings, V. J., & Wilkinson, M. R. (1996). Scale‐dependent recolonization: The role of sediment stability in a dynamic sandflat habitat. *Ecology, 77*(8), 2472-2487. https://doi.org/10.2307/2265747

Tilman, D., Reich, P. B., & Knops, J. M. H. (2006). Biodiversity and ecosystem stability in a decade-long grassland experiment. *Nature, 441*(7093), 629-632. https://doi.org/10.1038/nature04742

Turner, S., & Schwarz, A.-M. (2006a). Management and conservation of seagrass in New Zealand: An introduction. *Science for Conservation, 264*, 1-90.

Turner, S. J., & Schwarz, A. M. (2006b). Biomass development and photosynthetic potential of intertidal *Zostera capricorni* in New Zealand estuaries. *Aquatic Botany, 85*(1), 53-64. https://doi.org/10.1016/j.aquabot.2005.12.006

Turner, S. J., Thrush, S. F., Hewitt, J. E., Cummings, V. J., & Funnell, G. (1999). Fishing impacts and the degradation or loss of habitat structure. *Fisheries Management and Ecology, 6*(5), 401-420. https://doi.org/10.1046/j.1365-2400.1999.00167.x

Underwood, A. J. (1997). Experiments in ecology: their logical design and interpretation using analysis of variance: Cambridge University Press.

Underwood, G. J. C., & Krompkamp, J. (1999). Primary production by phytoplankton and microphytobenthos in estuaries. In D. B. Nedwell & D. G. Raffaelli (Eds.), *Estuaries* (Vol. 29, pp. 93-). Great Britain: Academic Press.

Vahtera, E., Conley, D. J., Gustafsson, B. G., Kuosa, H., Pitkänen, H., Savchuk, O. P., . . . Wulff, F. (2007). Internal ecosystem feedbacks enhance nitrogen-fixing cyanobacteria blooms and complicate management in the Baltic Sea. *AMBIO: A Journal of the Human Environment, 36*(2), 186-194, 189. https://doi.org/10.1579/0044-7447(2007)36[186:IEFENC]2.0.CO;2

Valdemarsen, T., Kristensen, E., & Holmer, M. (2010). Sulfur, carbon, and nitrogen cycling in faunated marine sediments impacted by repeated organic enrichment. *Marine Ecology Progress Series, 400*, 37-53. http://doi.org/10.3354/meps08400

Valone, T. J., & Barber, N. A. (2008). An empirical evaluation of the insurance hypothesis in diversity–stability models. *Ecology, 89*(2), 522-531. https://doi.org/10.1890/07-0153.1

Van Colen, C., Rossi, F., Montserrat, F., Andersson, M. G. I., Gribsholt, B., Herman, P. M. J., . . . Middelburg, J. J. (2012). Organism-sediment interactions govern post-hypoxia recovery of ecosystem functioning. *PLoS ONE, 7*(11), e49795. https://doi.org/10.1371/journal.pone.0049795

Vermaat, J. E., Agawin, N. S. R., Fortes, M. D., Uri, J., Duarte, C. M., Marbà, N., . . . Van Vierssen, W. (1997). The capacity of seagrasses to survive increased turbidity and siltation: The significance of growth form and light use. *Ambio, 26*(8), 499 - 504.

Vermaat, J. E., & Verhagen, F. C. A. (1996). Seasonal variation in the intertidal seagrass Zostera noltii Hornem.: coupling demographic and physiological patterns. *Aquatic Botany, 52*(4), 259-281. https://doi.org/10.1016/0304-3770(95)00510-2

Villnäs, A., Norkko, J., Lukkari, K., Hewitt, J., & Norkko, A. (2012). Consequences of increasing hypoxic disturbance on benthic communities and ecosystem functioning. *PLoS ONE, 7*(10), e44920. https://doi.org/10.1371/journal.pone.0044920

Villnäs, A., Perus, J., & Bonsdorff, E. (2011). Structural and functional shifts in zoobenthos induced by organic enrichment — Implications for community recovery potential. *Journal of Sea Research, 65*(1), 8-18. https://doi.org/10.1016/j.seares.2010.06.004

Virnstein, R. W., Mikkelsen, P. S., Cairns, K. D., & Capone, M. A. (1983). Seagrass beds versus sand bottoms: The trophic importance of their associated benthic invertebrates. *Florida Scientist, 46*(3/4), 363-381.

Vizzini, S., Di Leonardo, R., Costa, V., Tramati, C. D., Luzzu, F., & Mazzola, A. (2013). Trace element bias in the use of CO_2 vents as analogues for low pH environments: Implications for contamination levels in acidified oceans. *Estuarine, Coastal and Shelf Science, 134*, 19-30. https://doi.org/10.1016/j.ecss.2013.09.015

Vopel, K., Del-Río, C., & Pilditch, C. A. (2018). Effects of CO_2 enrichment on benthic primary production and inorganic nitrogen fluxes in two coastal sediments. *Scientific Reports, 8*(1), 1035. http://doi.org/10.1038/s41598-017-19051-w

Wahl, M., Saderne, V., & Sawall, Y. (2015). How good are we at assessing the impact of ocean acidification in coastal systems? Limitations, omissions and strengths of commonly used experimental approaches with special emphasis on the neglected role of fluctuations. *Marine and Freshwater Research, 67*(1), 25-36. http://doi.org/10.1071/MF14154

Walker, B., Kinzig, A., & Langridge, J. (1999). Original Articles: Plant attribute diversity, resilience, and ecosystem function: The nature and significance of dominant and minor species. *Ecosystems, 2*(2), 95-113. https://doi.org/10.1007/s100219900062

Wallace, R. B., Baumann, H., Grear, J. S., Aller, R. C., & Gobler, C. J. (2014). Coastal ocean acidification: The other eutrophication problem. *Estuarine, Coastal and Shelf Science, 148*, 1-13. http://dx.doi.org/10.1016/j.ecss.2014.05.027

Walter, L. M., & Burton, E. A. (1990). Dissolution of recent platform carbonate sediments in marine pore fluids. *American Journal of Science, 290*(6), 601 - 643.

Wang, Q., Li, Y., & Wang, Y. (2011). Optimizing the weight loss-on-ignition methodology to quantify organic and carbonate carbon of sediments from diverse sources. *Environ Monit Assess, 174*(1-4), 241-257. http://doi.org/10.1007/s10661-010-1454-z

Waycott, M., Duarte, C. M., Carruthers, T. J. B., Orth, R. J., Dennison, W. C., Olyarnik, S., . . . Williams, S. L. (2009). Accelerating loss of seagrasses across the globe threatens coastal ecosystems. *Proceedings of the National Academy of Sciences, 106*(30), 12377-12381. 10.1073/pnas.0905620106

Webb, A. P., & Eyre, B. D. (2004). Effect of natural populations of burrowing Thalassinidean shrimp on sediment irrigation, benthic metabolism, nutrient fluxes and denitrification. *Marine Ecology Progress Series, 268*, 205-220. https://doi.org/10.3354/meps268205

Welsh, D. T. (2003). It's a dirty job but someone has to do it: The role of marine benthic macrofauna in organic matter turnover and nutrient recycling to the water column. *Chemistry and Ecology, 19*(5), 321-342. https://doi.org/10.1080/0275754031000155474

Weston, D. P. (1990). Quantitative examination of macrobenthic community changes along an organic enrichment gradient. *Marine Ecology Progress Series, 61*(3), 233-244.

Widdicombe, S., Beesley, A., Berge, J. A., Dashfield, S. L., McNeill, C. L., Needham, H. R., & Oxnevad, S. (2013). Impact of elevated levels of CO_2 on animal mediated ecosystem function: The modification of sediment nutrient fluxes by burrowing urchins. *Marine Pollution Bulletin, 73*(2), 416-427. http://doi.org/10.1016/j.marpolbul.2012.11.008

Widdicombe, S., Dashfield, S. L., McNeill, C. L., Needham, H. R., Beesley, A., McEvoy, A., . . . Berge, J. A. (2009). Effects of CO2 induced seawater acidification on infaunal diversity and sediment nutrient fluxes. *Marine Ecology Progress Series, 379*, 59-75. 10.3354/meps07894

Winfree, R., & Kremen, C. (2009). Are ecosystem services stabilized by differences among species? A test using crop pollination. *Proceedings of the Royal Society B: Biological Sciences, 276*(1655), 229-237. https://doi.org/10.1098/rspb.2008.0709

Woodin, S. A., Volkenborn, N., Pilditch, C. A., Lohrer, A. M., Wethey, D. S., Hewitt, J. E., & Thrush, S. F. (2016). Same pattern, different mechanism: Locking onto the role of key species in seafloor ecosystem process. *Scientific Reports, 6*, 26678. http://doi.org/10.1038/srep26678

Wootton, J. T., Pfister, C. A., & Forester, J. D. (2008). Dynamic patterns and ecological impacts of declining ocean pH in a high-resolution multi-year dataset. *Proceedings of the National Academy of Sciences of the United States of America, 105*(48), 18848-18853. http://doi.org/10.1073/pnas.0810079105

Worm, B., Barbier, E. B., Beaumont, N., Duffy, J. E., Folke, C., Halpern, B. S., . . . Watson, R. (2006). Impacts of biodiversity loss on ocean ecosystem services. *Science, 314*(5800), 787-790. https://doi.org/10.1126/science.1132294

Yachi, S., & Loreau, M. (1999). Biodiversity and ecosystem productivity in a fluctuating environment: The insurance hypothesis. *Proceedings of the National Academy of Sciences, 96*(4), 1463-1468. https://doi.org/10.1073/pnas.96.4.1463

Yallop, M. L., de Winder, B., Paterson, D. M., & Stal, L. J. (1994). Comparative structure, primary production and biogenic stabilization of cohesive and non-cohesive marine sediments inhabited by microphytobenthos. *Estuarine, Coastal and Shelf Science, 39*(6), 565-582. https://doi.org/10.1016/S0272-7714(06)80010-7

Ysebaert, T., Meire, P., Herman, P. M. J., & Verbeek, H. (2002). Macrobenthic species response surfaces along estuarine gradients: prediction by logistic regression. *Marine Ecology Progress Series, 225*, 79-95. https://doi.org/10.3354/meps225079

Zuur, A. F., Ieno, E. N., Walker, N. J., Saveliev, A. A., & Smith, G. M. (2009). GLM and GAM for Count Data *Mixed effects models and extensions in ecology with R* (pp. 209-243). New York, NY: Springer New York.

APPENDICES

Appendix 1: A method for the experimental measurement of CO_2 flux in emerged intertidal habitats

Introduction

Water-column turbidity prevents light from reaching benthic autotrophs, thus reducing benthic primary production (PP) during submergence (Pratt *et al.*, 2014b). In Chapter 2, I wanted to determine the proportion of total benthic PP that was contributed by periods of emergence in intertidal habitats to investigate their resilience to increasing site turbidity. An effective way to determine rates of PP is to measure the flux of photosynthetic (O_2) or aerobic respiration (CO_2) products across the sediment surface (Hopkinson & Wetzel, 1982; Dalsgaard, 2003). Release of O_2 from sediments or uptake of CO_2 in the presence of light indicates photosynthesis and is a measure of net PP (NPP), as photosynthesis and community respiration occur in unison. Uptake of O_2 or release of CO_2 by sediments under darkness indicates community metabolism (or sediment oxygen consumption), as photosynthesis is blocked. Combining light and dark fluxes provides an estimate of gross PP (GPP). Whilst closed-chamber methods for measuring submerged fluxes of dissolved O_2 are well-developed and tested (e.g. Gladstone-Gallagher *et al.* (2016), Lohrer *et al.* (2013), Needham *et al.* (2011) and Tait and Schiel (2010)), there has been a relatively minor focus on comparable methods for measurement during emergence (Streever *et al.*, 1998; Migné *et al.*, 2002; Bulmer *et al.*, 2015b). This has resulted in a large knowledge gap regarding the functioning of intertidal environments during emergence.

Developing generic guidelines for measuring emerged gaseous fluxes in intertidal environments is complicated by the variety of habitats that occur there (e.g. sand, mud, seagrass, saltmarsh) and the fluctuating nature of sediment water content, which varies with time since emergence (due to tidal processes and evaporation) and weather (e.g. rainfall). These characteristics also mean that protocols developed for terrestrial soils (for example Kanemasu *et al.* (1974) and Pumpanen *et al.* (2004)) cannot simply be applied to marine sediments. In all environments the key physical principles that influence gaseous fluxes are associated with diffusion and mass flow: diffusion is controlled by the concentration gradient of a given gas, and mass flow by the pressure fluctuations at the sediment surface (Geankoplis, 2003). It is important that chamber designs and field protocols do not unintentionally disrupt these processes to ensure accurate and precise measurements.

To date, only Migné *et al.* (2002) have published guidelines on how to effectively measure fluxes of CO_2 in emerged intertidal environments (see Table A1.1 for a review of their study). Their aim was to illustrate whether a dynamic closed-chamber methodology could be used to measure net primary production and respiration of emerged estuarine sediments, a method which was already popular in terrestrial field studies (Norman *et al.*, 1992; Ray & Svejcar, 1999; Mielnick & Dugas, 2000). They succeeded in demonstrating the efficacy of benthic chamber incubations for measuring stable CO_2 fluxes, however only unvegetated sediments were addressed.

Table A1.1 Summary of study by Migne *et al.* (2002) that developed methods for measuring intertidal CO_2 flux during emergence.

Chamber specifications & incubation protocol		
Closed-chamber CO_2 flux system using infrared gas analyser. Whole community flux (sediment, phyto- and zoobenthos combined).	Circular steel base with 40 cm dia. (0.126 m^2 surface area) and domed acrylic lid. Volume of 25 L when inserted 10 cm into sediment.	20–30 min incubation duration.
Trials		
Habitats: - exposed sandy beach, low Chl *a* (2.5 mg m^{-2}) and macrofauna (<1 g AFDW m^{-2}) biomass - estuarine muddy sand, high Chl *a* (200 mg m^{-2}) and macrofauna (~10 g AFDW m^{-2}) biomass	1 trial conducted at exposed beach location. 3 trials conducted at estuarine location.	Each estuarine trial was conducted at different time of day: 10:00–10:25 am 11:15–11:45 am 8:00–8:25 pm
Key results		
Significant differences in respiration and gross community production were detected between habitats.	Longer stabilisation periods were required during dark incubations than light, assumed to arise from physiological processes in the sediment.	CO_2 fluxes differed significantly between each estuarine trial due to differences in light availability.

Although there are now several examples of these closed-chamber methods being implemented *in situ* in intertidal habitats (see Leopold *et al.* (2013), Ouisse *et al.* (2011) and Otani *et al.* (2010) for examples), guidelines for their use in vegetated sediments are still missing from the literature. Additionally, considerations around how these methods should be incorporated into sound experimental designs are absent, limiting their applicability. For example, the number of replicate flux measurements needed to detect differences between habitats is unknown, and recommendations for sampling designs when simultaneous measurements are not possible (due to equipment limitations) have not been described. The ability to increase the spatial scale of flux measurements is also of importance to benthic ecologists, as this allows more of the spatial heterogeneity characteristic of intertidal sediments to be incorporated into community flux measurements (Blanchard & Bourget, 1999; Hewitt *et al.*, 2007). Therefore, to achieve the objectives of Chapter 2, answers to the following outstanding questions were sought:

1. Are different measurement protocols needed for vegetated versus unvegetated habitats?
2. Can the principles underlying small-scale measurements be applied at larger scales?
3. What level of replication is required to detect differences between habitats, and how can this be optimised for experimental designs?
4. How should sampling across treatments be conducted when simultaneous measurements are not possible?

Methods

The LI-COR LI-8100A (Nebraska, USA) is a gas analyser that measures CO_2 fluxes using infrared gas analysis. The instrument detects the ppm of CO_2 in a continuous air sample circulated from an attached LI-COR Survey Chamber designed for use in terrestrial environments. Here, the LI-COR LI-8100A infrared gas analyser (IRGA) was connected to custom-built chambers designed for measurement of CO_2 fluxes in emerged intertidal habitats.

Chamber design & materials

The custom chambers were initially modelled on the existing 10 cm dia. LI-COR chamber so comparison between the two could confirm whether the custom chamber design was effective, thus validating its use in developing measurement and experimental protocols. Clear acrylic (0.5 cm thickness) was used to construct two cylindrical chambers (14 cm dia., 20 cm height) with flat, square lids (22 x 22 cm) (Figure A1.1). Cylindrical chambers were used to minimise the obstruction of air flow by sharp corners which may lead to the formation of dead-air spaces and affect gas diffusion (Holmes & Mahall, 1982; Eklund, 1992). The bottom edges of the chambers were bevelled to allow easy and smooth insertion into the sediment. Simple thermocouples (constructed by twisting together a short length of type K wire (NiCr-Ni)) were attached to the centre (inside the chamber) and outer edge of each lid to measure air temperature and connected to the IRGA auxiliary block. An air-in and air-out port were located on opposing sides of the thermocouple, constructed from rigid 0.3 cm dia. nylon tubing. Air-tight push-in connectors were attached and secured to the end of each port for attachment to the IRGA via a further length of nylon tubing. All fixtures and ports were secured with waterproof sealant and one chamber was completely covered with black electrical tape to create a complementary transparent and opaque pair.

After initial field tests and protocol development, it was decided larger chambers (the same as are commonly used by the University of Waikato marine ecology group for measuring submerged solute fluxes) should be developed to allow the direct comparison of PP during submergence and emergence. These chambers consist of a square aluminium base (50 cm L x 50 cm W x 15 cm H) with a clear acrylic domed lid and encapsulate approximately 41 L when inserted 5 cm into the sediment (Figure A1.2). The same modifications were made to the lid as for the smaller chambers, and the only fundamental difference when operating these larger chambers was the insertion of a small computer fan to promote the gentle mixing of air. A comparison between the small and large custom chamber was performed under dark conditions to check whether the modification affected CO_2 flux.

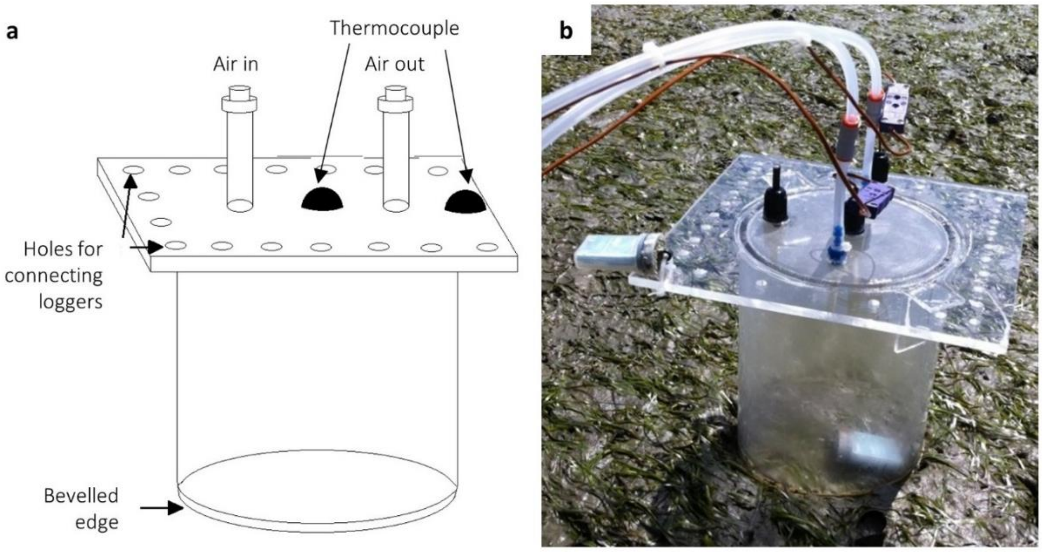


Figure A1.1. a) Diagram and b) photograph of custom-built incubation chamber.

Figure A1.2. Large custom benthic incubation chamber (Kaipara Harbour, 2016).

Incubation protocol

Operation of the IRGA followed manual instructions and the LI-8100A Application Software v4.0.0 was used to communicate with the instrument in the field. The “dead band” is the period of time after the initial sealing of the chamber during which CO_2 measurements are excluded from flux calculations, as this period is expected to demonstrate highly variable CO_2 concentrations while the system equilibrates and recovers from the disturbance of chamber insertion. A dead band of 10 s was used based on visual inspection of preliminary CO_2 concentration measurements which showed a reduction in the scatter of points around the line of best fit after this period. IRGA data were recorded to an on-board memory logger (LI-COR Li-1400) with a logging frequency of every second.

Prior to incubations a HOBO Pendant light logger (Onset; Massachusetts, USA) logging every 30 s was attached to the chamber lid to measure external light intensity, and another was placed inside the chamber to measure the light penetrating through to the photosynthesising organisms. The thermocouples were connected and the chamber was inserted 5 cm into the sediment (encapsulating 2.3 L of air in the small chamber). Finally, the air-in and air-out ports were connected to form a closed system and CO_2 measurements were initiated.

In situ testing

Testing of custom chambers was conducted between 9th October and 24th November 2015 (austral spring) on clear, sunny days encompassing a range of environmental conditions (photosynthetically active radiation (PAR): 2753–4078 μmol photon m^{-2} s^{-1}; temperature: 19–31 °C). For each incubation chambers were moved to a new position to avoid pseudoreplication (i.e. replicates not being statistically independent (Hurlbert, 1984; Heffner *et al.*, 1996)), and ensure any effects of sediment disturbance on CO_2 flux due to chamber insertion were consistent. The small dark custom chamber and 10 cm dia. LI-COR survey chamber were initially used to carry out alternating flux measurements in a terrestrial grass habitat to see whether the custom chambers could obtain fluxes comparable to the purpose-built LI-COR chamber. The same procedure was also conducted to compare CO_2 fluxes obtained from the small and large custom chambers in an intertidal sandflat habitat.

Time is an important factor limiting *in situ* intertidal experiments and affects the level of replication that can be achieved. Different duration incubations (5 and 3 min), based on existing terrestrial and saltmarsh guidelines (Streever *et al.*, 1998; Mielnick & Dugas, 2000), were tested to see whether similar CO_2 fluxes could be obtained with each, thus enabling the use of short incubations that increase replication potential.

Flux measurements were taken in sand, muddy sand and seagrass habitats under light and dark conditions in Raglan (37°47’43.89” S, 174°52’17.49” E) on the west coast and Tuapiro Point (37°29’15.64” S, 175°57’17.26” E) on the east coast of New Zealand to see whether differences between habitats could be detected (sand and muddy sand habitats were dominated by microphytobenthos (MPB)). The effect of chambers on environmental conditions that may affect CO_2 flux (e.g. temperature and light availability) were also investigated.

Finally, the effect of pre-shading sediments on dark CO_2 fluxes was investigated in sand and seagrass habitats, as it was suspected that photosynthetic processes may not cease instantly when dark conditions are imposed, as indicated by the findings of Migné *et al.* (2002). The sandflat was shaded for 0, 5 and 10 mins and seagrass habitat for 0, 5, 10, 30 and 60 mins prior to measuring CO_2 flux.

Data analysis

Regressions of CO_2 concentration against time and the coefficient of determination (r^2) associated with software-calculated fluxes (µmol m^{-2} h^{-1}) were inspected in the LI-COR File Viewer 8100 3.1.0. Only fluxes with an $r^2 > 0.8$ were used in further analyses, and flux data from all trials were pooled to include the effects of environmental variability.

SPSS Statistics (v24) was used to perform all statistical analyses. All data were checked for normality (via inspection of Q–Q plots) and homogeneity of variance (using Levene’s test) to guide the use of parametric (assumptions met) or non-parametric (either assumption violated) analyses. Initially independent t-tests looked at the difference between CO_2 fluxes measured by the small custom chamber and the LI-COR survey chamber (n = 4), and the small and large custom chamber (n = 3). Fluxes obtained from 5 versus 3 min incubations (n = 9) were then compared. Several environmental variables that had the potential to affect CO_2 flux were analysed via independent t-tests: temperature (light n = 14; dark n = 12) and light intensity (n = 9) inside versus outside chambers and internal chamber temperature at the start versus the end of incubations (n = 8). Finally, one-way ANOVA and post-hoc pairwise tests (if main tests were significant) identified differences in CO_2 fluxes between habitat types (n = 3) under both light and dark conditions and detected any effect of pre-shading habitats for different durations on CO_2 flux (n = 3).

Results & Discussion

The range of CO_2 flux values obtained using the 14 cm dia. dark custom chamber (839–2513 μmol m^{-2} h^{-1}) was similar to that obtained using the 10 cm dia. LI-COR chamber (731–2290 μmol m^{-2} h^{-1}) in a terrestrial grass habitat, and no significant difference between the mean CO_2 flux was detected (t(6) = 1.05, p = 0.33) (Figure A1.3a). LI-COR chambers use a pressure valve to ensure the pressure inside the chamber is equal to that outside, as the pressure inside the chamber may be elevated compared to ambient conditions otherwise. This could cause less CO_2 to leave the sediment and the overall flux to be underestimated. Despite not having a pressure valve in the custom chambers, fluxes measured by the custom chamber were comparable with those measured using LI-COR's 10 cm chamber.

Adjustment of the small custom chamber to a larger model was later required to enable direct comparisons of community production and respiration between emerged and submerged conditions. When measured under conditions of darkness, similar mean CO_2 flux values were obtained from an intertidal sandflat using the small (768 μmol m^{-2} h^{-1}) and large (612 μmol m^{-2} h^{-1}) custom chambers (Figure A1.3b) and no statistically significant difference was found (t(4) = -0.71, p = 0.51), supporting the use of the large chambers in experimental applications. All further trials were performed using the small custom chambers.

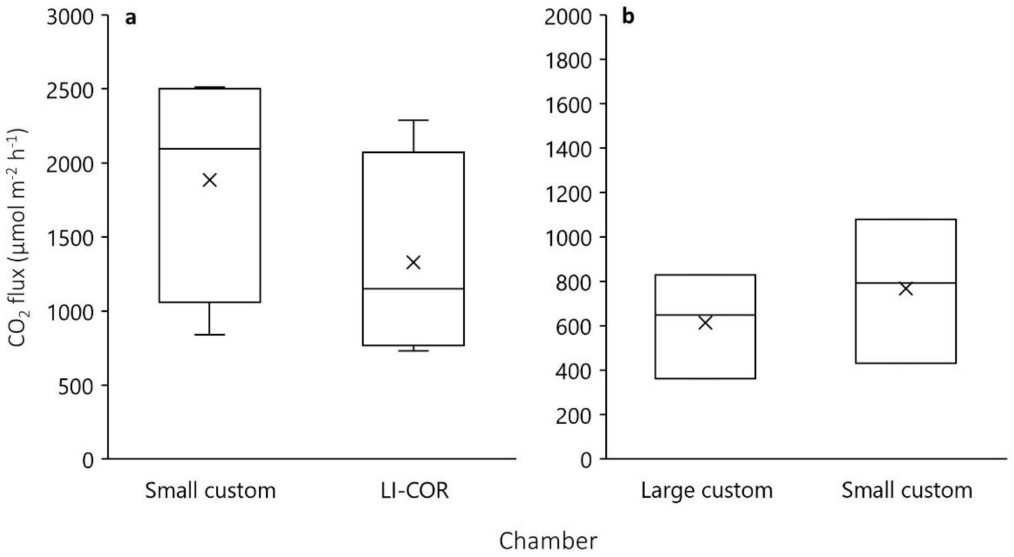


Figure A1.3. Comparisons of CO_2 fluxes obtained with a) the small (14 cm dia.) custom chamber and the LI-COR (10 cm dia.) chamber (n = 4) from grassland and b) the large and small custom chambers (n = 3) from emerged intertidal sandflat. Whiskers indicate the non-outlier maximum and minimum values. Box limits represent 25th and 75th percentiles, lines intersecting boxes show median values and crosses are means.

Time since emergence was assumed to affect fluxes according to changing sediment water content, as demonstrated by Migné *et al.* (2016). To account for this, all trials (and consequent measurements in Chapter 2) were conducted within a 2 h period following low tide. It is worth noting, however, that variable relationships between CO_2 efflux and soil water content have been found in terrestrial systems, with Fang and Moncrieff (2001) finding no obvious influence of water content (ranging from 20 to 50 % by volume) on farmland soil respiration, yet Mielnick and Dugas (2000) showing water content accounted for 26 % of the variability in CO_2 flux variance in prairie tallgrass. For future campaigns it may be appropriate to also collect data on sediment water content to help explain variability in emerged CO_2 fluxes, especially if conducting measurements at multiple sites and times of day. To increase the comparability of fluxes within studies, study sites should have similar tidal elevations and durations of exposure and a timeframe should be selected within which all measurements are taken; this will minimise any effects that differences in sediment water content may have on CO_2 flux.

Stable linear changes in CO_2 concentration were measured using the custom chambers in intertidal habitats (see Figure A1.4 for a seagrass habitat example). Under darkness, CO_2 concentrations increased steadily due to benthic respiration

(Figure A1.4a), and under ambient light conditions CO_2 concentrations decreased due to photosynthetic uptake (Figure A1.4b). There was no apparent period of highly variable measurements immediately after sealing chambers, but a precautionary “dead band” period of 10 s was used to minimise the effect on flux calculations if this was to occur.

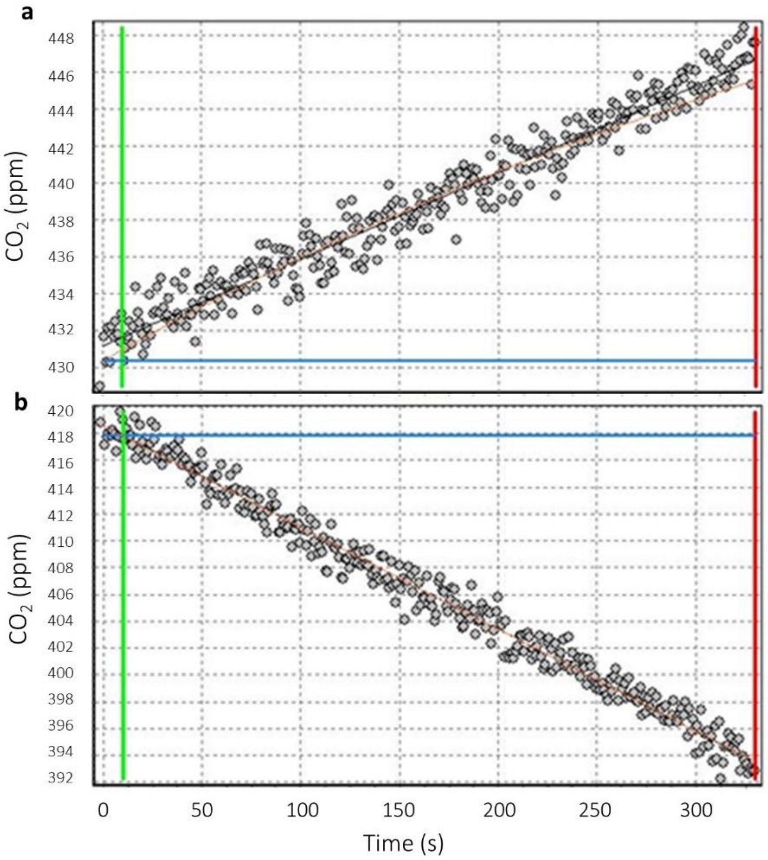


Figure A1.4. Regression of raw CO_2 concentration measured in an emerged intertidal seagrass-dominated habitat in a) darkness and b) ambient light conditions over a 5 min (300 s) incubation. The green line indicates the end of the “dead band” period, where data was excluded from flux calculations.

There was slightly more variability between flux measurements when incubations were 3 min in duration (ranging from -6120 to 11880 µmol m^2 h^{-1}) than 5 min (-5976 to 10332 µmol m^2 h^{-1}), but this had little effect on the average CO_2 flux and no significant difference was detected (t(22) = -0.09, p = 0.93) (Figure A1.5). The high water content associated with intertidal sediments meant moisture build-up in both the chamber and the tubing between the chamber and the IRGA was a factor limiting incubation duration. Moisture build up may impede the diffusion of CO_2 from the sediment (Geankoplis, 2003) and can reduce the amount of light penetrating through the chamber walls due to increased opacity, which may impact benthic photosynthesis. For this reason, it is beneficial to use as short an incubation duration as possible and 3 min incubations are recommended.

These incubation durations are substantially shorter than those trialled and recommended by Migné *et al.* (2002), who suggest 10 min incubations may be acceptable in highly productive sediments but that a minimum of 20 min is required in low productivity sediments, or during dark measurements. I have shown this is not necessary and in fact advise against conducting incubations for any longer than 5 min in intertidal habitats, due to changes in internal environmental conditions (e.g. moisture and CO_2 build-up) which may affect CO_2 diffusion (Jensen *et al.*, 1996; Kabwe *et al.*, 2002). This also has the benefit of vastly increasing the number of measurements that can be conducted in a given time, which is an important consideration for experimental studies where replication or multiple measurements are required.

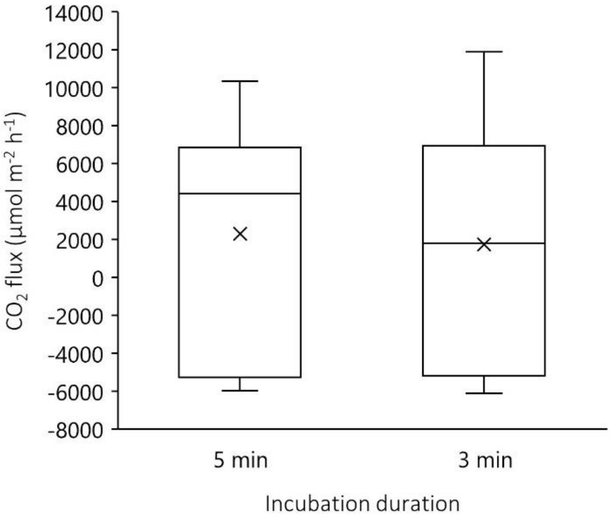


Figure A1.5. Comparison of CO_2 fluxes measured via incubations of different durations (n = 9) in emerged intertidal habitats under light and dark conditions. Whiskers indicate the non-outlier maximum and minimum values. Box limits represent 25th and 75th percentiles, lines intersecting boxes show median values and crosses are means.

During light incubations, the air temperature inside chambers was on average 3.7 °C higher than outside and constituted a significant difference (t(26) = -2.19, p = 0.04). Contrastingly, during dark incubations the internal temperature was 2.5 °C lower, although this difference was not significant (t(25) = 0.58, p = 0.57) (Figure A1.6). The change in temperature inside the chamber between the start and end of light incubations also differed significantly from zero (t(24) = 3.03, $p < 0.01$), however the average absolute increase was minor (2.2 °C). An increase in temperature has been shown to exponentially increase rates of soil respiration in terrestrial systems (Fang & Moncrieff, 2001), but the effects in intertidal habitats are less clear. Migné *et al.* (2002) observed no change in MPB photosynthetic rate even with a temperature increase of 5.4 °C on a muddy sandflat, whereas Blanchard and Guarini (1996) demonstrated that temperature was an important factor determining MPB PP in intertidal muds. These contrasting results indicate that temperature may exert changeable levels of control on CO_2 flux; it will be important to consider this when comparing flux measurements between studies conducted at different times and locations. It seems a slight increase in temperature is inevitable when sealing transparent chambers in the field under sunny conditions, but keeping incubation durations to a minimum should help to minimise any effects this may have.

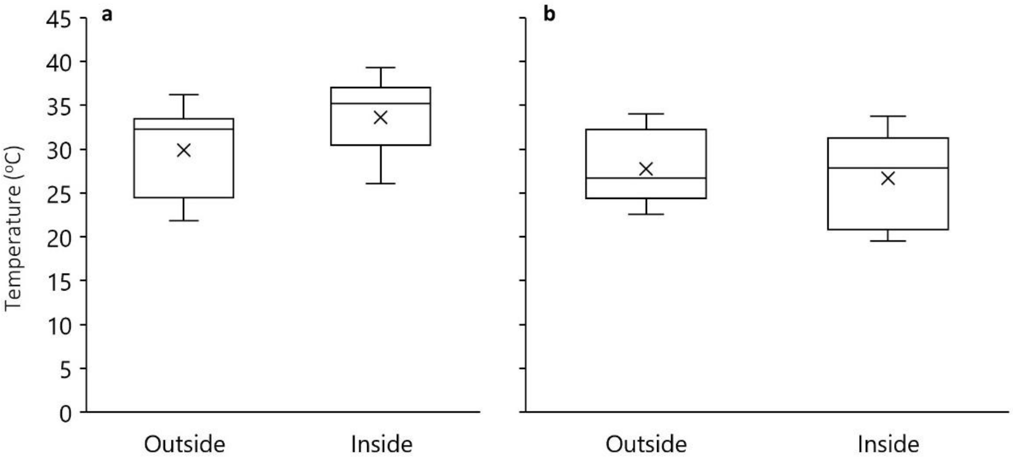


Figure A1.6. Mean temperature inside and outside chambers during a) light (n = 14) and b) dark (n = 12) incubations. Whiskers indicate the non-outlier maximum and minimum values with circles highlighting outliers. Box limits represent 25th and 75th percentiles, lines intersecting boxes show median values and crosses are means.

There was a greater range in average light intensities outside chambers (1360 µmol photons m^{-2} s^{-1}) than inside (885 µmol photons m^{-2} s^{-1}), which was expected due to the obstruction of light by the chamber walls, however this was driven largely by two outliers (Figure A1.7) and there was no significant difference between mean PAR values (t(28) = -0.18, p = 0.86). An unavoidable consequence of conducting experiments *in situ* is that light availability is unlikely to remain constant during flux measurements, as changes in cloud cover and the position of the sun affect this over fairly short time-scales (Anthony *et al.*, 2004). To reduce the effect this may have on the ability to interpret trends it is advised that field campaigns are conducted on clear sunny days and light levels inside chambers must be measured at a frequency of at least every 5 s to help explain trends retrospectively. Also see the discussion below on how sampling can be structured to reduce the effects of temporally variable environmental characteristics.

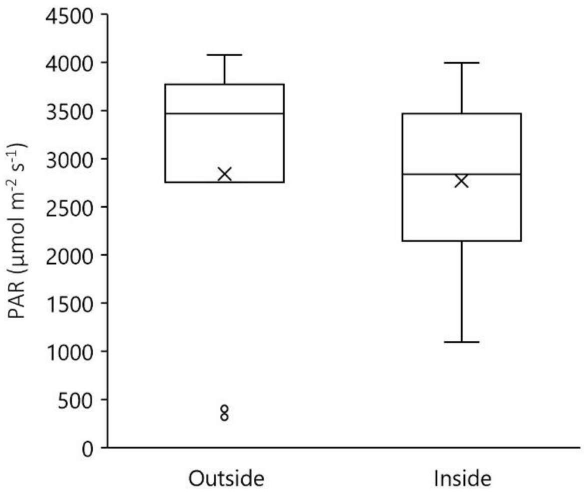


Figure A1.7. Mean photosynthetically active radiation (PAR) (n = 9) inside and outside chambers during light incubations. Whiskers indicate the non-outlier maximum and minimum values with circles highlighting outliers. Box limits represent 25th and 75th percentiles, lines intersecting boxes show median values and crosses are means

The greatest CO_2 fluxes were measured in seagrass-dominated habitats (-3792 and 3312 µmol m^{-2} h^{-1} in dark and light conditions, respectively), and the lowest in muddy sand habitats (-492 and 324 µmol m^{-2} h^{-1} in light and dark) (Figure A1.8). There was high variability around the mean CO_2 flux obtained from seagrass habitats but not in the unvegetated sediments, indicating PP and community metabolism is more spatially heterogeneous in seagrass habitats. The implication of this is that a greater level of replication may be required in vegetated versus unvegetated habitats to encompass this heterogeneity and reduce the variability among replicates. In all habitats significant differences were found between light and dark CO_2 fluxes (Table A1.2), indicating that the effect of photosynthesis on CO_2 flux was detected using this method. When measured under light conditions, the average CO_2 flux in both MPB-dominated habitats differed significantly to that in seagrass-dominated habitat, but not from each other (Table A1.3). When measured under darkness, a significant difference could only be detected between muddy sand and seagrass habitats.

The increased ability to detect differences between habitats in light versus dark conditions, i.e. when photosynthesis occurred, is not unexpected, as higher rates of PP have previously been documented in seagrass than MPB habitats due to the greater photosynthesising biomass associated with them (Moncreiff *et al.*, 1992). It was more difficult to detect differences between habitats in terms of community metabolism (dark CO_2 flux), which is dependent on the respiration of flora and fauna associated with the habitat as well as the chemical O_2 demand of the sediment. These trials aimed to determine whether the chosen methods could be used to detect differences between habitats but not to identify the reasons for any differences, or lack thereof; in future studies that implement this method it will therefore be essential that photosynthetic and faunal abundance/biomass is also measured to aid interpretation of such patterns. Nevertheless, this method has the ability to detect differences in CO_2 flux between some habitats despite low levels of replication ($n = 3$), although greater replication may be required to tease apart differences between sandflat and muddy sandflat habitats. Variance among the 3 replicate measures from these habitats was low, however (indicated by small error bars), suggesting the lack of a difference between the two may be accurate rather than a statistical artefact.

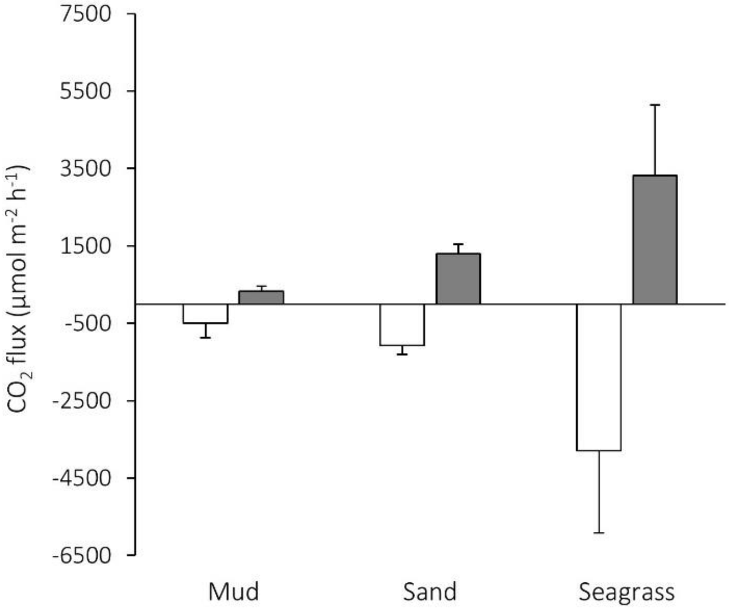


Figure A1.8. Comparison of CO$_2$ fluxes (n = 3) obtained in emerged intertidal muddy sandflat, sandflat and seagrass-dominated habitats measured under light (white bars) and dark (grey bars) conditions. Bars are means and standard deviation bars are shown.

Table A1.2. Results of t-tests comparing CO$_2$ fluxes measured in light versus dark conditions in different intertidal habitats. Significant results (α < 0.05) are highlighted in bold.

	df	t	p	Pairwise comparisons
Mud	4	3.51	**0.03**	L < D
Sandflat	4	12.4	**<0.01**	L < D
Seagrass	4	4.39	**0.01**	L < D

Table A1.3. Results of ANOVA comparing CO$_2$ fluxes (n = 3) from different habitats (sand, muddy sand and seagrass) under light and dark conditions. Significant results (α < 0.05) are highlighted in bold.

	df	F	p	Pairwise comparisons
Light	2	5.91	**0.04**	Mud = Sandflat Mud ≠ Seagrass Sand ≠ Seagrass
Dark	2	6.12	**0.04**	Mud = Sandflat Mud ≠ Seagrass Sand = Seagrass

Pre-shading MPB-dominated sandflat habitats prior to dark incubations had no effect on CO_2 flux (Figure A1.9a; Table A1.4). In seagrass-dominated habitats, however, the CO_2 flux measured varied depending on the duration of pre-shading (Table A1.4). There was no consistent relationship between the duration of pre-shading and CO_2 flux (Figure A1.9b), with a particularly high flux being measured after 10 min (5436 µmol m^{-2} h^{-1} versus an average of 2626 µmol m^{-2} h^{-1}), although there were significant differences between all consecutive durations of pre-shading apart from 30 and 60 min, and replicate measures in the 60 min treatment exhibited the lowest variability (smallest error bars) (Figure A1.9b). This indicates that CO_2 fluxes measured under darkness from seagrass-dominated habitats are unpredictable and highly variable when measured during daylight conditions, but this is reduced following 30 min of pre-shading. It is therefore recommended that field studies conducted during daylight hours pre-shade seagrasses for a minimum of 30 min prior to dark incubations, and studies that investigate both sandflat and seagrass habitats should ensure consistency and pre-shade all habitats even though this was not deemed necessary in sandflat environments; this will help prevent any additional effects of pre-shading (such as reduced sediment temperature) from contributing to differences between habitats.

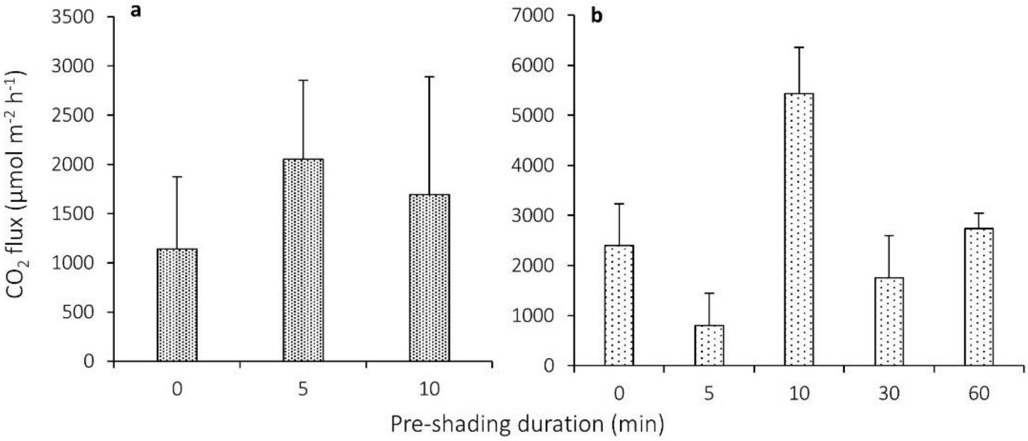


Figure A1.9. Comparison of CO_2 fluxes (n = 3) measured in a) sandflat and b) seagrass habitats after different durations of pre-shading. Bars are means and standard deviation bars are shown.

Table A1.4. Results of ANOVA comparing CO_2 fluxes measured under darkness after different durations (min) of pre-shading in microphytobenthos-dominated sandflat and seagrass habitats. Significant results ($\alpha < 0.05$) are highlighted in bold and results of post-hoc pairwise comparisons shown.

	df	F	p	Pairwise tests
Sandflat	2	0.72	0.52	
Seagrass	4	16.3	**<0.01**	0 > 5 5 < 10 10 > 30 30 = 60

In cases where field studies are interested in differences in CO_2 fluxes between treatments (for example different habitats or experimental manipulations) and have access to a single IRGA only, it will be essential that a sampling strategy is implemented that prevents any temporal changes in environmental conditions from confounding interpretation of the results (Hewitt *et al.*, 2007). For example, if there are multiple treatments it is advised that replicate measurements are conducted alternately between them, rather than completing all measurements in one treatment before moving onto the next. This prevents a temporal separation occurring between the sampling of distinct treatments, thus allowing them to be compared with the confidence that environmental variables that change temporally and may affect CO_2 flux (such as sediment water content, temperature and light intensity) will have had an equal effect on each treatment.

Conclusions

Small (0.06 m^2) and large (0.25 m^2) custom benthic incubation chambers were developed for use with the LI-COR LI-8100 CO_2 gas analyser for the purpose of measuring CO_2 fluxes across the sediment–air interface in estuarine intertidal environments, with which I obtained stable CO_2 flux measurements in three habitats (seagrass-dominated and MPB-dominated sandflat and muddy sandflat). Large chambers incorporate more seafloor heterogeneity and may therefore be of greater value to ecological studies.

Air temperature increased significantly inside sealed transparent chambers and there is contrasting evidence around the effects of this in the literature. By keeping incubation durations short any potential effects should be minimised, however further investigation is warranted. Light availability, however, was not significantly affected by the presence of chambers.

I have shown that stable CO_2 flux measurements can be obtained via 3 min incubations which allow a maximum of 20 measurements to be taken per hour with a single instrument, enabling the design of well-replicated studies. Significant differences in net PP were detected between vegetated versus unvegetated habitats under light conditions with only 3 replicate flux measurements, however this was not sufficient to detect differences between sandflat and muddy sandflats. Where differences between such habitats are of interest, trial measurements should be conducted *in situ* to determine the level of CO_2 flux variability within each habitat and use this to inform an appropriate level of replication.

The following are key methodological recommendations that have arisen from these trials:

1. Incubations should be conducted within a pre-defined period following low tide and study sites should have similar tidal elevations to ensure changing sediment water content does not introduce substantial variability between fluxes.
2. Incubation durations should not exceed 5 min.
3. Seagrass-dominated sediments should be pre-shaded for a minimum of 30 min prior to conducting dark measurements (this is not required for sandflat habitats).
4. Where multiple treatments exist, consecutive flux measurements should alternate between treatments so temporal changes in environmental conditions do not confound interpretation of results.

Appendix 2: Sediment O_2 consumption during emergence (Chapter 2)

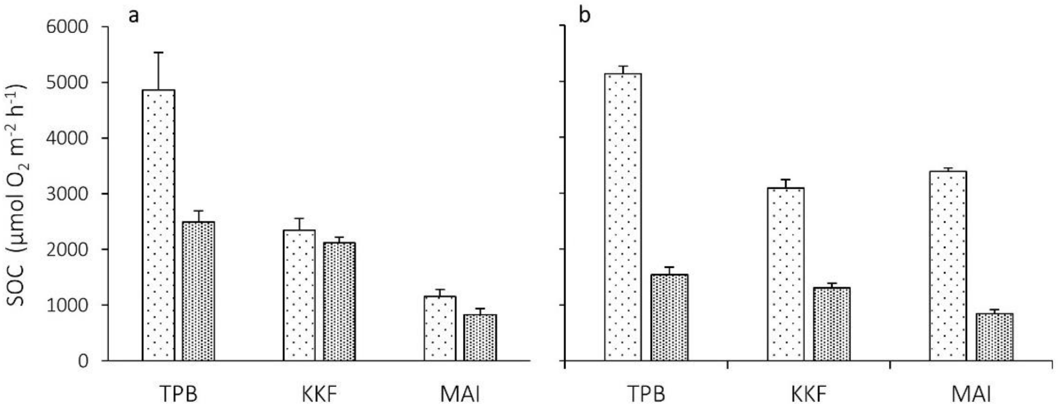


Figure A2.1. Sediment oxygen consumption (SOC) during a) submerged and b) emerged tidal conditions in seagrass and sandflat habitats within Kaipara Harbour. Site turbidity increased from TPB to MAI. Bars represent means (n = 5) with standard error bars displayed.

Table A2.1. Results of three-way PERMANOVA comparing sediment oxygen consumption (SOC) as a function of site (3 levels; TPB, KKF and MAI), habitat (2 levels; seagrass and sandflat) and tide (2 levels; submerged (Sub) and emerged (Em)). Significant effects (p(perm) < 0.05) are given in bold, and post-hoc pairwise tests are shown for significant interactions. Main effects are ignored in the presence of a significant interaction.

				Pairwise tests		
Term	df	Pseudo-F	p (perm)	Site	Habitat	Tide
SOC (raw)						
Site x Habitat	2	20.11	**<0.01**	**Seagrass** TPB > KKF > MAI **Sand** TPB > KKF > MAI	**TPB** Seagrass > Sand **KKF** Seagrass > Sand **MAI** Seagrass > Sand	
Site x Tide	2	11.09	**<0.01**	**Emerged** TPB > KKF = MAI **Submerged** TPB > KKF > MAI		**TPB** Em = Sub **KKF** Em = Sub **MAI** Em > Sub
Habitat x Tide	1	38.49	**<0.01**		**Emerged** Seagrass > Sand **Submerged** Seagrass > Sand	**Seagrass** Em > Sub **Sand** Em < Sub
Site x Habitat x Tide	2	1.19	0.32			

Appendix 3: Comparison of control treatments (Chapter 3)

The experiment in Chapter 3 utilised three different control treatments to account for potential effects of experimental procedures; a procedural control to mimic the disturbance associated with treatment setup, a $CaCO_3$ control (+$CaCO_3$ (–OM)) to identify effects of the physical presence of $CaCO_3$, and un-manipulated ambient plots that acted as reference for comparison.

Seventy days after establishment $CaCO_3$ control plots had a higher $CaCO_3$ content than the ambient and procedural control plots, as expected due to the addition of $CaCO_3$, and phaeopigment concentrations were higher in ambient than procedural and $CaCO_3$ controls (Table A3.1). When tested using one-way PERMANOVA, no surface sediment characteristics (median grain size, organic and mud content, chlorophyll a, phaeopigments and porewater pH) differed significantly (p(perm) > 0.05) between control treatments (results not shown), nor did a multivariate measure combining these variables (Table A3.2). There was a marginally significant difference in the abundance of adult *Austrovenus stutchburyi* between control treatments (Table A3.2) driven by a lower abundance in PC plots; however, there was no such effect on *Macomona liliana*; total abundance and the number of taxa were also comparable between treatments (Table A3.1).

There was no consistent variation in measures of ecosystem function and nitrogenous solute fluxes between control treatments (Table A3.1). Although net primary production (NPP) was greatest in PC treatments, and sediment O_2 consumption (SOC) was highest in $CaCO_3$ controls, high varibility (consistent with the dynamic nature of the sandflat) meant there was no significant differences between treatments (NPP: Pseudo-F = 2.80, p(perm) = 0.12; SOC: Pseudo-F = 1.58, p(perm) = 0.22). There was little difference in the average flux of nitrogenous solutes between control plots (Table A3.1) and a single multivariate measure of ecosystem function (consisting of NPP, SOC and nitrogenous solute fluxes) did not differ between treatments (Table A3.2).

Overall, no consistent differences were detected between control treatments with respect to sediment characteristics, macrofaunal community composition or ecosystem function. Therefore, apart from raising sediment $CaCO_3$ content, the procedure of removing and replacing sediment and incorporating $CaCO_3$ did not substantially affect the response variables measured in this study.

Table A3.1. Surface sediment characteristics (0–2 cm unless stated otherwise), macrofaunal community indices and solute fluxes in ambient, procedural control (PC) and $CaCO_3$ control plots. Means (and standard deviation) are shown. Abbreviations and units: Organic content (OC), $CaCO_3$ content and mud content, %; Chlorophyll *a* and phaeopigments, µg g^{-1} dw sediment; median grain size, µm; macrofauna community indices, no core^{-1}; all ecosystem functions, µmol m^{-2} h^{-1}.

	Ambient (n = 3)		PC (n = 4)		$CaCO_3$ (n = 4)	
Sediment characteristics						
OC	1.7	(0.1)	2.1	(0.1)	1.9	(0.1)
OC (2–5 cm)	2.0	(0.2)	2.0	(0.4)	2.2	(0.1)
$CaCO_3$ (2–5 cm)	0.5	(0.1)	0.7	(0.4)	1.1	(0.4)
Mud content	3.0	(1.5)	4.2	(1.4)	3.1	(1.1)
Chlorophyll *a*	11.5	(2.3)	10.8	(2.6)	11.3	(1.3)
Phaeopigments	5.0	(0.6)	4.1	(1.1)	3.3	(0.5)
Median grain size	188	(2)	190	(4)	192	(2)
Porewater pH (at 2 cm depth)	7.4	(0.2)	7.3	(0.3)	7.3	(0.2)
Macrofauna community						
Total abundance	133	(31)	109	(25)	113	(33)
Total taxa	23	(1)	23	(3)	22	(1)
A. stutchburyi (>5 mm)	9	(2)	5	(2)	8	(2)
M. liliana (>5 mm)	4	(1)	3	(1)	4	(1)
Ecosystem functions						
NPP	682	(34)	1440	(621)	988	(102)
SOC	471	(145)	302	(111)	776	(601)
$NH_4^{+}{}_{\text{light}}$	17	(17)	6	(22)	-8	(14)
$NH_4^{+}{}_{\text{dark}}$	19	(15)	17	(13)	31	(24)
$NO_2^{-}{}_{\text{light}}$	5	(5)	7	(3)	2	(3)
$NO_2^{-}{}_{\text{dark}}$	2	(4)	-6	(4)	-2	(3)
$NO_3^{-}{}_{\text{light}}$	-108	(23)	-89	(14)	-88	(17)
$NO_3^{-}{}_{\text{dark}}$	-139	(36)	-116	(48)	-131	(31)

Table A3.2. Results of one-way PERMANOVA comparing multivariate sediment characteristics and ecosystem function, and univariate macrofaunal community indices between control (ambient, procedural and $CaCO_3$) treatments.

	df	Pseudo-F	p(perm)
Sediment characteristics*	2	0.92	0.45
Macrofauna community			
Total abundance	2	0.48	0.65
Total taxa	2	0.43	0.69
A. stutchburyi >5 mm	2	4.51	0.05
M. liliana >5 mm	2	0.91	0.39
Ecosystem function*	2	2.11	0.08

*Sediment characteristics and ecosystem function encompass all corresponding variables included in Table A3.1.

Appendix 4: Macrofaunal community response to calcium carbonate addition (Chapter 4)

Macrofauna abundance data were averaged from two cores per plot. A non-metric multidimensional scaling (nMDS) ordination, based on untransformed data and the Bray–Curtis similarity index (Bray & Curtis, 1957), was used to visualise the separation between plots that had been enriched with $CaCO_3$ (+$CaCO_3$) and those that had not (–$CaCO_3$) in terms of community composition. This was paired with a preliminary permutational ANOVA (PERMANOVA) where $CaCO_3$ treatment was a fixed factor and OM treatment was included as a continuous covariable. Data points were interspersed with no separation between $CaCO_3$ treatments in the nMDS plot (Figure A4.1), and PERMANOVA detected no significant effect of $CaCO_3$ treatment on community composition either directly (pseudo-F = 1.07, p(perm) = 0.38) nor via its interaction with OM treatment (pseudo-F = 0.93, p(perm) = 0.51).

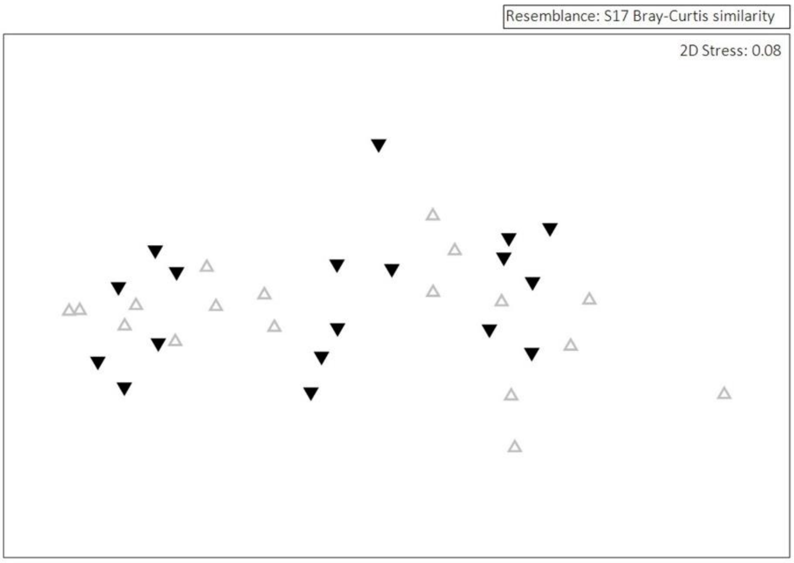


Figure A4.1. Non-metric MDS ordination showing $CaCO_3$ treatment (filled triangles = –$CaCO_3$, open triangles = +$CaCO_3$) effects on benthic macrofauna community composition. Each point represents a plot.

Appendix 5: Cluster analysis of macrofauna community according to organic matter enrichment (Chapter 4)

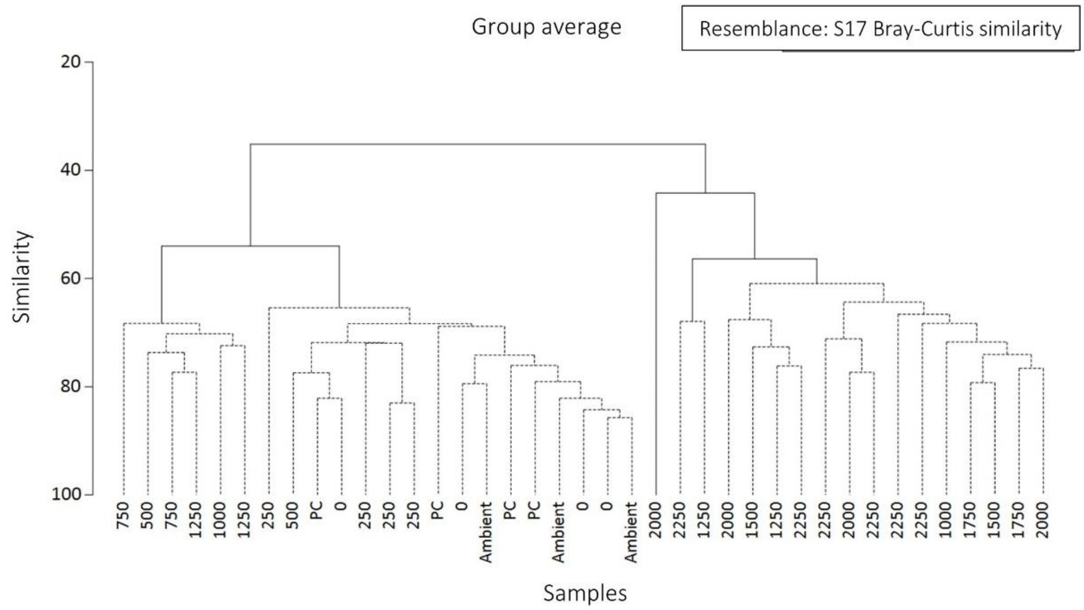


Figure A5.1. Preliminary cluster analysis dendrogram illustrating separation of taxonomic macrofauna community composition according to organic matter enrichment treatment (g dw m^{-2}) to inform categorical analyses in Chapter 4. Sample labels correspond to organic matter enrichment treatments (g dw m^{-2}). PC = procedural control.

Appendix 6: Species accumulation curve (Chapter 4)

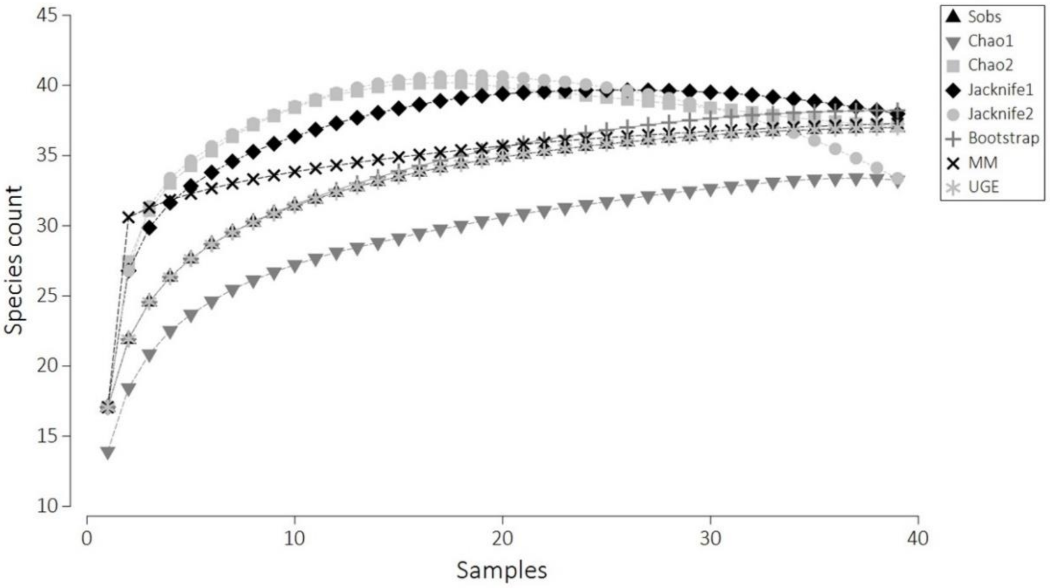


Figure A6.1. Species accumulation curve calculated using multiple diversity indices to show the number of replicate cores needed to sample all taxa in Chapter 4. Sobs = actual observations.

Appendix 7: Presence & absence of macrofauna (Chapter 4)

Table A7.1. Presence (*) and absence of taxa with increasing levels of organic matter (OM) enrichment treatment. Abbreviations: Biv = Bivalvia; Pol = Polychaeta; Gas = Gastropoda; Dec = Decapoda; Ant = Anthozoa; Cum = Cumacea; Amp = Amphipoda; Ses = Sessilia.

		OM treatment (g dw m^{-2})									
		0	250	500	750	1000	1250	1500	1750	2000	2250
Biv	*Austrovenus stutchburyi*	*	*	*	*	*	*	*	*	*	*
Pol	*Ceratonereis* sp.	*	*	*	*	*	*	*	*	*	*
Biv	*Macomona liliana*	*	*	*	*	*	*	*	*	*	*
Pol	*Nicon aestuariensis*	*	*	*	*	*	*	*	*	*	*
Pol	*Perinereis vallata*	*	*	*	*	*	*	*	*	*	*
Pol	*Prionospio aucklandica*	*	*	*	*	*	*	*	*	*	*
Pol	*Scoloplos cylindrifer*	*	*	*	*	*	*	*	*	*	*
Gas	*Zeacumantus lutulentus*	*	*	*	*	*	*	*	*	*	*
Pol	*Aonides trifida*	*	*	*	*	*	*	*		*	*
Biv	*Lasaea parengaensis*	*	*	*	*	*	*	*	*		*
Pol	*Scolecolepides benhami*	*	*	*	*	*	*	*	*		*
Pol	*Capitella* sp.	*	*	*	*			*	*	*	*
Pol	*Heteromastus filiformis*	*	*	*	*	*		*		*	*
Dec	*Hemiplax hirtipes*	*	*	*	*	*	*	*			*
Gas	*Diloma subrostrata*		*	*	*	*	*	*	*		*
Ant	*Anthopleura hermaphroditica*	*	*	*	*		*		*	*	*
Cum	*Colurostylis lemurum*	*	*	*	*	*				*	*
Biv	*Arthrtica bifurca*		*	*	*	*	*			*	*
	Oligochaeta	*	*	*	*	*					
Amp	*Paracalliope novizealandiae*		*		*	*		*			*
Gas	*Notoacmea scapha*	*		*		*		*			
Gas	*Cominella glandiformis*	*							*		*
Amp	Lysianassidae	*	*							*	
Ses	*Austrominius modestus*		*					*			*
Dec	*Austrohelice crassa*	*								*	*
Dec	*Halicarcinus whitei*	*									*
Pol	*Aricidea* sp.	*				*					
	Mysidae	*	*								
Pol	*Boccardia syrtis*			*						*	
	Nemertea		*			*					
Pol	*Magellona dakini*	*									
Pol	*Orbinia papillosa*	*									
Biv	*Paphies australis*	*									
Amp	*Phoxocephalidae*									*	
Dec	*Hemigrapsus* sp.					*					
Gas	*Potamopyrgus estuariensis*							*			
Gas	*Euterebra tristis*	*									

Appendix 8: Univariate response of macrofaunal community to organic matter enrichment (Chapter 4)

Table A8.1. Results of one-way PERMANOVA comparing univariate indices (*H′* = Shannon-Wiener diversity; *J′* = Pielou's evenness) of taxonomic and functional group (FG) community composition between low (0–250 g dw m^{-2}), medium (500–1250 g dw m^{-2}) and high (1500–2250 g dw m^{-2}) organic matter enrichment treatments. Significant (p(perm) < 0.05) results are indicated in bold.

			Pair-wise tests		
Variable	Pseudo-F	p(perm)	Groups	t	p(perm)
Total abundance	65.7	**<0.01**	Low, Med.	5.99	**<0.01**
			Med., High	3.96	**<0.01**
			Low, High	11.8	**<0.01**
Taxonomic					
Richness	16.4	**<0.01**	Low, Med.	3.29	**<0.01**
			Med., High	2.25	**0.04**
			Low, High	5.92	**<0.01**
H′	1.63	0.2			
J′	25.3	**<0.01**	Low, Med.	3.85	**<0.01**
			Med., High	2.18	**0.04**
			Low, High	8.25	**<0.01**
FG					
Richness	13.9	**<0.01**	Low, Med.	3.62	**<0.01**
			Med., High	2.01	0.06
			Low, High	4.98	**<0.01**
H′	0.89	0.42			
J′	26.6	**<0.01**	Low, Med.	3.11	**<0.01**
			Med., High	3.29	**<0.01**
			Low, High	8.32	**<0.01**

Appendix 9: Probability of occurrence models (Chapter 4)

Sufficient data were available to model 16 of the taxa found in control plots in the organic matter enrichment study of Chapter 4. Juvenile and adult *A. stutchburyi* and *M. liliana* were modelled separately, resulting in 18 models. Of these, two were not significantly related to organic matter enrichment (juvenile *Austrovenus stutchburyi* and *Perinereis vallata*). For the remaining 16 models, four (Lysianassidae, *Scolecolepides benhami*, *Nicon aestuariensis* and *Ceratonereis* sp.) had a reasonable predictive accuracy as indicated by concordance statistics (value between 0.6–0.7), and the remaining 12 had good (0.7–0.8), strong (0.8–0.9) or very strong (>0.9) predictive power.

The relationships between probability of occurrence and organic matter enrichment took one of five forms: threshold decrease (adult *A. stutchburyi*, *P. aucklandica, Scoloplos cylindrifer*, adult *M. liliana*, *Ceratonereis* sp. and *N. aestuariensis*), sigmoidal decrease (*Anthopleura hermaphroditica, Aonides* sp., *Heteromastus filiformis, Lasaea parengaensis* and juvenile *M. liliana*), linear decrease (*S. benhami)*, logarithmic decrease (Oligochaeta, *Colurostylis lemurum* and Lysianassidae), or logarithmic increase (*Z. lutulentus*) (Figure A8.1) (Thrush *et al.*, 2008). The critical quantity of organic matter for taxa exhibiting a threshold decrease was ~1250 g dw m^{-2}, beyond which there were rapid declines in probability of occurrence (Figure A8.1a), whereas taxa with a sigmoidal decrease relationship exhibited sharp declines by 1000 g dw m^{-2} (Figure A8.1b). Of the taxa modelled only 5 (gastropod *Z. lutulentus*, adult bivalves *A. stutchburyi* and *M. liliana*, and Nereid polychaetes *Ceratonereis sp.* and *N. aestuariensis*) had a greater than 50% chance of occurring in the 2250 g dw m^{-2} plots (Figures A8.1a & d). The form of the relationship could not apparently be summarised according to functional group, as taxa within the same functional group frequently displayed differing relationships. For example, *S. cylindrifer* and *S. benhami* are both in FG 12 but the former was very resilient (defined by a threshold (~1000 g dw m^{-2}) decrease relationship) whereas the latter was highly sensitive (defined by a linearly decreasing relationship) to organic matter enrichment.

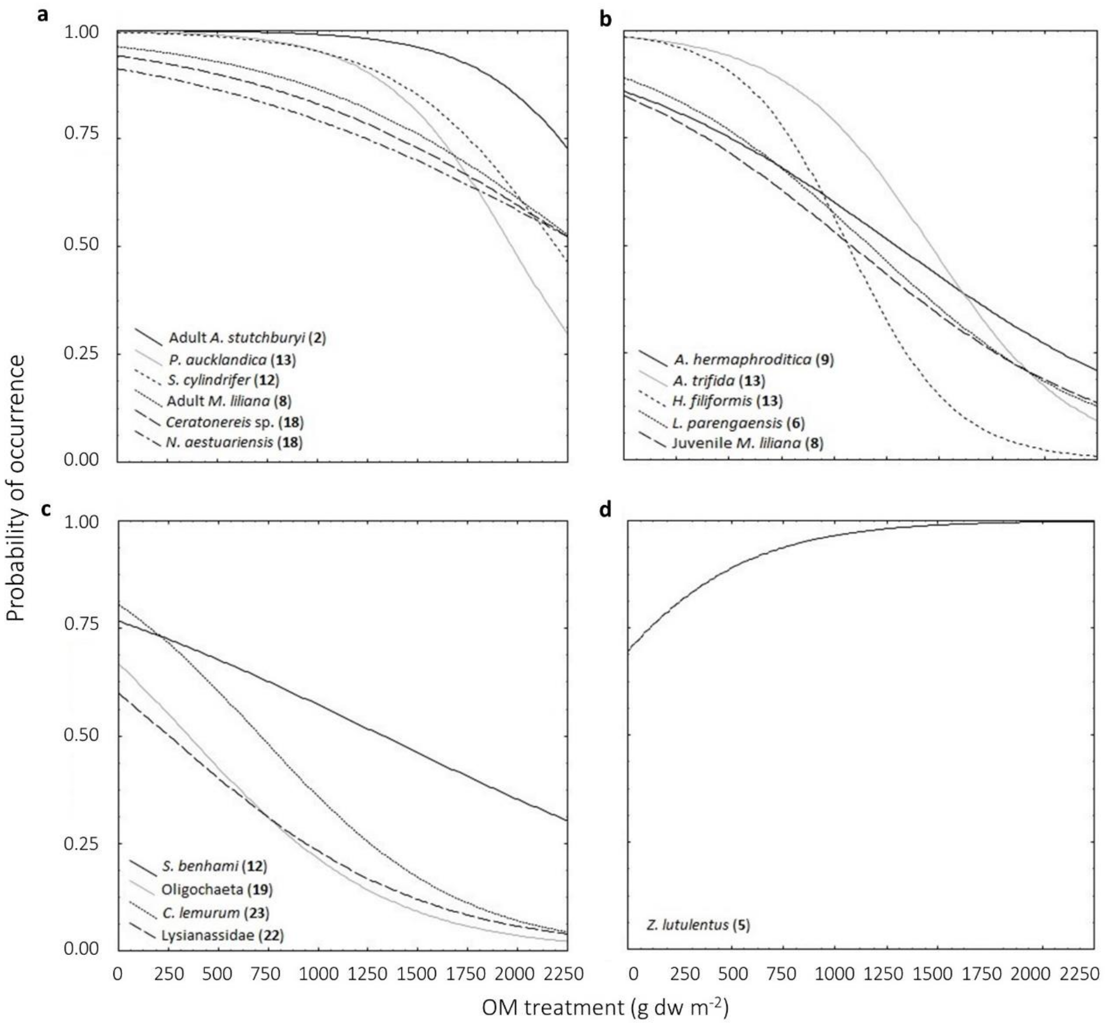


Figure A8.1. Probability of occurrence of common taxa (and their functional group; see Chapter 4, Table 4.2) with increasing organic matter (OM) enrichment treatment. Models are separated according to relationship form: a) threshold decrease, b) sigmoidal decrease, c) linear and logarithmic decrease and d) logarithmic increase.

Appendix 10: Two-way PERMANOVA results testing for response diversity (Chapter 4)

Table A9.1. Results of two-way PERMANOVA testing interactions between organic matter (OM) enrichment treatment (fixed factor, 3 levels: low, medium and high) and taxa (fixed factor) within functional groups (FG). Significant interactions are followed up with post-hoc pairwise tests for the OM enrichment factor only. Significant (p(perm) < 0.05) results are highlighted in bold and marginally significant results are in italics. See Chapter 4, Table 4.2 for FG descriptions.

FG	Source	df	Pseudo-F	p(perm)	Pairwise tests Taxa	Groups	t	p(perm)
2	OM	2	7.94	<0.01				
	Taxa	1	922	<0.01				
	OMxTaxa	2	7.89	**<0.01**	*A. stutchburyi*	Low, Med	0.62	0.55
						Low, High	3.69	**<0.01**
						Med, High	3.62	**<0.01**
					P. australis	Low, Med	0.91	1
						Low, High	0.11	1
						Med, High	0.84	1
6	OM	2	33.2	<0.01				
	Taxa	1	97.3	<0.01				
	OMxTaxa	2	34.3	**<0.01**	*L. parengaensis*	Low, Med	5.27	**<0.01**
						Low, High	9.30	**<0.01**
						Med, High	1.57	0.11
					P. estuariensis	Low, Med	-	
						Low, High	0.92	1
						Med, High	0.84	1
13	OM	2	35.9	<0.01				
	Taxa	5	66.7	<0.01				
	OMxTaxa	10	13.5	**<0.01**	*A. trifida*	Low, Med	3.27	**<0.01**
						Low, High	10.29	**<0.01**
						Med, High	3.96	**<0.01**
					Aricidea sp.	Low, Med	0.13	1
						Low, High	1.08	0.47
						Med, High	1.19	0.40
					Capitella sp.	Low, Med	0.31	0.89
						Low, High	0.36	0.83
						Med, High	0.62	0.61
					H. filiformis	Low, Med	1.48	0.14
						Low, High	3.98	**<0.01**
						Med, High	1.97	*0.06*
					M. dakini	Low, Med	0.91	1
						Low, High	1.08	0.46
						Med, High	-	
					P. aucklandica	Low, Med	3.63	**<0.01**
						Low, High	7.14	**<0.01**
						Med, High	2.81	**<0.01**

Table A9.1 continued.

					Pairwise tests			
FG	Source	df	Pseudo-F	p(perm)	Taxa	Groups	t	p(perm)
18	OM	2	13.7	<0.01				
	Taxa	3	42.5	<0.01				
	OMxTaxa	6	5.21	**<0.01**	*Ceratonereis sp.*	Low, Med	4.37	**<0.01**
						Low, High	6.06	**<0.01**
						Med, High	1.52	0.12
					Nemertea	Low, Med	0.19	0.96
						Low, High	2.00	0.09
						Med, High	1.63	0.17
					N. aestuariensis	Low, Med	0.92	0.39
						Low, High	2.81	**<0.01**
						Med, High	1.65	0.10
					P. vallata	Low, Med	0.26	0.92
						Low, High	0.93	0.38
						Med, High	0.77	0.48
22	OM	2	4.32	0.01				
	Taxa	3	8.09	<0.01				
	OMxTaxa	6	3.72	**<0.01**	Lysianassidae	Low, Med	2.82	**<0.01**
						Low, High	2.91	**<0.01**
						Med, High	0.34	1
					Mysidae	Low, Med	1.23	0.48
						Low, High	1.47	0.21
						Med, High	-	
					P.novizealandiae	Low, Med	0.81	0.47
						Low, High	1.14	0.26
						Med, High	1.68	0.11
					Phoxocephalidae	Low, Med	-	
						Low, High	0.92	1
						Med, High	0.84	1