Subtidal Mussel Restoration: A Tool for Recovering Ecosystem Services

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“Rebuilding marine life represents a doable Grand Challenge for humanity, an ethical obligation and a smart economic objective to achieve a sustainable future.”

Duarte et al. (2020) *Nature*

Abstract

In an attempt to address globally relevant environmental crises, the United Nations declared 2021-2030 as “the UN decade on Ecosystem Restoration” (UN Resolution 73/284). Restoration initiatives are especially timely for coastal ecosystems which deliver a myriad of benefits to mankind yet are subject to multiple anthropogenic stressors with cumulative effects. As the combined effects of these stressors have led to concurrent declines in global shellfish ecosystems, there is growing interest in the ability of bivalve restoration projects to revitalise the coastal ecosystems shellfish historically inhabited.

In response to anthropogenically-induced population collapse, coastal restoration utilising endemic green-lipped mussels (*Perna canaliculus*) is currently underway in New Zealand, with ecosystem service provision considered a primary justification for the continuation and upscaling of restoration projects. However, there is little information available to quantify service value associated with mussel restoration efforts, nor is there information regarding how ecological functions and processes underpinning these services might differ with contrasting environmental properties exhibited at various restoration locations.

This thesis investigates the role of mussel restoration in recovering ecosystem functionality and service value relevant to the biodiversity, eutrophication, and climate crises (chapters 2, 3, and 4 respectively). Through a variety of mechanisms, mussel beds are shown to generally increase species richness and abundance of associated organisms, which in turn influence regulating services (nitrogen and carbon cycling) explored in later data chapters. I show that enhanced nitrogen removal rates observed in mussel beds are consistent despite patchiness in restored beds, highlighting the upscaling-role of mussels in seafloor biogeochemistry. Finally, I take an ecosystem-based approach to create a carbon budget for restored mussel bed systems, discussing implications for coastal carbon cycling through space and time. Throughout the thesis I consistently demonstrate that the magnitude and extent of service provision varies as a result of environmental conditions experienced; therefore, restoration location should be crucially considered for its impact on the generation and delivery of ecosystem services.

Preface

This thesis is divided into five chapters. The general introduction (Chapter 1) provides context for the project—notably, the rationale for mussel restoration in New Zealand and the use of the Ecosystem Services framework. The general discussion (Chapter 5) outlines the significance of produced research and places acquired knowledge within a wider social-ecological setting, discussing implications for future restoration efforts. The bulk of this thesis is made up of three data chapters (Chapters 2-4) comprised of original research examining different ecosystem services provided by restored, subtidal mussel beds:

Chapter 2– Biodiversity

This chapter has been published in *Scientific Reports*:

Sea, M. A., Hillman, J. R., & Thrush, S. F. (2022). Enhancing multiple scales of seafloor biodiversity with mussel restoration. *Scientific Reports*, 12:5027.

Chapter 3 – Nitrogen Removal

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Sea, M. A., Thrush, S. F., & Hillman, J. R. (2021). Environmental predictors of sediment denitrification rates within restored green-lipped mussel (*Perna canaliculus*) beds. *Marine Ecology Progress Series*, 667, 1-13.

Chapter 4– Carbon Cycling

This chapter has been published in *Global Change Biology*:

Sea, M. A., Hillman, J. R., & Thrush, S. F. (2022). The influence of mussel restoration on coastal carbon cycling. *Global Change Biology, 00*, 1-14.

As all three data chapters are published in peer-reviewed journals, there is unavoidable repetition in the phrasing of some sections.

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This one is for you.

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Contents

List of Tables

List of Figures

List of Abbreviations

%	percent
°C	degrees Celsius
±	plus or minus standard error
ANOVA	analysis of variance
Ar	argon
C	carbon
$CaCO_3$	calcium carbonate
CO_2	carbon dioxide
chl *a*	chlorophyll a
cm	centimetre
d	day
DISTLM	distance-based linear model
DNF	denitrification
e.g.	exempli grata [Latin: “for example”]
eq.	equation
et al.	et alia [Latin: “and others”]
FIA	flow injection analyser
Fig.	figure
FNU	formazin nephelometric unit

g	gram
h	hour
HCO_3^-	bicarbonate
i.e.	id est [Latin: “that is”]
IPA	isopropyl alcohol
km	kilometre
L	litre
m	meter
mm	millimetre
M	molar
m^2	square metre
MIMS	membrane inlet mass spectrometer
min	minute
mL	millilitre
MPB	microphytobenthos
N	nitrogen
n	number of samples
nm	nanometre
N_2	di-nitrogen gas
NH_4^+	ammonium
N_2O	nitrous oxide

NO_2^-	nitrite
NO_3^-	nitrate
NO_x	nitrate + nitrite
O_2	oxygen
p	p-value
PO_4^{3-}	phosphate
R^2	correlation coefficient
ROMA	rapid organic matter assay
SOM	sediment organic matter
vs.	versus
α	alpha, significance level
µg	microgram
µm	micrometre
µmol	micromole
yr	year

Chapter 1

General Introduction

1.1 | The Need to Restore Coastal Ecosystems

Estuaries and coastal marine environments are complex, heterogeneous ecological systems that act as critical transition zones between fresh and saltwater habitats (Levin et al., 2001). With this unique positioning at the land-sea interface, coastal environments perform many important biological, cultural, and economic roles; they influence complex biogeochemical cycles (Wollast 1993, Herbert 1999), provide raw food and materials, sequester and detoxify heavy metals and other harmful pollutants (Thrush et al. 2013), and serve species-specific nursery functions (Vasconcelos et al. 2011) which support recreational and commercial fisheries worldwide (Seitz et al. 2014).

While coastal environments are widely acknowledged for the myriad of benefits they provide mankind, sustained migration of human populations to these regions has resulted in devastating environmental impacts (Tibbetts 2002, Lotze et al. 2006). Anthropogenic pressures on coastal marine habitat are many and severe (e.g. Halpern et al. 2008, Greene et al. 2015), and their cumulative effects not well understood (but see Thrush et al. 2021b). However, terrestrial sedimentation, habitat modifying fishing practices, nutrient loading, and climate change have been linked to both historic and future declines in coastal shellfish populations (Lotze et al. 2006, Parker et al. 2013, Gillies et al. 2018), losses which likely exacerbate further coastal degradation as a result of ensuing water quality effects, lack of suitable fish habitat, and loss of shoreline protection and sediment stability, with subsequent social and economic costs as well (Grabowski et al. 2012, zu Ermgassen et al. 2016a, Gillies et al. 2018). Despite their many important ecological functions, coastal bivalve populations have suffered extensive declines, with an estimated 85% of oyster reefs lost globally (Gillies et al. 2018). Relative abundances of mussel populations have similarly dropped by 50% or more in many regions, including the Wadden Sea, Adriatic Sea, Chesapeake Bay, San Francisco Bay, and Moreton Bay (Lotze et al. 2006), highlighting the spatial magnitude of this issue and a growing necessity to conserve and restore bivalve systems worldwide.

Reflected in the United Nations announcement that 2021-2030 is “the UN decade on Ecosystem Restoration” (UN Resolution 73/284), there is an urgent need to protect and restore these important coastal ecosystems. While human pressures on marine systems have generally increased over the past 200 years, evidence suggests our impacts are still reversible (Worm et al. 2006) and that recovery is achievable on decadal timescales with intentional, impactful interventions (Duarte et al. 2020). Known as active restoration, these interventions accelerate the recovery of the system of interest and are “designed to give nature a helping hand to overcome barriers to recovery” (Thrush et al. 2021a). The modern restoration concept has evolved to include both natural and social sciences, importantly moving beyond the ‘how it’s done’ to a greater focus on ‘why it’s done’ (Martin 2017) as various stakeholder groups typically have different reasons for engaging in the restoration process. While the answer to the question ‘why restore?’ is highly contextual, the response typically involves maximising shared values and beliefs (Martin 2017). Restoration to enhance shared social and ecological values in coastal marine environments is relatively new (Thrush et al. 2021a), owing to insufficient long-term data and a historically-limited understanding of the species/systems we seek to revitalise. However, clearly identifying and communicating multiple social and ecological benefits resulting from coastal restoration will be crucial for the continuation and long-term success of these projects, increasing both marine stewardship by active participants and the likelihood of sustainable ocean outcomes into the future.

The number and scale of shellfish restoration projects have rapidly increased over the past decade, with the majority of efforts utilising eastern oysters (*Crassostrea virginica*) in the United States and focused on the recovery of multiple social-ecological benefits (e.g., https://billionoysterproject.org/), but—as coastal restoration efforts are intensifying globally—other bivalves (namely mussels, clams, and other oyster species) are now being used in restoration works throughout the world (zu Ermgassen et al. 2020, Toone et al. 2021). In addition to studying the distribution and ecology of threatened and restored bivalve populations, Australia has created a Shellfish Reef Restoration Network for restoration practitioners (Gillies et al. 2018). Experimental reefs utilising juvenile and adult mussels have also been trialed in the Netherlands (e.g. de Paoli et al. 2015, Schotanus et al. 2020). There is growing interest in determining the capacity of these restored bivalve beds to revitalise the coastal environments they inhabit, thus simultaneously achieving restoration and sustainability goals set by the United Nations (UN Resolution 73/284; Sustainable Development Goal 14).

1.2 | Green-lipped Mussels in New Zealand

New Zealand's endemic, green-lipped mussel (*Perna canaliculus*) is a mytilid bivalve once historically distributed throughout the country's rocky shores, shallow estuaries, and soft-sediments, reaching depths of up to 50 m (Powell 1979). These mussels commonly inhabited coastal sediments until dredging, overharvesting, and other anthropogenic stressors resulted in population collapse; the North Island's Hauraki Gulf shellfishery was first to fail in the early 1960s, followed by the Marlborough Sounds and smaller North Island operations in the late 1970s and early 1980s (Paul 2012). While cultivation of *P. canaliculus* supports an impressive aquaculture industry (predicted to surpass NZ$1 billion in annual sales by 2025; Ministry for Primary Industries 2019), natural subtidal beds remain functionally extinct, with increased sedimentation rates, lack of suitable substrate, pollution, and predation pressures all predicted to contribute to the lack in natural recovery (Cummings & May 2010, Paul 2012, Alder et al. 2021).

Green-lipped mussel historical abundance and geographic distribution data are limited for the Hauraki Gulf region, although collected anecdotal evidence suggests that the entire Firth of Thames was effectively dredged in pursuit of mussels from 1920-1980 with over 46,000 tonnes of green-lipped mussels removed from the Gulf during this time (a conservative estimate as records of landings were voluntarily reported by fishermen in the 20s and 30s and this approximation does not consider poaching in later years; Paul 2012). Other bivalve species have experienced similar declines in New Zealand (e.g. horse mussels, scallops, and cockles; Bull 1989, Hayward et al. 1999, Tricklebank et al. 2021), but green-lipped mussels—now internationally identified as a species of restoration interest (zu Ermgassen et al. 2020)—seem overwhelmingly preferred in ongoing restoration projects, likely a result of their historical abundance in the Gulf, strong habitat forming capabilities, and the financial and logistical ease with which large quantities can be obtained from regional long-line aquaculture operations. Data is generally scarce and variable, but historical shellfish beds are estimated to have covered ~1500 km^2 of regional seafloor (Revive Our Gulf 2022), and the Hauraki Gulf Forum has recently set an ambitious goal to re-establish beds comprising 1000 km^2 of the Gulf (Auckland Council 2019). To my knowledge, however, there is no clearly anticipated timeline for this goal and considerable ambiguity regarding how the Forum will define success and measure returned value on mussel restoration projects.

1.3 | Conceptualising Ecosystem Services from Mussel Restoration

Successful green-lipped mussel restoration in the Hauraki Gulf will likely generate benefits to society, as mussels support a wide range of uses and values to mankind (e.g. Smaal et al. 2019). The benefits that humans freely obtain from mussel beds are defined here as ecosystem services (Millennium Ecosystem Assessment 2005, Barbier et al. 2011), although other frameworks have been proposed to conceptualise and acknowledge the many ways in which natural systems support humans (e.g. NCP, TEEB; Kumar 2012, Díaz et al. 2018). The ecosystem services framework links human welfare benefits to natural capital and importantly reframes the way we view our relationship with nature (with natural assets essential to creating a sustainable future; Costanza et al. 2014). The framework can aid in quantifying potential losses associated with degradation and extraction over time (relevant even for those who do not utilise a system directly; Costanza et al. 1997), but can also showcase the value of a healthy, functioning natural system to society. More recently, the framework has been used to highlight benefits associated with restoring specific species or ecosystems (Vermaat et al. 2016, zu Ermgassen et al. 2020), making it a useful tool for valuing and prioritising coastal restoration efforts.

Understanding benefits generated by green-lipped mussel restoration is crucial as service generation is considered a primary justification for upscaling restoration efforts in New Zealand. Below, I utilise the ecosystem services framework (Fig. 1.1) to briefly summarise notable services generated by mussels (for a full review refer to Smaal et al. 2019, zu Ermgassen et al. 2020). I use species-specific support where possible, but little research has focused on ecosystem functionality in green-lipped mussel beds, highlighting the need for data generation in this space.

1.3.1 | Provisioning Services

Tangible products (raw goods/materials) derived from the system of interest are defined as provisioning services. In the contexts of mussels, this most commonly involves consumption and distribution of mussel tissue. Bivalves are a major aquaculture product with an estimated 16 million tonnes harvested in 2015 (and a market value over $USD 17 billion; FAO 2016). New Zealand export revenues from *P. canaliculus* have steadily increased since the late 1990s and exceeded $NZ250M in 2018 alone (Ministry for Primary Industries 2019). While a goal of current *P. canaliculus* restoration projects is to promote services that do not involve the direct exploitation of mussel beds, there is undeniable potential for self-sustaining beds to provide a high-protein, low-fat food source for human consumption (potentially connected to lost cultural values as well, discussed below). This value however would have to be weighed against original restoration costs and the cost of disturbing soft-sediment ecosystem functionality established at restoration sites; others have shown that recovering these costs is not feasible with allowed harvesting (Grabowski et al. 2012). New Zealand’s restored mussel beds could instead provide additional larval supply for the aquaculture industry which almost exclusively relies on spat of unknown origin (> 80%) known to intermittently wash up with algal debris on a singular beach (Alfaro et al. 2010), thus reducing substantial risks involved with current long-line culturing methods. Mussel restoration projects in the Marlborough Sounds are financially and logistically supported by the mussel industry (e.g. The Marine Farming Association; https://www.marinefarming.co.nz/) and can therefore help maintain social license and sustainability of commercial operations.

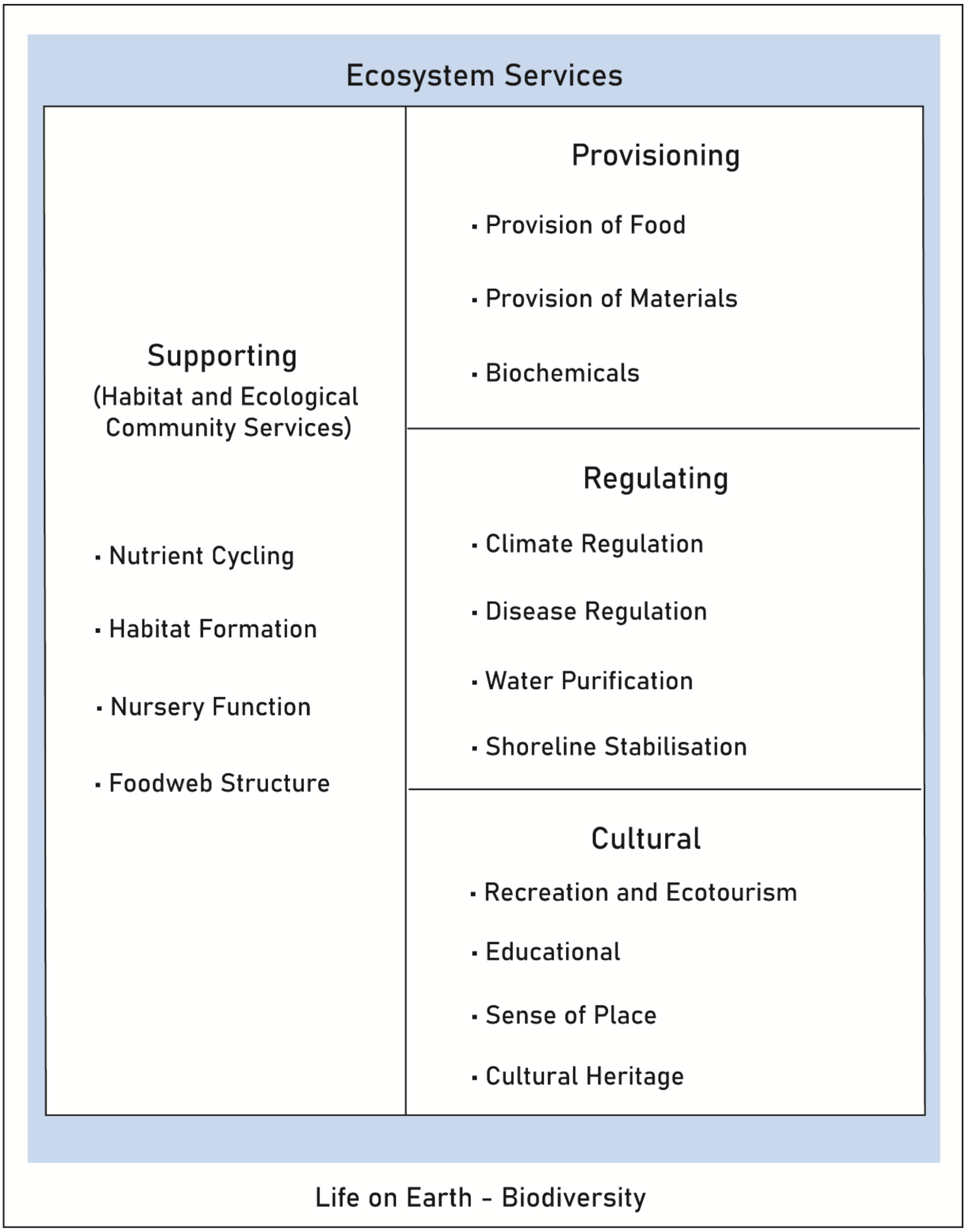

Figure 1.1 List of identified ecosystem services provided by restored mussel beds, categorised as supporting, provisioning, regulating, or cultural services. Framework structure and classifications adapted from the Millennium Ecosystem Assessment (2005). Note the placement of biodiversity outside the framework, highlighting its role in underpinning all other provided services.

Provisioning services also include genetic or biochemical products. A vast number of medicinal compounds are derived from nature, and restored beds could be an additional source of raw materials used to produce medical supplements that promote joint health and mobility in global users. Clinical trial results are varied, but there appears to be mild support for pain relief from rheumatoid arthritis and osteoarthritis with consumption of green-lipped mussel-derived products (Bierer & Bui 2002, Cobb & Ernst 2006). Independent studies have shown that omega-3 fatty acids (found in green-lipped mussel extracts) can help decrease pain intensity, stiffness, and tender joints in patients with rheumatoid arthritis (Goldberg & Katz 2007), likely contributing to the success of mussel-derived health products.

1.3.2 | Regulating Services

Regulating services are not consumed by humans but rather encompass the life supporting capabilities of a system that stem from the regulation of ecosystem processes (Millennium Ecosystem Assessment 2005); such services can include the maintenance of air quality, regulation of climate, or other outputs that alter the performance and activities of effected individuals and their communities. Most regulating services provided by mussels are related to their filtration capabilities. Mussels act as biological pumps, filtering nutrients, photosynthetic algae, and suspended sediments out of the water column and depositing waste products on the seafloor in the form of faeces and pseudofaeces (herein referred to as biodeposits; Newell 2004). This benthic-pelagic coupling (the exchange of energy and nutrients between the water column and the seabed) is ultimately responsible for many of the ecosystem services provided by mussels but is especially linked to the ability of green-lipped mussels to purify surrounding water bodies. Under optimal food and temperature conditions mussels are generally predicted to filter between 60 and 115 L of water per day

(Schulte 1975, Kittner & Riisgård 2005), but reported green-lipped mussel clearance rates are as high as 15 L h^{-1} (Hawkins et al. 1999). As green-lipped mussel clearance rates are typically half this value (Hawkins et al. 1999) and filtration rates vary substantially between individuals (Hatton et al. 2005), non-profit organisations invested in green-lipped mussel restoration currently maintain a single mussel filters roughly "a bathtub full of water" every day (or ~ 150-200 L; Revive Our Gulf 2021). The upscaling effects of thousands of mussels on water purification are thus substantial, with historical beds estimated to filter the entire Firth of Thames (over a billion liters of seawater) in a single day; by contrast, currently remaining remnant reefs require over two years to filter the same volume of water (Revive Our Gulf 2020).

Another result of active filter feeding is that mussels are capable of accumulating biochemicals and heavy metals throughout ontogeny (e.g. McDougall et al. 2019) and can serve as effective bio-indicators for chemical contaminants (Phillips 1985, Richardson et al. 2008). This, in combination with their sedentary lifestyle, availability, and geographic distribution makes mussels useful in many biomonitoring programs (Viarengo et al. 2007). Biomonitoring applications have been applied to green-lipped mussels, specifically for identification of trace metals in coastal environments (Chandurvelan et al. 2012, Chandurvelan et al. 2015) and toxic algal blooms linked to human mortality upon consumption of paralytic shellfish toxins (e.g. Marsden et al. 2015).

Nutrient removal similarly falls under the category of regulating services (Rullens et al. 2019), and bivalves are well-known influencers of the nitrogen cycle (Kellogg et al. 2014), potentially mitigating coastal eutrophication and the proliferation of harmful algal blooms either through direct nitrogen storage and removal upon human consumption (e.g.

Songsangjinda et al. 2000) or by stimulating microbially-mediated denitrification processes (Kellogg et al. 2013, Ray & Fulweiler 2021). However, very few studies have verified this removal of biologically available nitrogen through the denitrification process in-situ, and the majority have utilised oysters in eutrophic systems (Cerco & Noel 2007, Kellogg et al. 2013, Humphries et al. 2016) or mussels from suspended aquaculture operations (e.g. Christensen et al. 2003), necessitating verification of populations restored to the seafloor to similarly perform this service under different environmental conditions. Hillman et al. (2021) recently illustrated this enhanced nitrogen removal capacity on restored green-lipped mussel beds, estimating an average nitrogen removal service value of nearly \$50,000 per bed y^{-1}.

Climate regulation is an ecosystem service of increasing importance to mankind, although the role of bivalves in sequestering anthropogenic carbon sources is not currently well understood. Bivalves create a calcium carbonate shell which can be stored deep within marine sediments, leading some to believe that bivalves act as carbon sinks; however, calcium carbonate formation actually leads to the production of carbon dioxide (Zeebe & Wolf-Gladrow 2001, Fodrie et al. 2017). Major uncertainties surround the appraisal of biogenic calcification to carbon budgets (e.g. Macreadie et al. 2017, Saderne et al. 2019) and have led some to disregard shell formation in ecosystem service evaluations completely (van der Schatte Olivier et al. 2020). It should also be considered that bivalves ingest carbon-fixing phytoplankton from the water column and deposit carbon-rich waste products on the seafloor in quantities disproportionate to surrounding bare sediments; this enhanced benthic pelagic coupling may result in bivalves acting as carbon sinks through their filtration processes (Fodrie et al. 2017). In contrast, mussels themselves are large heterotrophic organisms which respire CO_2 (and support benthic communities which also respire). The complexity and interactions of these processes need to be considered in tandem to ultimately determine the capacity of bivalves to sequester carbon. Such ecosystem based approaches are rarely employed (but see Filgueira et al. 2015).

Other important regulating services provided by bivalves include sediment stabilisation and enhanced coastal defence (Piazza et al. 2005, Scyphers et al. 2011). Relevant service valuations involving the species of interest are currently absent from the literature (again dominated by oyster research), but researchers have identified blue mussels as potentially relevant to wave attenuation in regions with small tidal amplitude (Bouma et al. 2014). These authors note that wave energy can be attenuated by any aboveground structure and that the rigidity of biogenic reefs can prove more beneficial than vegetation in some cases (Borsje et al. 2011, Bouma et al. 2014). Creating restored beds in the intertidal zone could therefore increase the capacity of restoration projects to serve as efficient breakwaters in New Zealand. Even at deeper depths it is likely that current reduction and sediment stabilisation are improved by mussel habitat, as large mussel clumps trap sediment and reduce physical erosion processes in ways which enhance local particle deposition (Meadows et al. 1998, van Leeuwen et al. 2010, Borsje et al. 2011). Subtidal mussel beds display enhanced microphytobenthic communities (Chapter 2), further increasing sediment stabilisation (through the production of polymers which alter sediment cohesion; Hope et al. 2020).

1.3.3 | Cultural Services

Cultural services are nonmaterial in nature and can enrich spiritually, cognition, or a person’s way of life. Examples include enhancing spiritual, traditional, or religious values, additional educational opportunities (for example, this PhD), increasing recreation or tourism, or developing a sense of place associated with the system. Considerably less research has involved bivalves and cultural services, although a recent literature review suggests bequest value (fulfilment in conserving a system for future generations) and existence value (satisfaction from knowing a system exists, even if not directly utilised) can be derived from oyster reefs (van der Schatte Olivier et al. 2020). There is also evidence that bivalves can help construct a person's sense of place (Carss et al. 2020). While difficult to quantify cultural services directly, it is possible to quantify taxpayers' willingness to pay for generated services (Grabowski & Peterson 2007), although such techniques have been criticised for their subjectivity (e.g. participant biases and differences in expendable income; Qu et al. 2021). Bivalve-related examples using this method that demonstrate modest improvements in water quality (~20%) have an estimated combined worth of US$222 million for sports fisherman, beach goers, and recreational boaters in the Chesapeake area alone (Grabowski & Peterson 2007). These estimates are likely conservative as they do not predict economic gains from additional tourism that might come from enhanced water quality, nor do they include value derived from user groups living outside the bay area, but they illustrate legitimate social benefits derived from restoration efforts.

Although contextually dependent, bivalves can be inspirational, iconic, and/or symbolic in nature; they adorn traditional and modern art pieces and are observed on sculptures and religious buildings worldwide (for examples, see Smaal et al. 2019, Carss et al. 2020). Havelock, New Zealand holds an annual mussel festival celebrating green-lipped mussels grown in the Marlborough Sounds (Fig. 1.2). Family-friendly events, live music, and competitions for best mussel floats, sculptures, and costumes are all part of this quirky, New Zealand tradition which exemplifies the cultural significance of green-lipped mussels to local people.

Restoration projects involving green-lipped mussels have a unique opportunity to engage with local iwi regarding traditional mahinga kai (natural resource cultivation) and food gathering practices. The customary capacity of Māori populations in New Zealand to provide kai moana (seafood) to family and guests has been greatly diminished over the past hundred years and represents a current restoration goal for tangata whenua (people of the land). On the North Island, for example, Ngāti Whātua Ōrākei, Ngāi Tai ki Tāmaki, Ngāti Awa, Te Whakatōhea, Ngāi Tūhoe, and Ngāti Manuhiri are actively involved in mussel restoration projects in Okahu Bay, East Auckland, Ōhiwa Harbour, and throughout the Warkworth region. Green-lipped mussels appear both physically in middens and symbolically in Māori legends (Fig. 1.2; Paul 2012, Smith 2013), illustrating a deep connection and substantial potential for restoration projects to reconnect indigenous populations with coastal environments. There will be an eventual need to balance restoration objectives differing between and within stakeholder groups (for example, participating in restoration to enhance extractive values or non-extractive values), ultimately altering where and how coastal restoration occurs in New Zealand. Customary fisheries have shown tentative success with other bivalves in New Zealand (Twist et al. 2016), and projects which develop sustainable harvesting practices could provide future management, restoration, and economic opportunities for local stakeholders.

Figure 1.2 Examples of cultural services provided by green-lipped mussels. Left: Māori middens excavated in 2020 from coastal islands of the Hauraki Gulf, featuring mussel shells. Photo with permission from Tāmaki Paenga Hira, Auckland Museum (“Excavating Otata Island: A Midden Revealed”). Right: Photos from the Havelock Mussel Festival. Photos from Stuff Limited (top), Emilee Benjamin (bottom right), and Marlborough 4 Fun Events (bottom middle).

1.3.4 | Supporting Services

Supporting services (e.g. nutrient cycling, primary production, and soil formation) underpin all other services and are usually characterised by indirect benefits to people (Millennium Ecosystem Assessment 2005). Potential overlap occurs with regulating services, but the effects of supporting services are typically felt over longer time scales. For example, the Millennium Ecosystem Assessment deems climate regulation a regulating service as related ecosystem changes are realised on timescales applicable to humans and their decision-making processes; in contrast, oxygen production through photosynthesis is deemed a supporting service, as impacts involving changes in the concentration of atmospheric O_2 are likely felt over much longer time periods. Also ambiguous in the ecosystem services discourse is the relationship of biodiversity and the fundamental role of habitat provisioning to the framework. These services are occasionally included in a box or umbrella which surrounds the entire framework (e.g. Fig. 1.1), evoking the idea that biodiversity resulting from the system of interest underpins all other categories of services it potentially generates (Lele et al. 2013). Some have chosen to include these concepts in the supporting services category (e.g. Rullens et al. 2019, Carss et al. 2020), while others opt to change the supporting services category, for example, to “habitat and ecological community services” (Thrush et al. 2013), acknowledging the diverse actors and networks of interactions which collectively influence the delivery of all other services.

Henceforth conceptualised as supporting services, increased biodiversity and habitat provisioning services provided by mussel beds are known to be significant. The three-dimensional, biogenic reef structure created by mussels provides a source of refuge for associated organisms and increases habitat diversity by providing hard substrate of otherwise limited availability for colonisation in soft-sediment habitats (Commito et al. 2008). However, previous work has not resolved how differences in bed spatial arrangement (e.g. dense beds vs. clumps and small patches) might affect biodiversity outcomes at varying scales of mobility (e.g. fishes vs. macrofauna), which would have implications for the delivery of other supporting services emanating from biodiverse communities on restored mussel beds. The majority of available studies generally support the idea that shellfish beds increase biodiversity (in the forms of species richness and abundance) compared to nearby soft-sediment locations (Norling & Kautsky 2008, Lejart & Hily 2011, McLeod et al. 2014, Norling et al. 2015). Studies in the US have documented a nursery function of oyster reefs, showing increased fish recruitment and production in these habitats (zu Ermgassen et al. 2016b). Others have acknowledged a similar potential for mussel bed systems as well (Seitz et al. 2014), but it is currently unclear how restoration might affect these outcomes.

Perhaps one of the most significant supporting services provided by coastal shellfish beds, mussels are involved in the recycling and transformation of nutrients in coastal soft-sediments (Dame 2012). Additionally, the bioturbation activities of local species (e.g. Lohrer et al. 2004, Meysman et al. 2006) may be influenced by the presence of these bivalves on the seafloor (e.g. Ysebaert et al. 2009). Microbial activity in surrounding sediments is stimulated through the creation of burrows and holes that modify sediment permeability, alter chemical gradients in pore water, and influence the rate of organic matter subduction and inorganic nutrient release to the water column. Coastal bivalve beds and the species which inhabit them therefore regulate and maintain the supply of carbon, nitrogen, and other nutrients that influence primary production rates and biogeochemical processes relevant to human well-being. The organisms and local environmental characteristics (e.g. sediment grain size, porosity, and concentrations of organic matter and microphytobenthos) involved in these processes tend to vary with location, and together influence service generation related to nutrient cycling. The influence of bivalves on complex biogeochemical cycles will ultimately determine how successful restoration projects are as a tool to recover ecosystem functionality and the extent to which regulating services (specifically nitrogen removal and carbon sequestration) can be reclaimed through physical intervention processes. Understanding feedbacks between mussels, associated biodiversity, and environmental characteristics influential to service provisioning will aid in the selection of restoration sites, allowing for future bed placement in locations which maximise the services we ultimately wish to enhance.

1.4 | New Zealand Restoration: Overview, Objectives, and Research Questions

Since 2013 over 200 tonnes of green-lipped mussels have been returned to the Hauraki Gulf through the combined efforts of local iwi stakeholders, nongovernmental organisations, the marine farming industry, and academics associated with the University of Auckland. Subtidal mussel restoration efforts are in their infancy globally, and we are in the preliminary stages of developing best science practices to support the success and upscaling of these projects, importantly identifying where and why these efforts should take place. Restoration sites were originally selected to capture variation associated with sediment grain size, local hydrodynamics, light penetration/suspended sediment concentration, benthic community composition, and other environmental factors. Restored mussels have formed both uniform and aggregated bed patterns by anchoring to the sediment, other mussel shells, and shell fragments through secretion of byssus threads. While the varying spatial distribution patterns seen in restored, green-lipped mussel beds likely result as a trade-off between predation susceptibility and intraspecific competition (e.g. Bertness & Grosholz 1985, Okamura 1986), it remains entirely unknown how that heterogeneity or the environmental conditions experienced at various restoration locations influence functions underpinning ecosystem services provided by green-lipped mussels. A better understanding in this regard will help us to answer both 'where' and 'why' questions related to our restoration efforts.

Current mussel restoration efforts are concentrated in shallow estuaries and coastal ecosystems which are dynamic, complex, and highly heterogeneous in nature. In addition to the various spatial arrangements observed on mussel beds, bed placement in uniquely diverse habitats means that mussels will both affect and be affected by different environmental conditions experienced. A major knowledge gap involves how bed spatial structure and environmental conditions influence ecosystem service provisioning, and whether or not these patterns are consistent across a variety of services. As ecosystem services are often generated by complex and interrelated ecosystem processes that vary over space and time, it is of current interest to understand how the direction and magnitude of provided services change in regards to spatial heterogeneity (both at the mussel patch scale and as a result of restoration location), thus informing the placement of future beds and addressing major knowledge gaps related to ecosystem functionality in restored mussel bed systems.

This thesis broadly evaluates the ability of green-lipped mussel restoration projects to revitalise historic ecosystem functionality in New Zealand's coastal soft sediments, taking into consideration the spatially heterogeneous effects of geographic location, mussel aggregation patterns, and local environmental characteristics on ecosystem service delivery. I explore the following questions:

- How effective are green-lipped mussel restoration projects in delivering valued ecosystem services? Are restoration sites more valuable than nearby soft-sediments in terms of service delivery?
- Where do we place mussel beds to maximise services we value? How important is spatial variation to this effect?
- How effective are mussel beds in regaining lost ecosystem functionality?

To answer these questions, I chose to explore the role of mussels in supporting three different ecosystem services across multiple restoration sites displaying different environmental characteristics (e.g. sediment grain size, local hydrodynamics, light penetration). Experimental design and sampling techniques varied in each data chapter to address specific questions relevant to the spatial scales I sought to consider (for example, taking multiple cores on and off mussel beds to survey macrofaunal communities at the bed-scale vs. benthic chamber techniques within individual beds to make comparisons at the mussel patch-scale; Fig. 1.3). To evaluate if bed spatial arrangement and placement consistently affected ecosystem service delivery, it was practical to consider multiple services. I chose three ecosystem services diminished by anthropogenic influence yet fundamental to ecosystem functioning (presented in the order they are addressed in the thesis):

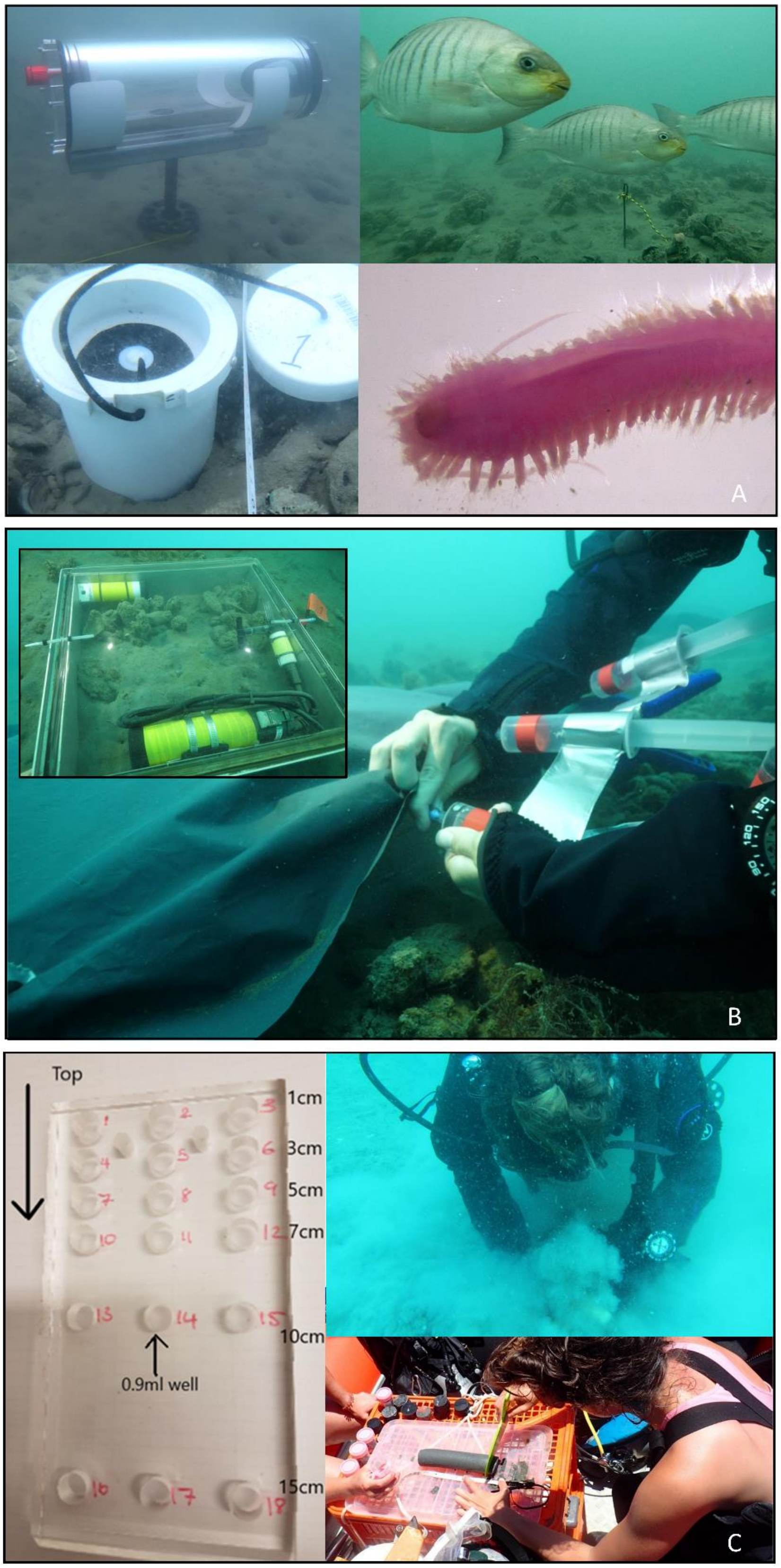

Figure 1.3 In-situ methodologies utilised in this thesis. Box A: unbaited remote underwater video setup (for mobile fish species; top) and sediment cores (contents sieved for macrofauna; bottom); Box B: an example benthic chamber (uncovered in inset to show contents) used in denitrification and carbon experiments; Box C: rapid organic matter assay plate (left) and a sediment core being recovered (right, top) and split into depth segments (right, bottom) for analysis of carbon content.

1. *Biodiversity enhancement.* Rapid declines in coastal species diversity have been recorded since the onset of industrialisation, and effects of the biodiversity crisis are being felt in marine ecosystems globally (Worm et al. 2006). Destruction of biogenic habitat such as mussel beds further exacerbates biodiversity loses in soft-sediment systems, as complex reef structures provide refuge and hard substrate necessary for colonisation by associated organisms.

Before examining other ecosystem services potentially provided by restored mussel beds, it is first worthwhile to demonstrate that green-lipped mussel restoration projects alter community composition in ways which would affect the provisioning of other ecosystem services. In addition to compositional changes, mussel restoration projects should illustrate increases in species richness and abundance of associated organisms relative to nearby unstructured habitats. Mussel restoration is theoretically predicted to increase biodiversity, as increases in habitat complexity are linked to enhanced biodiversity (Tokeshi 2009); however, the bivalve literature typically focuses on one aspect of associated diversity (e.g. epifauna or macrofauna exclusively; Saier 2002, Ysebaert et al. 2009), and few studies examine biodiversity in restored mussel beds over soft-sediments. It is also relevant then to determine how biodiversity effects compare across scales of mobility, as it is likely that mobile species (elasmobranchs and fishes) differ from less mobile species (e.g. macrofauna) in their utilisation of restoration sites. Understanding mechanisms that drive changes at different levels of mobility will then allow us to create future beds in locations which maximise specific communities and functions we wish to enhance. To date, one known study (McLeod et al. 2014) considers changes in diversity associated with green-lipped mussel beds. These sites however have either largely disappeared (‘relic reefs’) or are adjacent to mussel farms, thus necessitating verification of biodiversity and habitat provisioning benefits from current mussel restoration projects.

In chapter 2, I therefore seek to answer the following questions:

- Do restored mussel beds increase biodiversity relative to nearby soft-sediments devoid of mussels?
- Which species are significantly influenced by mussel restoration (positively or negatively)? How might reef utilisation relate to their mobility and lifestyle strategies?
- To what extent do restored mussel bed systems alter associated biological community structure? Does the magnitude of this effect vary with scales of mobility (e.g. sedentary invertebrates versus large mobile fishes)?
- How does restoration location influence diversity (species richness, abundance) and community composition?

2. *Nitrogen removal.* To meet a growing population’s demands, humans have significantly increased food and energy production, and consequently, the amount of nitrogen biologically available in marine systems, with anthropogenic nitrogen creation rates currently ten-times higher than they were during the preindustrial era (Galloway et al., 2004). It is well known that anthropogenic nitrogen loading has resulted in extensive coastal eutrophication, stimulating excessive plant growth (Anderson et al., 2002) and impacting the balance of organic matter production and metabolism in coastal ecosystems (Cloern 2001). As a result, nitrogen pollution has been deemed “one of the greatest consequences of human-

accelerated global change on the coastal oceans of the world" (Howarth and Marino, 2006).

Removal of bioavailable N through the denitrification process is therefore a crucial service in many coastal systems. While denitrification occurs both at land and sea, aquatic sediments are "far more important nitrogen sinks than terrestrial ones" (Bonaglia et al. 2014), with denitrification removing up to half of all nitrogen inputs into estuaries (Seitzinger 1988, Higgins et al. 2013). Coastal sediments are specifically optimal for this microbially-mediated process as they are typified by ample organic matter, low oxygen levels, and abrupt transitions between oxic and anoxic zones (Seitzinger et al. 2006).

Hillman et al. (2021) recently demonstrated that restored, green-lipped mussel beds enhance sediment denitrification and denitrification efficiency relative to nearby soft-sediment locations devoid of mussels. Questions remain regarding our ability to upscale reported denitrification rates given the heterogeneous spatial arrangements of mussels and the notable variability in denitrification rates observed at different restoration sites. It is therefore beneficial to further refine our understanding of nitrogen removal in these mussel beds by examining denitrification rates at the patch scale (within individual mussel beds) and by identifying notable environmental factors which significantly contribute to nitrogen removal rates observed across restoration sites.

In chapter 3, I answer the following questions:

- Does the presence/absence of mussels alter measured nitrogen fluxes at the patch scale?
- Where are nitrogen removal rates the greatest, and to what extent do local environmental conditions (grain size, local hydrodynamics, etc.) affect measured denitrification rates?
- Which environmental factors are most influential in predicting measured nitrogen fluxes?

3. *Carbon cycling.* Largely driven by the combustion of fossil fuels, atmospheric carbon dioxide concentrations have increased by over 40% since the pre-industrial era (Trenberth 2018). Human-induced climate change is a wicked, inter-generational problem with predicted global consequences in the form(s) of extreme weather events, flooding/sea level rise, and an increased loss of species which provide life-sustaining services to humankind (Trenberth 1998, Bosello et al. 2007, Rahmstorf & Coumou 2011, Nunez et al. 2019).

   Coastal ecosystems are dynamic zones of carbon transformation and change, obtaining large quantities of carbon and other nutrients from both riverine and open-ocean inputs; this allows coastal systems to support an abundance of life while significantly impacting the global carbon cycle (Chen & Borges 2009, Najjar et al. 2018). The role of coastal ecosystems in preserving and storing anthropogenic carbon sources is of current scientific interest (e.g. Duarte et al. 2005, Mcleod et al. 2011, Macreadie et al. 2019), yet virtually no work has elucidated the role of restored mussel populations in climate change mitigation strategies; significant research efforts are therefore needed to disentangle the complex, interrelated processes connecting bivalves to carbon cycling in coastal environments.

I seek to answer the following questions in chapter 4:

- How do mussel beds influence carbon cycling at the ecosystem level?
- Do the combined effects of shell formation, respiration, and biodeposition collectively make mussel beds sources or sinks of carbon?
- What are the operational time scales of relevant biological and biogeochemical processes and what capacity does mussel restoration have to influence these processes?

The majority of these questions were explored in-situ, through field experiments involving underwater surveying techniques (chapter 2), benthic incubation chambers (chapters 3 and 4), and sediment coring (chapter 4) at multiple restoration locations under varying environmental conditions (Fig. 1.3). Chapter 4 further expands on field data by combining literature data and other experiments to produce a conceptual model and first-order carbon budget. The combination of methods and techniques used here addresses current knowledge gaps related to the delivery of coastal ecosystem services at various spatial scales and allows us to evaluate the role of mussel restoration in generating benefits to society.

Chapter 2

Enhancing multiple scales of seafloor biodiversity with mussel restoration[*]

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2.1 | Abstract

Restoration projects are underway internationally in response to global declines in shellfish beds. As diverse biological assemblages underpin a variety of ecosystem services, understanding broader changes in biodiversity associated with mussel restoration becomes increasingly valuable to scientists and restoration practitioners. Studies generally show bivalve beds increase species richness and abundance, but results are scale-dependent and conditional on the mobility of specific communities observed. We examined biodiversity at multiple scales to determine how communities with varying levels of mobility are influenced by subtidal mussel restoration. Significant changes in assemblage structure were observed in both mobile fish and epifaunal communities, with enhanced species richness and total abundance of associated individuals. In contrast, we observed site-dependent effects of bivalve restoration on macrofaunal community structure and composition, with sheltered, harbour, mussel bed communities numerically dominated by detritivores accustomed to organically enriched, muddy sediments. Sediment organic matter significantly increased within mussel beds, and distance-based linear models showed that sediment organic matter was an important predictor of macrofaunal assemblage structure on mussel beds, highlighting the significance of benthic-pelagic coupling and biodeposition to soft-sediment communities. This study contributes novel methods and ecological insights on the role of species mobility and site selection in structuring restoration outcomes, better informing future mussel restoration efforts aimed at emphasising functionally-driven ecosystem services.

2.2 | Introduction

The United Nations' Decade on Biodiversity has recently ended (UN Resolution 65/161) and has shifted international focus towards restoration efforts (UN Resolution 73/284), yet a wide-spread biodiversity crisis continues to plague much of the planet (Driscoll et al. 2018, Díaz et al. 2019, Lees et al. 2020) with significant detrimental effects on marine ecosystems (Jackson et al. 2001, Sala & Knowlton 2006, McCauley et al. 2015). A vast ecological literature reaffirms that diversity in the marine realm (in the form of species richness, genetic variability, observed functional traits, etc.) is of great economic, scientific, and ecological value to mankind (Hooper et al. 2005, Worm et al. 2006, Beaumont et al. 2007). Biodiversity—simply considered here at the level of species richness and community composition—has also been deemed an ecosystem service in its own right for more intrinsic, abstract principles related to cultural, aesthetic, recreational, and existence value (Turpie 2003, Beaumont et al. 2007, Ruiz-Frau et al. 2013).

Biodiversity losses are predicted with increasing homogenisation of marine soft-sediment systems due to elevated disturbance regimes (Thrush et al. 2006). Such losses are exacerbated by declines in available biogenic habitat. Increasing anthropogenic disturbance has led to the global decline of shellfish beds (Gillies et al. 2018), known to form large, complex beds that provide refuge and resources for other organisms (Commito et al. 2008) and increase associated biodiversity due to increased habitat complexity (Tokeshi 2009). Especially in soft-sediment habitats, complex biogenic reef structures provide hard surfaces and a three-dimensional structure above the sediment surface, increasing habitat diversity in areas otherwise comprised of sand and mud. This bed structure becomes extremely important in predominantly soft-sediment ecosystems like

New Zealand’s Hauraki Gulf, where restoration projects utilising the green-lipped mussel (*Perna canaliculus*) are currently underway in response to their functional extinction due to anthropogenically-driven population collapse in the 1960s (Paul 2012).

Although negative impacts on community structure have been reported in instances where blue mussels outcompete other organisms for space and resources (Enderlein & Wahl 2004), a vast majority of studies demonstrate that shellfish habitats increase species richness and diversity of macrofauna, epifauna, or fish as compared to nearby bare sediments (Norling & Kautsky 2008, Lejart & Hily 2011, McLeod et al. 2014, Norling et al. 2015). In addition, it is known that a variety of coastal habitats (e.g. seagrasses, mangroves, kelp forests) serve as nursery grounds for commercially exploited species (Seitz et al. 2014). As a result of documented enhancement in fish recruitment and production, oyster reefs have been labelled ‘threatened nursey habitat’ in the United States (zu Ermgassen et al. 2016b). While a potential nursey function of mussel habitats has been recognised (Seitz et al. 2014), the role of mussel restoration in augmenting this nursery function has yet to be fully resolved.

Fundamental ecological interest lies in determining how community structure might change based on species mobility. It is likely that mobile species (e.g. elasmobranchs and fishes) will differ from less mobile species (e.g. sea cucumbers, whelks, or infaunal worms) in their utilisation of restored beds. For post-larval life stages, mobility is often correlated with body size. Piscivorous fishes and elasmobranchs (observed up to 2.5 m in these coastal waters) are considered to be the most mobile species in these soft-sediment systems, likely to utilise mussel beds temporarily to consume reef residents (Grabowski 2002). While also capable of covering notable distances in short periods of time, some smaller reef fishes (blennies, triplefins) are known to establish home ranges and exhibit site fidelity upon location of preferential habitat (Harding et al. 2020) and likely use mussel beds for greater periods of their lifetime. Mussel bed utilisation can also be tied to specific portions of the fish lifecycle (e.g., the documented association of juvenile snapper, *Chrysophrys auratus*, with *Atrina zelandica* beds/other structured estuarine habitats versus dramatic offshore movements during adulthood; Parsons et al. 2014). Together, these different lifestyle strategies make it difficult to predict changes in mobile fish community structure that may result from restoration efforts.

In contrast, smaller scales of mobility are observed in communities of epibenthic invertebrates (e.g. gastropod snails and sea cucumbers; 2-20 cm) which might take weeks to months to travel across estuaries in search of food or refuge, while comparable distances are achieved by mobile fish species in a fraction of this time. Similarly constrained by mobility, small encrusting invertebrate species (e.g. barnacles and ascidians; 1-10 cm) will settle on acceptable substrate throughout their adult life. The combined organic matter provisions and additional hard substrate provided by mussels likely attracts members of this less-mobile, epifaunal community, although the strength of this effect across environmental gradients has yet to be resolved.

Macrofaunal communities composed mainly of polychaetes and molluscs typically exhibit minimal mobility and patchy distributions. Distinct macrofaunal assemblages have been identified beneath shellfish beds, dominated by species that thrive in high organic matter and hypoxic sediment conditions typified by mussel reefs (Callier et al. 2009, Ysebaert et al. 2009). Our restoration sites exhibit a range of sediment grain sizes, porosity, organic matter, and chlorophyll content, leading to context dependent impacts of mussel beds on sediment-dwelling communities.

Utilising sampling techniques with various levels of resolution, this study aims to document entire biological communities, containing elements separated by ranging capacity (mobile fishes and elasmobranchs, epifaunal invertebrates, and macrofauna) to determine how mussel restoration projects affect biodiversity. Surveys were repeated on multiple mussel beds and at nearby control soft-sediment locations without mussels, allowing for meaningful comparisons to be made between restoration locations while ultimately evaluating the ability of current mussel restoration projects to re-establish complex biological assemblages. We hypothesised that: 1. as mussel beds modify local environmental conditions experienced by resident organisms, shifts in community structure should be most evident at the least-mobile, macrofaunal scale (as opposed to changes seen in mobile fish communities); 2. mussel restoration would significantly enhance epifaunal communities as a result of additional settlement substrate above soft-sediments; and 3. variations to the sediment environment and spatial aggregation patterns observed in mussel beds would result in significant changes in community structure across restoration sites.

2.3 | Methods

2.3.1 | Study Area

Between 2016 and 2019, adult green-lipped mussels (*Perna canaliculus*) were collected from mussel farms and transplanted to soft-sediment locations of similar depth (5-15 m). Chosen sites varied in terms of sediment composition, ranging from muddy sands to fine and medium sands (Table 2.1). Mussels were unloaded off a barge and sunk to the seafloor, creating multiple beds in the Hauraki Gulf on the north-eastern side of New Zealand's North Island (Fig. 2.1). The majority of beds were created using 10-20 tonnes of mussels (~ 80-100 mm shell length) which clumped over time to form restored mussel beds ~ 20-30 m^2 in size. Three beds were located near the mouth of Mahurangi Harbour (Pukapuka, Lagoon Bay, and New Lagoon Bay; PP, LB, and NLB respectively), and two were located adjacent to coastal islands in Kawau Bay (Motuora and Motoketekete; MR and MK). Beds displayed various spatial aggregation patterns at the time of study (Sea et al. 2021), ranging from small clumps at Kawau Bay sites (~ 10-15 individuals m^2) to generally larger clumps with smaller gaps at LB and PP (~ 25-75 m^2). Restoration sites utilised in this study therefore varied in terms of observed sediment characteristics and mussel spatial aggregation patterns.

Table 2.1 Mean and range of environmental characteristics measured at each site, separated by restoration status (inside vs. outside mussel beds). Mud is comprised of silt + clay (< 63 μm). Coarse sand > 500 μm. SOM = sediment organic material. Sites arranged from inner Mahurangi Harbour to outer Kawau Bay. MK sediment data from Hillman et al., 2021. Pukapuka = PP, Lagoon Bay = LB, New Lagoon Bay = NLB, Motuora = MR, and Motoketekete = MK.

Site	Date Established	Temp (ºC)	Salinity (ppt)		Mud Content (%)	Coarse Sand (%)	SOM (%)	Chl *a* Content (ug g^{-1})
PP	November 2018	21	33.3	in	21.9 (17.4-34.2)	1.3 (1.0-2.4)	3.5 (2.8-4.8)	8.3 (5.7-10.0)
				out	21.4 (18.0-23.8)	1.1 (0.3-1.3)	2.9 (2.0-3.9)	5.7 (5.2-6.4)
LB	November 2018	21	32.6	in	22.8 (16.6-33.9)	0.7 (0.0-3.4)	3.9 (3.3-4.6)	6.6 (4.6-7.7)
				out	22.2 (15.0-27.1)	0.5 (0.0-1.1)	2.9 (2.5-3.2)	4.5 (3.9-5.2)
NLB	July 2019	21	32.4	in	24.2 (14.6-44.1)	1.7 (0.7-4.4)	5.9 (4.1-8.0)	10.9 (5.2-14.0)
				out	17.4 (13.7-20.6)	2.6 (0.3-5.0)	2.8 (2.5-3.2)	2.7 (1.5-3.3)
MR	November 2017	21	31.5	in	4.6 (2.3-7.1)	3.7 (1.9-4.4)	2.8 (2.5-3.3)	4.4 (2.9-6.4)
				out	3.6 (2.7-6.3)	5.0 (2.4-7.5)	2.2 (1.8-2.6)	4.6 (3.4-6.1)
MK	October 2016	21	32.8	in	3.6 (3.2-3.7)	7.5 (6.4-8.7)	2.0 (1.8-2.1)	8.6 (8.2-8.9)
				out	3.3 (2.7-3.9)	5.7 (5.1-6.2)	1.8 (1.7-1.9)	7.4 (7.0-7.9)

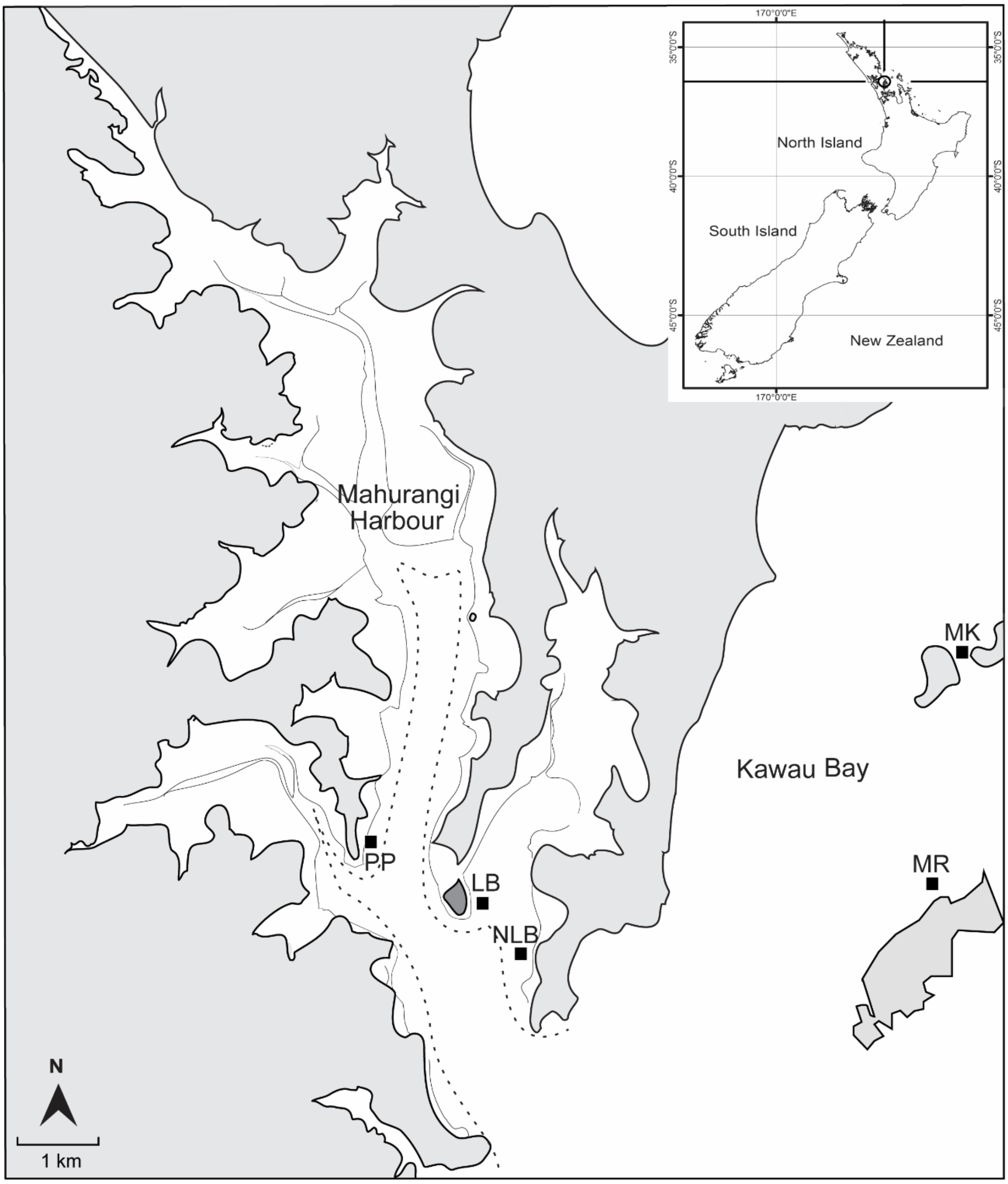

Figure 2.1 Each rectangle represents a paired study site, including a mussel reef and soft-sediment control (~ 5m away), located either in Mahurangi Harbour or Kawau Bay, New Zealand. Pukapuka = PP, Lagoon Bay = LB, New Lagoon Bay = NLB, Motuora = MR, and Motoketekete = MK.

To investigate changes in diversity and biological community structure associated with restoration efforts, three separate methods were utilised at each site which sampled at scales relevant to the ranging capacities of each community of interest. Un-baited remote underwater video methods used to observe mobile species (fishes, sharks, rays) were conducted at all five beds. For logistical reasons, video transects and macrofauna cores (to visualise epifauna and macrofauna respectively) were conducted at four of the mussel beds (PP, LB, NLB, and MR). Methodologies were repeated ~ 5 m away from each mussel bed in nearby soft-sediments devoid of biogenic structure so that each site had a mussel bed and control location for comparison purposes. All surveys were conducted between November 2019 and February 2020.

2.3.2 | Un-baited Remote Underwater Video Methodology

To observe mobile species, 1-2 video recording devices (GoPro Hero7 Silver, resolution 1440p) were situated in the middle of each mussel bed and placed in an underwater housing system (height = 0.35 m). Such visual census methods are non-extractive, reduce size/species selectivity of other fish sampling techniques (Wells et al. 2008, Bacheler et al. 2013), and avoid behavioural changes associated with diver surveys (Emslie et al. 2018). Recent fish community research on various Australian coastal habitats detected no assemblage differences between baited and un-baited video surveys (Piggott et al. 2020), and no bait was used in the present study. Our video cameras were connected to voltaic USB battery packs and set to record continuously during daylight hours. Resulting footage was trimmed so that all videos were the same length (just over 5 hours) and each video started 1-2 hours ahead of the turn of the tide.

To account for differences in water clarity between sites, a peg 14 cm high was inserted 0.75 m away from the face of the camera housing. Species that crossed in front of this peg were identified and their abundances recorded by Citizen Science volunteers. These volunteers were given the same instructions and training on underwater species identification. 3-5 independent volunteers coded each video. All data resulting from the same video were examined simultaneously for discrepancies in species identification, number of individuals observed, and position relative to the counting peg. Any discrepancies above 40% (e.g., more than 1/3 or 2/5 Citizen Scientists) were flagged and the time-point in question reviewed by the lead author to obtain a master dataset used in later analysis. Total abundance (the sum of all observed individuals of one species) and species richness (total number of taxa identified over the sampling period) were recorded and used in data analysis.

2.3.3 | Video Transect Methodology

To quantify benthic invertebrates and other epifaunal species, perpendicular transects, intersecting the bed centrally (c 20 m long), were placed across each restored mussel bed. With the aid of a plumb line (height = 20 cm) defining the distance from the camera lens to the reef, scuba divers videoed each transect with the camera lens at a 45-degree angle. For consistency, visible organisms from video footage were counted by a single, trained citizen scientist (the resulting dataset reviewed in its entirety by the lead author) and total abundances recorded for each transect.

Taxa were classified to family or species level where possible. Although frequently observed along video transects, species of small fish from the family Tripterygiidae (triplefins) were more easily seen in static videos and less likely biased by diver presence (Emslie et al. 2018) and were excluded in this portion of the analysis.

2.3.4 | Macrofaunal Sampling Methodology

At each mussel bed, eight sediment cores (10 cm diameter, 10 cm deep) were randomly taken along the longest transect used in the diver video sampling to obtain a representative sample of the macrofaunal community. Cores were sieved (500 µm mesh) and sieve contents preserved in 70% isopropyl alcohol and stained with rose bengal. Macrofauna were sorted and classified at the lowest taxonomic group possible.

To characterise sediment characteristics at each site, two small sediment cores (1.9 cm diameter, 3 cm deep) were collected next to each of the eight macrofauna cores. One sediment core was used for chlorophyll *a* and grain size analysis while the other was used for sediment porosity and organic matter content. All sediment samples were kept on ice in the dark and frozen at the laboratory until later analysis.

Percentage sediment organic matter (SOM) was determined by loss on ignition (Dean 1974). Sediments were left in a 60 °C oven until fully dried, and then weighed before and after combustion at 500 °C for five hours. Sediment porosity was calculated as the difference between wet and dry weight (g), divided by core volume (cm^3). Samples for sediment grain size (~20 g each) were digested with 6% H_2O_2 and rinsed after 48 hours. Roughly 15 mL of 5% Calgon was added to each sample prior to analysis with a Malvern Mastersizer (ATA Scientific). Chlorophyll *a* was extracted from 1 g of freeze-dried sediment with 3 mL 90% acetone. Optical density of extracts were measured at 664, 665, and 750 nm with a UV-Vis spectrophotometer (Thermo Scientific, Multiskan Sky) before and after hydrochloric acid acidification (0.1 mL of 0.1M HCl). Values at 750 nm were subtracted from values at 664 and 665 nm to correct for turbidity. Sediment chlorophyll *a* content was calculated using equations based on 90% acetone extraction (Lorenzen 1967).

2.3.5 | Multivariate Statistical Analysis

Our strategy of sampling different size/mobility levels of seafloor biodiversity resulted in three separate multivariate data sets: mobile species data (from 1-2 remote videos at each mussel bed and control location), epifaunal species data (two diver transects for each mussel bed/control pair at sites PP, LB, NLB, and MR), and soft-sediment macrofauna data (from eight cores for each mussel bed and each control at sites PP, LB, NLB, and MR). Multivariate data consisted of counts from a total of 18, 18, and 88 taxa for un-baited remote underwater videos, transects, and macrofauna cores, respectively.

For all three levels of the biological community, we used permutational multivariate analysis of variance (PERMANOVA; Anderson 2001) to determine if community structure significantly varied by ‘Site’ (PP, LB, NLB, MR, and MK) and by ‘Status’ (mussel bed or control). For each dataset we created a Bray-Curtis similarity matrix calculated from square-root transformed abundance data and tested statistical significance using 9999 permutations of residuals under a reduced model (chosen significance level of $\alpha = 0.05$). When Status (mussel bed vs. control) was a significant main effect or interaction term, similarity percentage analysis (SIMPER, One-Way Design; Factor = Status) was used to determine the percent of similarity of samples and to identify which species most highly contributed to observed differences between groups. Community data resulting from each of the three methods was visualised in multivariate space using nonmetric multi-dimensional scaling (nMDS), based on the same Bray-Curtis resemblance matrix constructed from the corresponding square-root transformed abundance data.

To determine if the best subset of environmental factors capturing multivariate macrofaunal assemblage structure varied between mussel bed and non-mussel bed locations, distance-based linear models (DISTLMs; McArdle & Anderson 2001) were ran separately on resemblance matrices constructed from separate mussel bed and control community data. Our models utilised environmental data (SOM, chlorophyll *a*, porosity, percentage mud content and percentage coarse sand) as explanatory variables, and the community resemblance matrix (obtained using the Bray-Curtis similarity measure on square-root transformed abundance data) from corresponding macrofauna cores as the dependant variable. Parsimonious models were informed by Akaike information criterion (AIC) and constructed using a backwards elimination procedure. Predictor variables were log-transformed as necessary to meet assumptions of normality, and correlation among explanatory variables was examined; due to high correlation ($R^2 > 0.85$) between percentage grain size parameters, percent medium sand was excluded from models. All multivariate statistical analyses were conducted in PRIMER v7 (Clarke & Gorley 2015) with the add-on package PERMANOVA+ (Anderson et al. 2008).

2.3.6 | Univariate Statistical Analysis

The effects of Site (MK, MR, NLB, LB, and PP) and Status (mussel bed or control) on univariate measures of species richness (total number of species) and abundance (total number of individuals) were investigated for all three biological communities using 2-way ANOVA. Q-Q plots, Shapiro-Wilk tests, and Levene’s tests were used to check assumptions of normality and homogeneity of variance, and values log-transformed where necessary. Significance levels were set to $\alpha < 0.05$, and standard errors (SE) were used to assess the precision of mean values. All ANOVA analyses were conducted using the R statistical package (version 4.1.0).

As we believed that the greatest changes in assemblage structure would be apparent in the macrofaunal community, we complimented our multivariate analysis of macrofauna with ranked species abundance plots. For each mussel bed and control, all eight replicate cores were pooled and the relative abundance (percentage of total abundance observed) plotted against the increasing log-ranked species (Clarke & Gorley 2015). This was done to visualise potential dominance of individual species, to compare relative evenness of macrofaunal communities, and to observe changes in the number of rare species (by comparing curve lengths) between sites. Dominance curves were created in PRIMER v7 (Clarke & Gorley 2015).

2.4 | Results

2.4.1 | Mobile Species

Communities of mobile species utilising mussel beds were distinctly different from those found on nearby bare sediments (PERMANOVA; $PsF_{1,11} = 4.40$; p = 0.031; Table 2.2; Fig. 2.2). The nMDS ordination revealed a clear separation of mussel bed and non-mussel bed communities across a single axis (Fig. 2.3A). The interaction between Site and Status was also significant (PERMANOVA; $PsF_{4,11} = 3.48$; p = 0.027), indicating mussel bed effects were site dependent. SIMPER analysis revealed a substantial dissimilarity (62.94 %) between mussel bed and non-mussel bed assemblages, a change largely driven by increases in snapper and triplefin abundance associated with mussel restoration (Table AI.1). All six taxa identified as having a major contribution to this dissimilarity (triplefins, snapper, parore, mackerel, mullet, and trevally; Tripterygiidae, *Chrysophrys auratus*, *Girella tricuspidata*, *Trachurus* spp., Mugilidae, and *Pseudocaranx dentex* respectively) were found in higher abundances on mussel beds than in nearby soft-sediments.

The total abundance of mobile individuals observed on mussel beds was significantly (up to 20x) higher than off the beds (2-way ANOVA; $F_{1,2} = 22.38$; $p = 0.042$; Table AI.3), with greatest abundances recorded within Mahurangi Harbour. An increase in species richness (the number of species observed) was also apparent with mussel restoration (2-way ANOVA; $F_{1,2} = 62.74$; $p = 0.016$), a pattern observed across all five sites (Table 2.3; Fig. 2.2).

Table 2.2 Permutational multivariate analysis of variance results based on the Bray-Curtis similarity measure for square-root abundance data of: (**a**) mobile species; (**b**) epifauna and benthic invertebrates; and (**c**) macrofaunal species. Statistically significant *p* values (α = 0.05) are bold. Tests conducted using 9999 permutations under a reduced model. MR= Motuora, NLB = New Lagoon Bay, LB = Lagoon Bay, and PP = Pukapuka.

Mobile species					
Source	*df*	SS	MS	F	P
Site	4	12872.0	3217.9	12.28	**0.0102**
Status	1	3974.0	3974.0	4.40	**0.0309**
Site x Status	4	3652.7	913.2	3.48	**0.0265**
Residual	2	524.2	262.1		
Total	11	21923.0			
Epifauna/ benthic invertebrates					
Source	*df*	SS	MS	F	P
Site	3	14343	4781.0	2.67	**0.0015**
Status	1	12839	12839.0	4.03	**0.0287**
Site x Status	3	9548.9	3183.0	1.78	**0.0465**
Residual	8	14338	1792.3		
Total	15	51069			
Macrofauna					
Source	*df*	SS	MS	F	P
Site	3	48559.0	16186.0	11.03	**0.0001**
Status	1	7561.3	7561.3	3.27	0.0712
Site x Status	3	6946.5	2315.5	1.58	**0.0079**
Residual	56	82158.0	1467.1		
Total	63	145220.0			

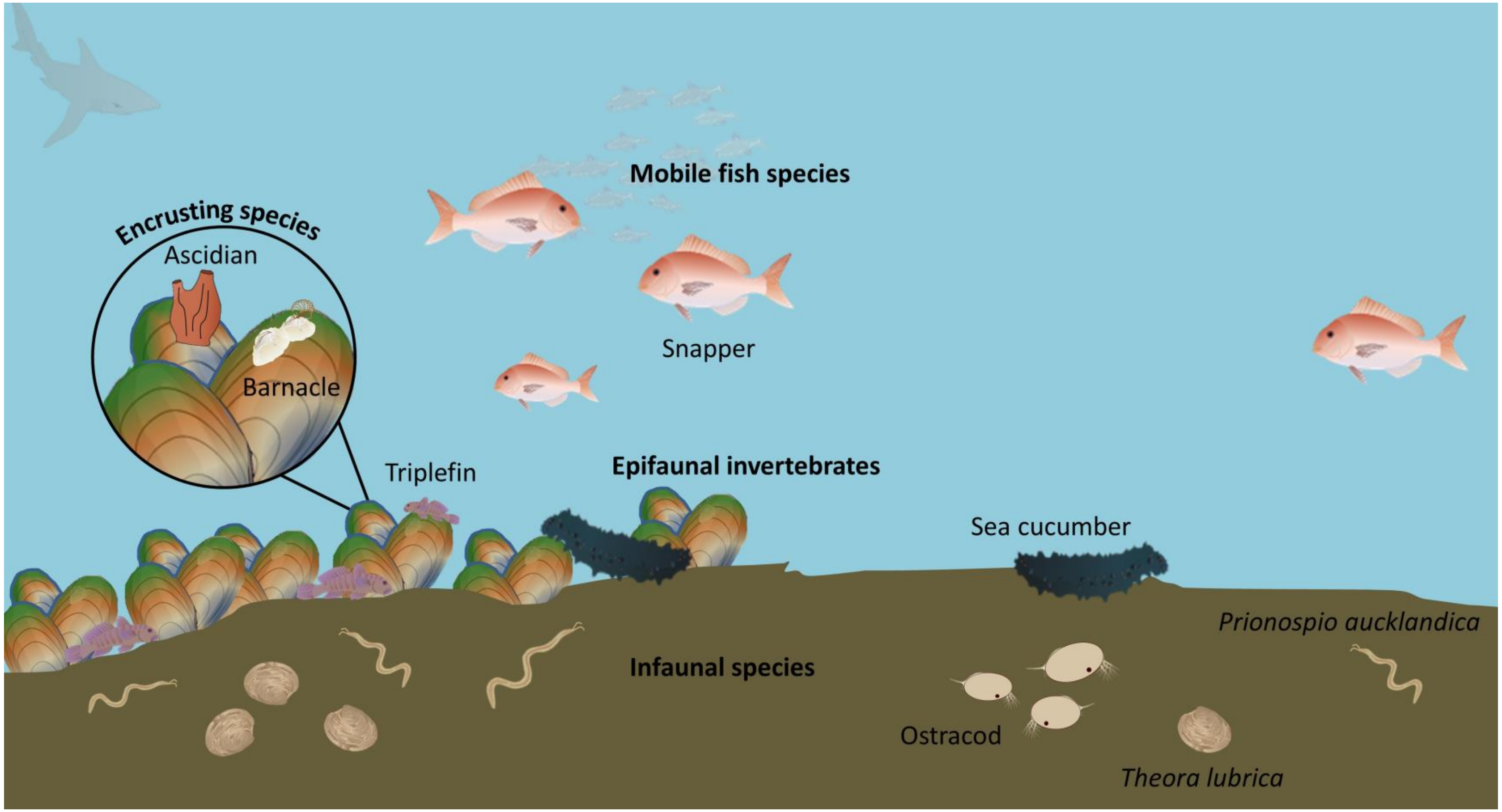

Figure 2.2 Conceptual diagram illustrating effects of subtidal mussel restoration on biological communities, differentiated by scales of mobility. Mobile communities of triplefins and commercially important fish species such as snapper (*Chrysophrys auratus*) are found in higher abundances on mussel beds than soft-sediment control sites, while highly transient elasmobranchs were not found to significantly differ with habitat type. Species richness and abundance of epifaunal invertebrates and encrusting species significantly increase with additional organic matter and hard substrate provided by mussels. Location-dependent changes in macrofaunal assemblage structure result from restoration efforts, with bivalve and polychaete detritovores more abundant beneath muddy, organically enriched mussel beds. Some symbols adapted from Integration and Application Network (ian.umces.edu/media-library).

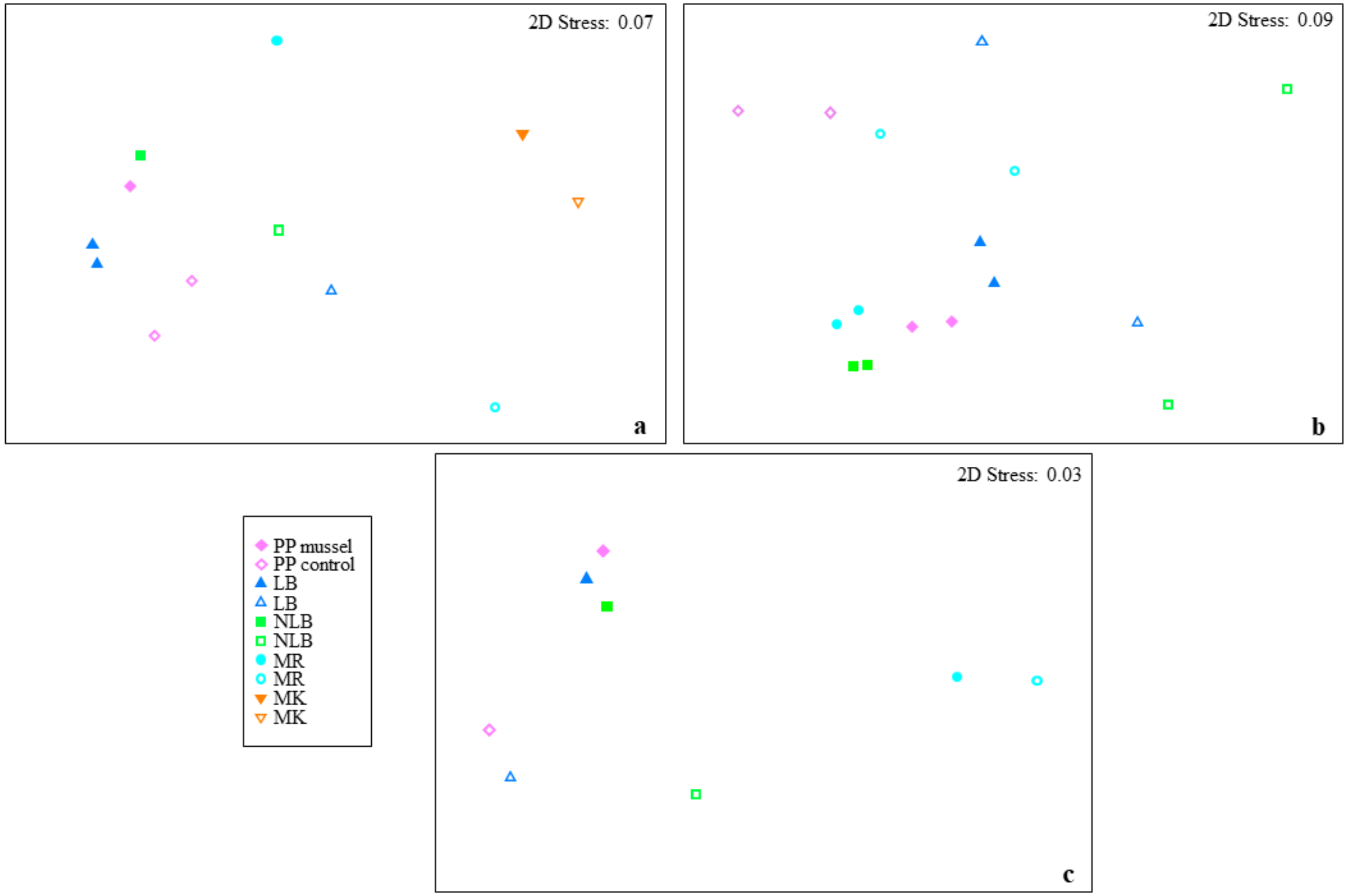

Figure 2.3 Two-dimensional non-metric multidimensional scaling (nMDS) plots for visualisation of differences in assemblage structure observed between sites (colour) and mussel bed status (fill). (a) Mobile community (points represent all available reps from un-baited video cameras); (b) epifauna/benthic invertebrate community (ordination showing two transects per site); and (c) macrofaunal community (calculated from the distance between centroids where n = 8 sediment cores for the combined factor SiteStatus). Ordinations created from Bray-Curtis similarity matrices on square-root transformed data using PRIMER v. 7 (Clarke & Gorley 2015; available at http://www.primer-e.com/).

2.4.2 | Epifauna and Benthic Invertebrate Diversity

Encrusting invertebrates and large epifaunal species were rare in soft-sediments without mussels. A significant increase in total abundance—over 100-fold in some cases—was observed at mussel bed locations (2-way ANOVA; $F_{1,8} = 32.24$; p = <0.001; Fig. 2.2; Table 2.3; Table AI.4). Mussel restoration resulted in significant increases in species richness (albeit less substantial; up to 5-fold) as well (2-way ANOVA; $F_{1,8} = 119.12$; $p < 0.001$). A total of 17 taxa were found across different mussel beds, while only 11 were found on control sediments.

Site and Status (bed vs. control) were both significant main effects in the PERMANOVA analysis ($PsF_{3,15} = 2.67$; p = 0.002; and $PsF_{1,15} = 4.03$; p = 0.029 respectively), suggesting that epifaunal communities on mussel beds were significantly different from those inhabiting surrounding bare sediments. A significant interaction between these main factors was also apparent (PERMANOVA; $PsF_{3,15} = 1.78$; p = 0.047; Table 2.2). These relationships are displayed in an nMDS plot (Fig. 2.3B), with transects from mussel bed communities markedly separated from controls. Communities from soft-sediment control locations were more spread apart in the ordination space, suggesting that the closely grouped restoration sites were, overall, more similar in assemblage structure. SIMPER analysis confirms that the similarity between mussel bed sites (47.23 %) was much higher than the similarity between control sites (13.28 %), and that species utilising the two habitat types were very different (88.76% dissimilar; Tables AI.1 and AI.2). This difference was largely driven by encrusting species such as barnacles and ascidians, but all taxa which contributed to the observed dissimilarity between habitat types were found in higher abundances on mussel beds.

2.4.3 | Macrofaunal Diversity

We found between 8.8 ± 1.3 and 25.5 ± 2.5 total macrofauna species at mussel bed locations, and 8.8 ± 0.9 and 26.1 ± 1.8 species at non-mussel bed locations. Species richness significantly changed with site (2-way ANOVA; $F_{3,56} = 53.52$; $p = < 0.001$), with more species found at MR than NLB, PP, and LB. The existence of mussel habitat, however, did not affect species richness (2-way ANOVA; $F_{1,56} = 1.72$; $p = 0.195$) or total abundance of counted individuals (2-way ANOVA; $F_{1,56} = 0.71$, $p = 0.403$) at the macrofaunal level.

PERMANOVA detected a significant interaction between Site and Status for macrofaunal assemblages ($PsF_{3,63} = 1.58$; $p = 0.008$). As reflected in the nMDS ordination, the way mussel beds affected macrofaunal assemblage structure varied by site; communities underneath beds at PP, LB, and NLB were distinctly different from their control counterparts, while the mussel bed assemblage at site MR—although highly distanced from PP, LB, and NLB in the ordination space—appeared to be more similar to its control sediments (Fig. 2.3C). A large number of species contributed to the overall dissimilarity (67.68 %) between mussel bed and control sediments (Table AI.1), with the bivalve mollusc *Theora lubrica* and polychaete worms from the family Spionidae notably responsible. Both taxa are well-accustomed to muddy, organically enriched sediments and were present in higher densities at mussel bed locations.

Table 2.3 Summary of univariate diversity indices for all three community data sets. Sites arranged over a decreasing mud gradient. Lagoon Bay = LB, Pukapuka = PP, New Lagoon Bay = NLB, Motuora = MR, and Motoketekete = MK. Asterisks (*) denote significant differences (2-way ANOVA, $p < 0.05$) between mussel beds and controls for the identified diversity index. Where applicable, data represent the mean ± SE.

		Mobile Species (Un-baited Remote Videos)		Epifauna/ Benthic Invertebrates (Video Transects)		Macrofauna (Sediment Cores)	
Site	Status	Species Richness*	Total Abundance*	Species Richness*	Total Abundance*	Species Richness	Total Abundance
LB	Mussels	7.0	212.5	5.5 ± 0.5	46.5 ± 5.5	8.88 ± 1.29	28.13 ± 5.98
	Control	5.0	34.0	1.5 ± 0.5	3.0 ± 2.0	8.88 ± 0.91	18.13 ± 3.44
PP	Mussels	6.0	316.0	10.0 ± 0.0	229.0 ± 97.0	12.37 ± 0.96	35.13 ± 3.71
	Control	5.5	126.0	2.5 ± 1.5	7.5 ± 0.5	11.88 ± 1.17	27.00 ± 4.68
NLB	Mussels	9.0	353.0	5.5 ± 0.5	537.0 ± 32.0	10.00 ± 1.34	37.00 ± 5.30
	Control	6.0	49.0	1.0 ± 1.0	3.0 ± 3.0	15.25 ± 0.96	49.38 ± 10.82
MR	Mussels	9.0	149.0	11.5 ± 0.5	446.0 ± 19.0	25.50 ± 2.46	100.38 ± 14.18
	Control	3.0	7.0	5.0 ± 0.0	13.0 ± 3.0	26.13 ± 1.80	99.63 ± 11.05
MK	Mussels	6.0	16.0	n/a	n/a	n/a	n/a
	Control	5.0	6.0	n/a	n/a	n/a	n/a

Differences in mud content (% < 63 µm) were apparent between sites (2-way ANOVA; $F_{3,56} = 148.05$, $p < 0.001$), with PP, LB, and NLB characterised by significantly muddier sediments than MR (Table AI.5). Percentage coarse sand (> 500 µm) was significantly higher at MR than at PP and NLB (all of which were significantly higher than LB). SOM significantly increased from 2.70 ± 0.08 % at control sediments to 4.01 ± 0.25 % at mussel beds, although the strength of this effect varied with site (2-way ANOVA; $F_{3,56} = 11.82$, $p < 0.001$). Similarly, chlorophyll *a* content significantly varied with mussel bed status (2-way ANOVA; $F_{1,56} = 55.45$, $p < 0.001$), but effect direction and strength varied with site (2-way ANOVA; $F_{3,56} = 22.11$, $p < 0.001$).

DISTLMs captured 39.18 % of the variation in macrofaunal community structure from soft-sediment control sites and 29.11 % of the variation from mussel bed communities (Table 2.4). Sediment grain size characteristics (percent mud and coarse sand content) were important explanatory variables in models for both mussel bed and non-mussel bed communities, but chlorophyll *a* was selected in soft-sediment control models while SOM was retained in mussel bed models. The 10.07 % decrease highlights that our chosen environmental parameters do a better job of explaining community variation in soft-sediment systems devoid of mussels.

Ranked species abundance plots revealed differences in macrofaunal assemblage evenness across sites and by mussel bed status (Fig. 2.4). Control sites at PP, LB, and NLB displayed higher evenness (or lower dominance, as indicated by a lower, more gradual slope) than their mussel bed counterparts. MR was the only mussel bed exhibiting higher evenness than nearby control sediments. All other mussel bed communities (PP, LB, and NLB) appeared to be numerically dominated by one or two species which represented between 38 and 55% of the total sample population, while the percentage dominance of the most abundant species at bed MR was comparable to all controls (around 20%). Abundance plot lengths suggest that restoration had a minimal, variable effect on the number of rare species observed, which instead varied as a function of site (MR exhibiting the greatest number of rare species).

Table 2.4 Summary table of distance-based linear model (DISTLM) results, showing chosen environmental predictors best fit to corresponding response communities. Results shown are for models with lowest AIC values. Analyses based on Bray-Curtis similarity measures for square-root transformed macrofauna abundance data, constructed separately from mussel bed and control sediment cores. SOM = sediment organic matter. Significant effects ($p < 0.05$) are shown in bold.

Response community	AIC	Total variation explained (%) by chosen model	Predictors chosen	Pseudo-F	p
Mussel beds	243.03	29.11	Percentage mud content	7.20	**<0.001**
			Log (coarse sand content)	4.32	**<0.001**
			SOM %	4.17	**0.001**
Control sediments	237.82	39.18	Percentage mud content	12.15	**<0.001**
			Log (coarse sand content)	6.68	**<0.001**
			Chlorophyll *a*	1.96	0.059

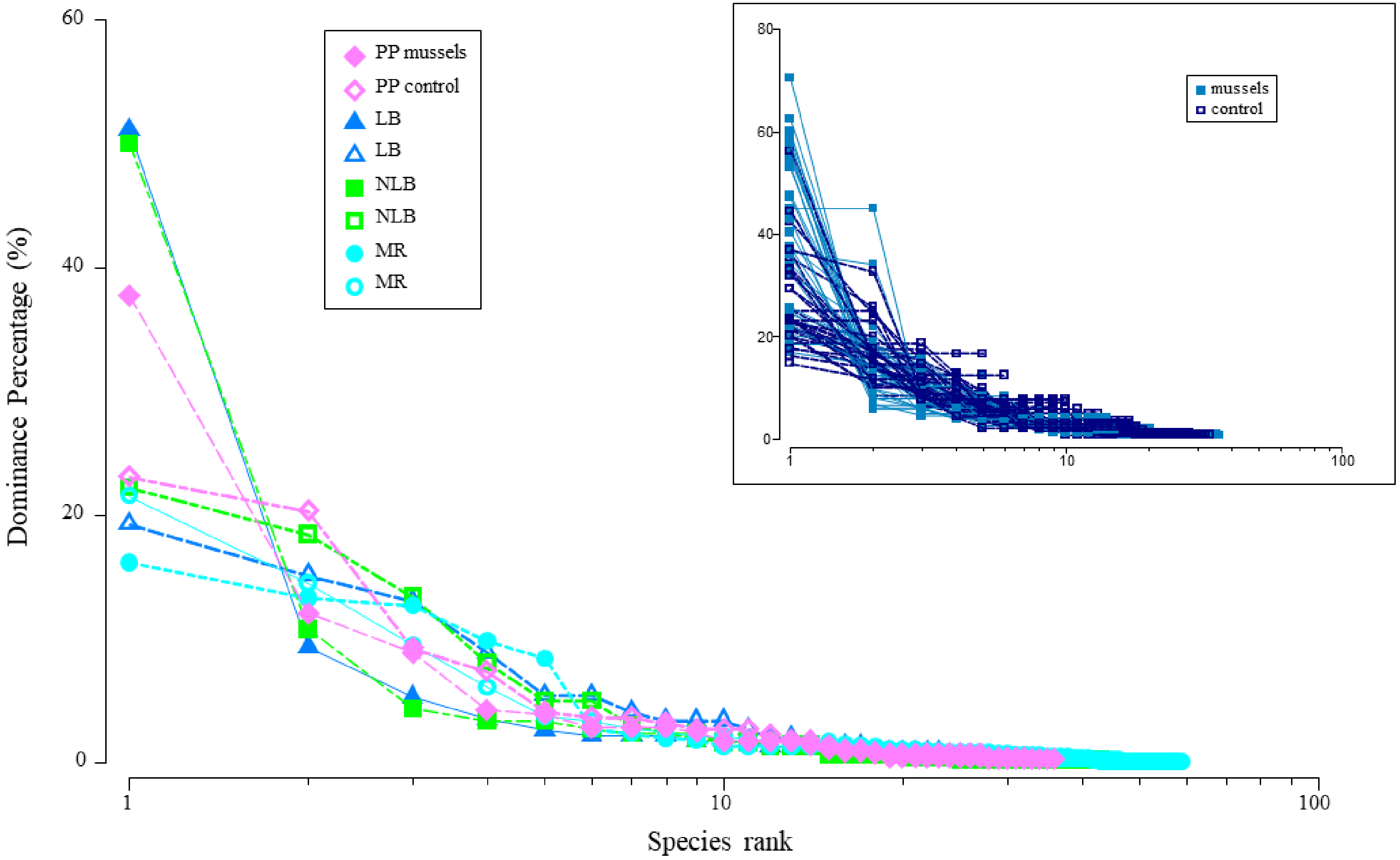

Figure 2.4 Ranked species abundance plot for pooled macrofauna cores (n = 8), split by site (colour) and mussel bed status (fill). Inset: dominance curves for all macrofauna cores (n = 64), split by mussel beds (light blue, filled) and soft-sediment controls (dark blue, open), highlighting increased dominance percentage in mussel bed communities. Labels for y-axis (relative abundance as a percentage of total abundance observed) and x-axis (species rank on log scale) are the same for both plots.

2.5 | Discussion

A majority of studies examining effects of benthic bivalves on associated species have focused on one aspect of the biological community (e.g. Saier 2002, Peterson et al. 2003, Callier et al. 2009, Ysebaert et al. 2009). This study documents changes in entire communities, highlighting that ranging capacity and site-specific environmental attributes differentially impact community structure at restoration sites. We documented significant changes in species richness, abundance, and assemblage structure in both mobile and epifaunal communities associated with subtidal, restored mussel beds, concluding that our restoration efforts have affected population and community level processes (summarised in Fig. 2.2). This in-situ study is first in reporting changes in species composition and assemblage structure on New Zealand's subtidal restored mussel beds, while describing methodologies to be utilised in future monitoring efforts aimed at evaluating restoration progress against ecological objectives.

Epifaunal communities with minimal ranging capacity were found to be directly linked to mussel resources, both physically (with mussels as a source of habitat) and trophically (with mussels as a source of organic carbon and nitrogen). Extensive increases in species richness and diversity in epifaunal communities are likely a result of shell surfaces providing surface complexity, settlement substrate, and a source of refuge to colonisers (Gutiérrez et al. 2003). Indeed, SIMPER analysis revealed that barnacles and ascidians (sessile and encrusting species almost never observed outside mussel habitat) highly contributed to the dissimilarity between mussel bed and non-mussel bed locations. Even for non-attached species, however, diversity increases in subtidal mussel beds have previously been documented (Saier 2002) and are a likely result of organic matter additions associated with bivalve biodeposition (Norkko et al. 2001, Commito et al. 2008, Norling et al. 2015). Sea cucumbers and small gastropod snails observed in this study were found in higher abundances on restored beds and also contributed significantly to the high dissimilarity (> 88 %) between mussel and control locations. The constrained mobility of these species together with pronounced differences in measured diversity indices suggests a clear coupling of highly diverse epifaunal communities to restoration efforts. The fundamental link between restored beds and biodiversity enhancement at the epifaunal scale ultimately influences resource provisioning to communities at other organisational scales (as observed epifaunal species support a variety of regional reef predators; Russell 1983) and is crucial in advancing public support and eventual upscaling of restoration efforts (Gillies et al. 2015).

The results of the current study demonstrate that mobile species—highly transient and (as opposed to the observed epifaunal communities) physically untied to mussel habitat—are disproportionately attracted to restoration sites compared to alternative soft-sediment habitats. Enhanced abundance of mobile species at restoration locations suggests that mussel beds are exploited for additional food resources, with highly mobile species utilising beds (albeit temporarily) to ingest both mussels and reef residents found in higher densities at restoration sites. High accumulations of mobile fish have been shown to deplete invertebrate communities in restored reefs elsewhere (Lenihan et al. 2001), and such trophic interactions plausibly result in community changes documented at other organisational scales in this study. For example, increased triplefin abundance (up to 16x higher on beds) may be partially responsible for lower overall abundances of known prey species (e.g., amphipods, ostracods) at restoration sites (Table AI.1). Further examination of predator-prey relationships on restored beds is required to determine the potential effects of specific fish species on local prey populations and to predict changes in ecosystem function that result from modifications in community structure.

While the current study design does not allow us to distinguish between mechanisms driving observed community changes (e.g., food availability vs. refuge) the most notable enhancements at the mobile scale were observed in smaller species highly coupled to mussel bed habitat. Enhanced abundances of smaller fishes (triplefins, juvenile snapper) suggest decreased mortality as a result of refuges provided by structurally complex reef habitat. These ideas are supported by others who have tied triplefin success to physical complexity in the surrounding environment (Connell & Jones 1991) and snapper settlement to structured estuarine habitat types such as other bivalve beds in the Hauraki Gulf (Usmar 2009, Parsons et al. 2014). Other regional studies have shown the abundance of small, cryptic reef fishes is strongly influenced by habitat structural complexity and predator effects (Willis & Anderson 2003), factors which likely impact abundance differences observed between mussel bed and non-mussel bed locations here. This study however does not resolve long-term survivorship of these species, which likely varies with life-style strategies. For example since resident demersal fish such as triplefins exhibit strong site fidelity (Thompson 1983), these fish are likely associated with mussel beds throughout their lifetime, and documented increases in abundance likely reflect true population increases resulting from restoration efforts. In contrast, juvenile snapper (representing over 90% of total observed snapper in this study) were positively correlated to mussel restoration sites, but their long-term fitness and survivorship will be influenced by complex ontogenetic movements (Parsons et al. 2014) and human predation. Our results indicate that the structural complexity generated by restored beds is important in maintaining biodiversity at the mobile scale (Thrush et al. 2002), but acknowledge the current study represents a snapshot of the community at a specific time point; given the transient nature of mobile communities, further spatial and temporal variation can be resolved with future monitoring efforts.

All six mobile taxa identified to highly contribute to the dissimilarity between mussel beds and control sediments were found in higher abundances on restored beds, and four of these taxa (snapper, mackerel, mullet, and trevally; *Chrysophrys auratus*, *Trachurus* spp., Mugilidae, and *Pseudocaranx dentex* respectively) have well-established commercial value in New Zealand. Although increases in economically valuable fish species were evident on mussel beds, the current study design does not allow us to definitively differentiate between new fish production (those that exist solely because new habitat was generated) and those fishes that, as a result of behavioural preferences, merely aggregated around new structure without increasing fish production or abundance (Pickering & Whitmarsh 1997). While difficult to quantify potential enhancement of fish production, Peterson and colleagues (2003) creatively combined growth and survivorship information from other published works to estimate that 10 m^2 of oyster reef returns an additional 2.6 kg of mobile species annually. More recent studies predict slightly larger (but variable) increases in production (~4 kg per 10 m^{-2} y^{-1}) as a result of increased fish recruitment on reefs (zu Ermgassen et al. 2016b). We predict similar or even increased trends in fish production as a consequence of current mussel restoration efforts, as these beds replace important nursery habitats (Usmar 2009) which have been severely degraded over the past decade. However, quantifying the magnitude and extent of this service was outside the current study's scope.

The habitat heterogeneity hypothesis has been extended to bivalve systems (Cranfield et al. 2004, Hanke et al. 2017, Karp et al. 2018) and suggests that complex habitats support greater abundances and more diverse communities of macrofauna. Perhaps less anticipated, this was not the case for our restored beds, as species richness and total macrofaunal abundance remained largely unchanged as a result of restoration. Many studies report increases in macrofaunal diversity and abundance associated with bivalves (Norling & Kautsky 2007, Commito et al. 2008, Norling & Kautsky 2008, Ysebaert et al. 2009, Lejart & Hily 2011), but some note that changes in sediment composition associated with beds (increased organic additions leading to sediment anoxia, production of sulphides, etc.) favour small, opportunistic species perhaps less valuable in terms of their functional role in soft-sediment systems. While we did observe significant changes in sediment conditions as a result of mussel restoration (e.g. SOM, porosity, chlorophyll *a*), such opportunistic species (namely oligochaetes, capitellids) did not appear to highly contribute to community differences observed in this study (< 3% each; Table AI.1).

Our ranked species abundance plots also suggest important compositional changes occur with mussel restoration despite traditional measures of macrofaunal diversity (richness, abundance) failing to differentiate ecologically relevant community changes between habitat types (Jaunatre et al. 2013). The three mussel beds in Mahurangi Harbour were numerically dominated by a few species (Fig. 2.4), typically the bivalve mollusc *Theora lubrica*, amphipods from the family Phoxocephalidae, and spionid polychaetes from the genus *Prionospio*. Detritivores and deposit feeders, these species thrive in muddy, organically enriched sediments typified by dense mussel beds and are important to local community-dynamics and sediment biogeochemistry (McCann 1989, O'Meara et al. 2020). In this study SOM inside mussel beds was found to be higher than in nearby soft-sediment controls, a likely result of biodeposition which supported higher abundances of these species, and is relevant to the delivery of other ecosystem services provided by restored beds (Sea et al. 2021). It is notable that ranked species abundance plots suggest a more even community structure at MR, a site composed of individual mussels and small clumps as opposed to dense beds observed within Mahurangi Harbour (PP, LB, NLB). Together, these observations suggest that mussel aggregation patterns have functional consequences on macrofaunal communities and the services they deliver, which vary with local sediment conditions experienced.

Similar conclusions can be drawn from PERMANOVA results, which indicated the effect of mussel habitat on macrofaunal assemblage structure varied with restoration location. This interaction can be visualised in the corresponding nMDS plot (Fig. 2.3C), showing a large shift in community structure (observed along the vertical axis in 2D space) between mussel bed and non-mussel bed communities within Mahurangi Harbour (PP, LB, NLB) and a more modest shift in community structure (observed along the horizontal axis) for the mussel bed/ control pair at the sandier MR site in Kawau Bay. This is surprising as one might predict that the creation/deposition of fine particles by mussels would result in greater infilling of interstitial spaces in coarse sands at MR, and thus have a greater impact on communities less acclimated to finer sediments (in contrast to macrofaunal communities already adapted to silty harbour sediments). These multivariate results should be viewed in tandem with community data at other organisational levels; unlike its macrofaunal assemblage, the epifaunal community established at MR is quite similar to inner-harbour sites (observed as a tight clustering of all mussel beds in multivariate space in the epifaunal data set; Fig. 2.3B). This suggests that a different mechanism drives community changes at the two organisational levels. At the epifaunal level, the addition of hard substrate results in colonisation by similar species, regardless of mussel bed location, while sediment modifications resulting from biodeposition affect macrofaunal communities in different ways depending on the local environmental conditions experienced. For example, it is possible that higher preservation of biodeposits occurs within the sheltered Mahurangi Harbour (where mussels have forms tightly packed, dense masses as compared to patchy Kawau Bay beds). These spatial differences likely influence organic matter available at the patch scale, which in turn affects macrofaunal assemblage structure. As community structure depends on the spatial configuration of biogenic habitat within the given environmental context, integration of spatial heterogeneity into future experimental designs will be pertinent in quantifying service value associated with varying macrofaunal assemblage structure.

The above insights are corroborated by the results of DISTLMs which suggest that SOM—shown here to be significantly higher within mussel beds—is a driving factor in altering macrofaunal communities. The 10% decrease in explanation of assemblage structure in mussel habitat is likely influenced by the site-dependent effect of mussel bed communities on macrofaunal assemblage structure. The way that increased SOM (as a consequence of mussel biodeposition) affects community structure is dependent on bed location and specific local environmental conditions (e.g. local hydrodynamics, sediment grain size, mussel aggregation patters, etc.), and creating a linear model which fully encompasses this interaction is expectedly more difficult. The DISTLM models include chlorophyll *a* concentration in the absence of mussels. As a proxy for the microphytobenthos, it seems intuitive that variations in the energetic foundation of most coastal food webs (Christianen et al. 2017, Hope et al. 2020) would be beneficial in the prediction of macrofaunal community structure. The substitution of chlorophyll *a* for SOM at mussel bed locations suggests the underlying macrofaunal community relies on biodeposition as a source of essential nutrients, and that other forms of organic matter will differentially affect community structure when biodeposits are unavailable. Others have separated the structural and functional role of mussels (Norling & Kautsky 2007) to determine that live mussels supply limiting resources (organic carbon and nitrogen) to sediment dwellers through biodeposition, which in turn increases the carrying capacity of these systems. Additional SOM inputs observed—while not shown to significantly increase macrofaunal diversity here—have been beneficial in the prediction of other ecosystem services associated with *P. canaliculus* restoration (Sea et al. 2021).

Others have importantly noted the “dynamic nature of mussel bed structure” (Commito & Dankers 2001). Bed structure can change as a result of mortality following extreme circumstances (e.g. severe weather events which can destroy entire beds), or more localised events (e.g. predation or dislodgement at the patch scale). In this study we were able to demonstrate significant changes in diversity across multiple scales of mobility at a specific time point; given the dynamic nature of beds through time, future changes in bed densities and the creation of additional restored beds will influence the magnitude and extent of observed community changes.

While mussel-associated communities can be influenced by local hydrodynamics (Arribas et al. 2014), it should be considered that, as ecosystem engineers, bivalves too can influence their environment (e.g. dampening wave energy, preventing sediment resuspension) even beyond the extent of reef boundaries (Walles et al. 2015), which would have implications for community structure at larger spatial scales. We can conclude that such effects are likely limited to a scale of < 5 m in this specific case, as significant differences in community structure on and off beds were observed at this distance; however, it would be of interest to determine how engineering effects on infaunal community composition diminish at increasing distances from restored beds. In addition, others have shown that mussel size and/or bed age can influence species composition (Tsuchiya & Nishihira 1986, Craeymeersch & Jansen 2019); however, as these restored beds varied in age by less than three years and were composed of similarly sized individuals, such ideas were not explored further here.

Our findings substantiate the importance of complex structural features in enhancing overall diversity (species richness, abundance) in soft-sediment habitats. These subtidal mussels modified their physical environment in ways that differentially impacted associated biological community structure at various organisational scales. Community responses to restoration varied with species mobility and lifestyle strategies, and examining assemblage data separately allowed us to disentangle various mechanisms driving observed community changes. Most notable effects derived from altered availability of resources; mussels generated organically enriched biodeposits which influenced local biogeochemistry and resultant macrofaunal communities directly tied to surrounding sediment conditions. Highly diverse epifaunal communities were supported by restoration, utilising mussels and their biodeposits for consumption and capitalising on hard substrate additions. Mussels and the epifaunal communities they supported then likely became a source of refuge and food to mobile species that supported predators at higher trophic levels. While we were able to link changes in community structure to the mobility of organisms at specified organisational scales, our results also highlight complex interactions between restoration effects and site selection on biological communities. Such context-dependency and strong location effects suggest that restored mussel beds should not be generalised as global hotspots of diversity (Buschbaum et al. 2009), and that critical site selection will influence biodiversity generated across scales of mobility. Determining the influence of subtidal mussel restoration on associated biological assemblages helps us better understand and evaluate ecosystem services underpinned by the diverse communities associated with restoration efforts.

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Chapter 3

Environmental predictors of sediment denitrification rates within restored green-lipped (*Perna canaliculus*) mussel beds[*]

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3.1 | Abstract

Benthic processes in coastal marine environments can enhance the natural removal of bioreactive nitrogen through denitrification (DNF), a valuable ecosystem service as nutrient over-enrichment intensifies globally. Enhancing ecosystem services is an important justification for restoring coastal ecosystems, and while it is known that epifaunal bivalves (oysters or mussels) are capable of influencing nitrogen cycling, empirical measurement of the role of specific species across a range of environmental conditions is missing. Bivalves within shellfish beds are not uniformly distributed and thus clumps and empty patches within restored beds may differentially impact DNF given the importance of local biogeochemistry. This study reports denitrification and respiration rates in four restored beds that vary in sediment composition, while identifying which ecological and biogeochemical factors best explain the observed variability in measured fluxes. We deployed benthic chambers in sediments with and without mussels at these sites, and measured net N_2 fluxes through membrane inlet mass spectroscopy. Sediment organic matter proved to be the most significant predictor of DNF rates in regression tree and random forest models, suggesting that biodeposition by green-lipped mussels enhances nitrogen removal at these sites and that these effects occur across beds despite differences in mussel density. Greatest DNF rates corresponded to lower sediment chlorophyll *a* concentrations and higher nitrate/nitrite effluxes measured within chambers. Determining the influence of subtidal mussel restoration on significant sediment processes informs future restoration efforts aiming to maximise this nitrogen removal service, while providing insights on underrepresented oligotrophic systems of the southern hemisphere.

3.2 | Introduction

Nitrogen loading is commonly associated with eutrophication and the degradation of coastal environments, as nitrogen typically limits algal growth and primary production (Howarth & Marino 2006), but in excess, can lead to toxic algal blooms (Paerl 1997, Anderson et al. 2002) and hypoxic conditions (Diaz & Rosenberg 2008). Anthropogenic nitrogen enrichment is currently accelerating at alarming rates (Galloway et al. 2004, Galloway et al. 2008, Kuypers et al. 2018), and therefore has growing consequences for coastal oceans and the services they provide (Nixon 1998, Howarth & Marino 2006). As humans continue to generate bioreactive nitrogenous compounds (e.g. ammonium and nitrate available for biological uptake) and fail to control excessive nitrogen inputs to ecological systems, the capacity of coastal environments to remove excess nitrogen becomes of even greater value to mankind. However, this process is currently not well understood. The fate of over 60% of global reactive nitrogen is unknown (Galloway et al. 2008), but a significant portion of this nitrogen is thought to be denitrified in coastal to continental shelf sediments (Middelburg et al. 1996, Seitzinger et al. 2006). It is thought that coastal marine environments are capable of removing half (Seitzinger 1988) to over 80% (Galloway et al. 2003) of terrestrial nitrogen inputs through the denitrification (DNF) process. Driven by facultative anaerobic bacteria, DNF converts bioavailable, inorganic nitrogen (in the forms of nitrite and nitrate) to molecular dinitrogen gas (N_2) and nitrous oxide (N_2O), resulting in the net removal of this nutrient from the system (Thrush et al. 2013, Humphries et al. 2016). It is estimated that estuaries and coastal shelf regions currently remove 24 Tg of reactive nitrogen each year (Galloway et al. 2004), making DNF a valuable service provided by coastal soft sediments.

Previous work has shown that bivalves are capable of enhancing the denitrification process (e.g. Christensen et al. 2003, Piehler & Smyth 2011, Carlsson et al. 2012, Kellogg et al. 2013, Humphries et al. 2016). However, bivalve populations are under pressure in many coastal ecosystems as a result of multiple stressors, including over-harvesting, physical disturbance, sedimentation, contamination and eutrophication (e.g. Peterson et al. 1994, Cook et al. 2000, Kirby 2004, McLeod et al. 2012, Thrush et al. 2021b). Restoration projects are underway worldwide to restore these populations and regain ecosystem services associated with shellfish reef formation (zu Ermgassen et al. 2020), and shellfish restoration could be an effective tool to enhance this nitrogen removal service limiting degradation in coastal ecosystems. In short, subtidal epifaunal bivalve populations remove resuspended microphytes and phytoplankton from the water column as a result of their filter feeding processes (Dame 2012). Bivalves then deposit this organic matter, sourced across a range of spatial scales depending on hydrodynamic regimes, onto the seafloor in the form of faeces and pseudofaeces (hereafter referred to as biodeposits; Newell 2004). The remineralisation of these biodeposits results in ammonium driving nitrification in surface-layer aerobic sediments (Kellogg et al. 2013). The nitrate produced from this process typically drives DNF at oxic/anoxic interfaces and results in the conversion of biologically reactive forms of nitrogen into inert N_2 gas, effectively removing nitrogen from the system. While enhanced sediment denitrification is predicted as a result of bivalve restoration projects in highly eutrophic systems (e.g. Cerco & Noel 2007), studies in oligotrophic systems are rare (Vieillard et al. 2020), and there is a severe lack of in-situ studies involving bivalves and denitrification.

Laboratory studies (Kellogg et al. 2013, e.g. Smyth et al. 2018) and one known in-situ study (Humphries et al. 2016) have shown that bivalve restoration projects on the seafloor can enhance sediment denitrification rates as compared to nearby sediments without any bivalve presence. While these studies determine how nitrogen removal varies spatially and/or temporally in sediments with and without the presence of shellfish, the complex interactions between environmental factors that drive enhanced DNF within these beds remain less well understood. In addition, the spatial structure of mussels within mussel beds are influenced by a multitude of factors, including location within an estuary, position along tidal height gradients, differential predation, local hydrodynamics, etc. (Commito & Dankers 2001). The role of these heterogeneous spatial arrangements of clumps and bare patches within mussel beds has yet to be explored, but is important in estimating the overall contribution of restored beds to DNF. We have observed this patchiness in the distribution of mussels at our restoration sites which led us to question how the quantity of trapped sediment organic matter (SOM) from both allochthonous sources and local biodeposits influences DNF rates within individual beds.

Bivalves involved in benthic-pelagic coupling and the resultant organic material accumulated in restored beds are predicted to enhance sediment DNF, but the effect of organic matter deposits on nitrogen mineralisation pathways ultimately depends on multiple factors, including the type and quantity of material deposited (Eyre et al. 2013), as well as local hydrodynamics and benthic mineralisation rates in coastal waters (Prins et al. 1998). In addition, differing sediment characteristics will likely influence the accumulation of organic-rich biodeposits and transport of nutrients, as changes in suspended sediment concentration (associated with a grain size gradient) affect bivalve clearance rates (Hawkins et al. 1999) and the quality of biodeposits produced (Iglesias et al. 1996). Species diversity and varying abundances of benthic macrofauna (typically polychaetes and molluscs) will also affect particle movement and water transport as a result of feeding activity and bioturbation (Quintana et al. 2007, Douglas et al. 2017, O'Meara et al. 2020). Together, these environmental variables and the complex interactions among them affect sediment biogeochemistry and likely regulate DNF in different restoration sites. A greater understanding of factors that significantly influence nitrogen removal rates will aid in our selection of future mussel bed restoration locations that seek to enhance this service.

These gaps in our understanding together with a paucity of research on restored mussel beds provide an opportunity to explore the complexities of nitrogen cycling in coastal ecosystems. In this study, we measured a suite of environmental factors associated with sediment DNF rates in two muddy and two sandy subtidal restoration sites of New Zealand's endemic green-lipped mussel (*Perna canaliculus*). To determine if DNF rates varied within the larger mussel bed complex, benthic chambers were deployed at each site enclosing mussel clumps or bare sediments between bivalve patches. Net gas fluxes across the sediment-water interface were measured utilising ratios of dinitrogen to argon (N_2/Ar) through membrane inlet mass spectroscopy (MIMS). The importance of a representative suite of environmental factors in explaining flux variability was then explored using decision trees and random forest algorithms. We hypothesised that organic-rich biodeposits trapped within mussel beds would be most important in stimulating microbial activity associated with enhanced sediment DNF, but that the strength of these effects would vary with site characteristics.

3.3 | Methods

3.3.1 | Study Area

Restored mussel beds were created by transplanting roughly 10 tonnes of adult green-lipped mussels sourced from a mussel farm to desired soft sediment locations at similar depths (5-10 m), forming beds approximately 10 m^2 in size. At the time of this study, beds were 2-3 years old and exhibited different spatial patterns (see supplemental text in Appendix II). Restoration sites were initially identified to capture environmental variation associated with sediment grain size, SOM, and macrofaunal assemblages. Two of these restored beds were located near the mouth of Mahurangi Harbour (Lagoon Bay and Pukapuka) and two outside the harbour adjacent to nearshore islands (Motuora and Motoketekete; Fig. 3.1). Our two harbour sites were characterised by finer, siltier sediments more easily resuspended in the water column, whereas the island sites exhibited a sandy substrate with less resuspended material. Chosen sites therefore provided differences in both a suite of environmental characteristics and the effect of mussel clumps.

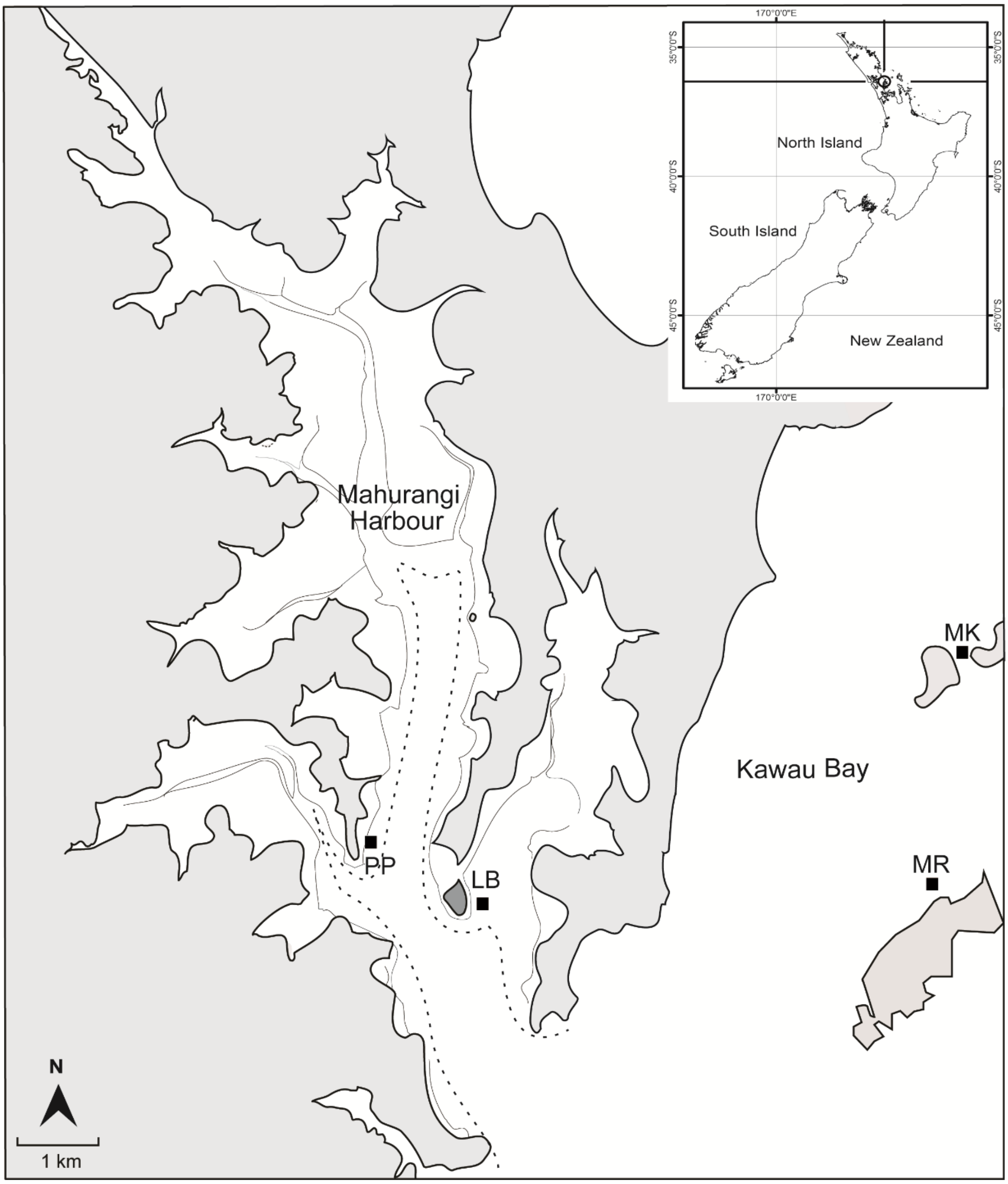

Figure 3.1 Location of study sites in Mahurangi Harbour and Kawau Bay, New Zealand. Lagoon Bay = LB, Pukapuka = PP, Motuora = MR, and Motoketekete = MK.

3.3.2 | Sample Collection

This study was conducted in March and April 2019. Using SCUBA, benthic chambers (e.g. Lohrer et al. 2004, O'Meara et al. 2020) were placed within restored beds over sediments containing mussel clumps. To capture spatial variability and account for bed patchiness, a number of chambers without mussels (yet within bed boundaries) were also deployed in this study. Chambers (0.25 m^2 each) were pushed into the sediment to a pre-marked line 7 cm from the chamber base, so that all chambers held 41 L of near-bed seawater at the start of the incubation. A total of 12 chambers were deployed at three sites (Motuora, Motoketekete, and Pukapuka; beds roughly 10 m^2 each), while 9 chambers were deployed at the smaller Lagoon Bay bed (~7 m^2). Photos were taken of each chamber and the number of mussels recorded (Table AII.1). One site was sampled per day.

Each benthic chamber was equipped with a miniDOT optical oxygen logger (PME, USA), which recorded dissolved oxygen in the chamber water every minute, and a pump which recirculated chamber water for 5 seconds at 30 second intervals. Chamber lids were carefully sealed while underwater to ensure there were no gas bubbles. Chambers were then covered with black polyethylene to omit incoming light. These dark incubations mean we omit the role of microphyte production, but this avoided complications associated with bubble formation in light chambers due to photosynthesis as well as complicating comparisons due to variation in photosynthetically active radiation due to changes in weather and water clarity.

To determine net fluxes across the sediment-water interface, samples for MIMS and dissolved inorganic nutrient analysis (nitrate/nitrite, reactive phosphorous, and ammonium concentrations herein referred to as NO_x^-, PO_4^{3-}, NH_4^+ respectively) were taken from the chamber port using two 60 mL syringes immediately after covering chamber lids, taking care to remove any air bubbles from the syringes beforehand. Water samples were taken again from each chamber at the end of the incubation period (approximately 3 hours). At the end of the incubation period the chamber lids were removed and 2 sediment syringe cores (3 cm length, 1.9 cm diameter) taken from within each chamber for chlorophyll *a* (Chl *a*), SOM, and grain size analysis. Avoiding mussel clumps, an additional 10 cm diameter, 10 cm deep sediment core was taken from within each chamber to assess dominant macrofauna present at each site.

Immediately after collection, water samples for nutrient analysis were filtered through Whatman GF/F filters (0.7 µm pore size) and stored on ice before being transferred to the lab and frozen. Each unfiltered MIMS sample was transferred into two gas-tight 12 mL exetainer vials (for a total of 4 per chamber) and poisoned with $ZnCl_2$ to stop biological activity. MIMS samples were stored below collection temperature, but above freezing. These samples were transferred to the lab and kept at 4° C pending analysis. Sediment samples for SOM, grain size, and Chl *a* content were kept on ice in the dark and frozen at the lab until later analysis. Macrofauna cores were sieved (500 µm mesh) with seawater and contents transferred into storage containers with 70% IPA stained with rose bengal. Macrofauna were later sorted and classified to the lowest practical taxonomic group. For each core, the total number of macrofauna present, total number of known bioturbators present (total bioturbators), and total number of species present (species richness), was recorded for use in later analyses.

3.3.3 | Sediment Characterisation

SOM was determined by loss on ignition (Ball 1964). Sediments were left in a 60 °C oven, dried for 48 hours, and then weighed before and after combustion at 500 °C for 10 hours.

To determine sediment grain size, homogenized sediment samples (~20 g) from each chamber were digested with 50 mL of 6% H_2O_2 and periodically mixed over a 48 hour time period. Samples were then rinsed of hydrogen peroxide and sat overnight in ~15 mL of 5% Calgon (a dispersing agent for sediment particles) prior to grain size analysis with a Malvern Mastersizer 3000 (ATA Scientific).

To assess the standing stock of the microphytobenthos (MPB), Chl *a* was extracted from 1 g of freeze-dried sediment samples with 3 mL 90% acetone. Optical density of extracts were measured at 664, 665, and 750 nm with a UV-Vis spectrophotometer (Thermo Scientific, Multiskan Sky) before and after hydrochloric acid acidification (0.1 mL of 0.1M HCl). Values at 750 nm were subtracted from values at 664 and 665 nm to correct for turbidity in samples. Sediment chlorophyll *a* content was calculated using equations from Lorenzen (1967) based on 90% acetone extraction (eq. 3.1):

$$\text{Chl } a \text{ content } (\mu\text{g Chl } a \text{ g}^{-1} \text{ sediment}) = \frac{11 * 2.43 * (E_{664b} - E_{665a}) * V_e}{D} \qquad \text{Eq. 3.1}$$

where $E_{664}b$ is the corrected absorbance at 664 nm before acidification, $E_{665}a$ is the corrected absorbance at 665 nm after acidification, V_e is the volume of the extractant (mL), and D is the dry weight of sediment (g).

3.3.4 | Flux Analysis

To determine if net DNF rates varied between mussel clumps and the spaces between them, seawater samples were analysed on a quadrupole membrane inlet mass spectrometer (with Pfeiffer Vacuum Prisma Plus QMG220 M1 QMS, Bay Instruments) using the MIMS technique (Kana et al. 1994). This high precision (< 0.05%) method utilises N_2/Ar ratios to determine a net flux of N_2 from all processes that form (denitrification, anaerobic ammonium oxidation) or use (nitrogen fixation) N_2. With this technique, a net positive N_2 flux indicates denitrification dominates, while a net negative N_2 flux indicates nitrogen fixation dominates. It should be noted that, while anaerobic ammonium oxidation produces N_2 gas, the contribution of this process to overall nitrogen removal in estuarine environments has been shown to be quite minor globally (5-14% on average; Hou et al. 2015) and goes undetected in high salinity, low nutrient sites (< 6 µM NO_3^-; Rich et al. 2008) similar to those studied here (see Table AII.2 for a summary of additional environmental characteristics recorded). We therefore assume that all nitrogen removal measured in this study is the direct consequence of DNF, but that further nitrogen removal (not estimated) may be occurring as a result of burial within sediments.

With known incubation times for each chamber, fluxes were calculated as the difference between initial and final concentrations and corrected for volume and surface area of the chamber to obtain a flux in μmol m^{-2} h^{-1} (e.g. Eyre et al. 2002, O'Meara et al. 2020). This was done for both N_2 and O_2 fluxes. O_2 fluxes calculated from the MIMS technique were then compared to data obtained from the oxygen loggers placed in benthic chambers. Dissolved oxygen data from these loggers were similarly graphed and the slope of the linear portion of the line used to calculate a flux rate that was also corrected for volume and area of the chamber space. Even though O_2 fluxes were found to be comparable, the manual truncation method described above to calculate O_2 fluxes has been critiqued for introducing subjectivity into analysis (see Olito et al. 2017), and data from the MIMS was instead used in further calculations. All chambers with mussels were examined for hypoxia (< 2.0 mg O_2 L^{-1}) at the end of the 3 hour incubation period, and chambers with oxygen concentrations below this threshold were discarded from further analysis.

Nutrient samples were analysed using a Lachat Autosampler (ASX-260 Series) flow injection analyser (FIA; method detection limits of 0.032 μmol L^{-1}, 0.05 μmol L^{-1}, and 0.071 μmol L^{-1} for PO_4^{3-}, NH_4^+, and NO_x^- respectively), with standard methods for analysis of seawater nutrients. Values were corrected using calibration standards, and net fluxes calculated by taking the difference between final and initial concentration values and dividing by chamber incubation time while accounting for volume and area of sediment enclosed by the chamber.

3.3.5 | Statistical Analysis

The effects of site, mussel presence/absence, and their interaction on measured fluxes and varying environmental characteristics were investigated using 2-way ANOVA, followed by Tukey's tests for post hoc comparisons (results summarised in Table AII.3). Assumptions of normality and homogeneity of variance were checked using the R statistical package s20x (version 3.6.1; R Core Team 2019). Q-Q plots, Shapiro-Wilk tests, and Levene's tests were used to check assumptions, and values log-transformed where necessary. Significance levels were set to $\alpha < 0.05$, and standard errors (SE) were used to assess the precision of mean values, unless otherwise stated.

To determine which of the measured environmental variables explained the most variation in measured fluxes within mussel beds, we used machine learning statistical techniques involving regression trees and random forest models. These non-parametric approaches have gained popularity in ecological sciences (e.g. Evans & Cushman 2009, Ray et al. 2020, Smith DiCarlo et al. 2020) and are valued for their ability to cope with complex interactions, non-linear relationships, and unbalanced experimental designs, while producing graphical outputs that are easily interpreted and reveal relationships that often go unrecognised using more traditional statistical techniques (De'ath & Fabricius 2000).

We used the ‘rpart’ package in R (version 3.6.1; R Core Team 2019) to create a decision tree which split the full data set into multiple groups (each split creating the lowest possible within-group variation) until predetermined end points were met (in this case, 4 minimum observations required in a node for a split to be attempted and at least 2 observations in any terminal node; complexity parameter = 0.014). Eleven explanatory variables were used to predict net N_2 fluxes in regression trees and random forests: SOM, oxygen fluxes, nutrient fluxes (NO_x^-, PO_4^{3-}, NH_4^+), total macrofauna abundance, total number of bioturbators, species richness, Chl *a* content, total number of mussels per chamber, and percentage mud content. Variables near the root (top) of the tree were considered most influential in classifying the response variable into various terminal nodes (in this case, net N_2 fluxes). Interactions between explanatory variables were conceptualised as subsequent splits, dependent on the results of decisions at nodes higher in the tree.

It is recognised that regression trees and related predictive mapping methods typically undergo a two-step process: first, modelling the relationship between a response variable and a suite of explanatory variables from field measurements or observations; and second, using the generated model to predict the response variable at new sites given known values for the explanatory variables. As the goal of this study was to determine which predictor variables could best explain N_2 flux variability in the single data set, it was only necessary to conduct the model creation process. We advise against the use of this descriptive model in predicting fluxes outside our wider study system, although conclusions gleaned from this investigation regarding the general importance of various environmental and biogeochemical factors can be used to inform the design of future experiments involving predictive modelling techniques.

A random forest algorithm (‘randomForest’ package, R Core Team, 2019) was used to rank the relative importance of explanatory variables used in this study. Random forest is an ensemble method resulting from the growth of hundreds to thousands of unique regression trees, each of which is created using a bootstrapped sample of the dataset and random subsets of explanatory variables at each node (Breiman 2001, Diesing et al. 2017). Random forest outputs a list of variable importance, determined by permuting each predictor variable and calculating the percentage error increase associated with the change. Higher values correspond to greater variable importance. Variable importance from the random forest was then compared to regression tree outputs. This study utilises a forest with 500 trees and tests 8 randomly selected variables at each split.

3.4 | Results

3.4.1 | Environmental Variables

Differences in mud content (% < 63 μm) were apparent between sites (2-way ANOVA; $F_{3,24} = 333.43$, $p < 0.001$), with LB and PP characterised by significantly muddier sediments than the sandier Kawau Bay sites (MR and MK; Table 3.1). A significant difference in percentage mud content was also detected between Kawau Bay sites, with MK exhibiting less silt and clay than MR. Moderate SOM was observed at all locations, with mean SOM ranging from 2.3 ± 0.02 % at LB to 3.4 ± 0.36 % at PP. Significant differences in Chl *a* content were observed between sites (2-way ANOVA; $F_{3,24} = 5.31$, $p < 0.01$), with site LB having higher Chl *a* content than sites PP and MR.

We observed differences in macrofaunal abundance between sites (2-way ANOVA; $F_{3,24} = 8.35$, $p < 0.001$), with sandier sediments at MR and MK exhibiting significantly higher macrofaunal abundance per core than PP and LB (Table 3.1). The total number of species identified per core increased from 10 ± 0.9 and 9.5 ± 1.2 at muddier sites LB and PP to 14.5 ± 1.2 and 14.5 ± 1.0 species at sandier sites MR and MK respectively, indicating an increase in species richness associated with sandier sites. While there was no significant difference in abundance of individual macrofauna present between the two sandier sites, a distinct separation in community structure was apparent, with higher numbers of gastropods and amphipods separating MK from MR (Fig. AII.1). Polychaetes were less abundant and more similar in species composition within the harbour sites.

Table 3.1 Summary of sediment characteristics between sites, separated by chambers with and without mussels. Mud is comprised of silt + clay (< 63 μm). Medium sand is between 250 and 500 μm. Coarse sand > 500 μm. SOM = sediment organic material. Sites arranged over a decreasing mud gradient. Lagoon Bay = LB, Pukapuka = PP, Motuora = MR, and Motoketekete = MK. Different letters denote significant differences between sites (post hoc Tukey's test, $p < 0.05$). Data represent the mean ± SE.

Site	Chamber Contents	Mud Content (%)	Medium Sand (%)	Coarse Sand (%)	SOM (%)	Chl *a* Content (ug g^{-1})	Total Number Macrofauna ($core^{-1}$)
PP	Mussels	22.4 ± 0.37^{a}	1.32 ± 0.13^{a}	1.87 ± 0.78^{a}	3.2 ± 0.33^{a}	4.1 ± 0.9^{a}	34.0 ± 2.5^{a}
	No mussels	24.0 ± 0.27^{a}	1.13 ± 0.16^{a}	2.31 ± 0.65^{a}	3.4 ± 0.36^{a}	3.8 ± 1.1^{a}	36.7 ± 24.2^{a}
LB	Mussels	22.9 ± 1.61^{a}	0.18 ± 0.03^{a}	0.18 ± 0.03^{a}	2.7 ± 0.38^{b}	5.6 ± 2.5^{bc}	34.0 ± 11.4^{a}
	No mussels	20.0 ± 1.56^{a}	0.42 ± 0.10^{a}	0.71 ± 0.18^{a}	2.3 ± 0.02^{b}	8.5 ± 1.1^{bc}	30.0 ± 6.3^{a}
MR	Mussels	7.0 ± 0.83^{b}	12.28 ± 1.04^{b}	0.89 ± 0.39^{a}	3.4 ± 0.30^{a}	4.9 ± 0.5^{ac}	107.3 ± 13.6^{b}
	No mussels	4.6 ± 0.10^{b}	12.14 ± 0.28^{b}	1.45 ± 0.69^{a}	2.9 ± 0.17^{a}	3.2 ± 2.7^{ac}	84.0 ± 1.0^{b}
MK	Mussels	3.3 ± 0.29^{c}	35.81 ± 0.67^{c}	11.17 ± 0.89^{b}	2.7 ± 0.08^{b}	6.1 ± 0.4^{c}	76.1 ± 10.6^{b}
	No mussels	3.1 ± 0.38^{c}	36.86 ± 0.45^{c}	10.98 ± 0.71^{b}	2.7 ± 0.11^{b}	6.8 ± 0.3^{c}	107.3 ± 28.1^{b}

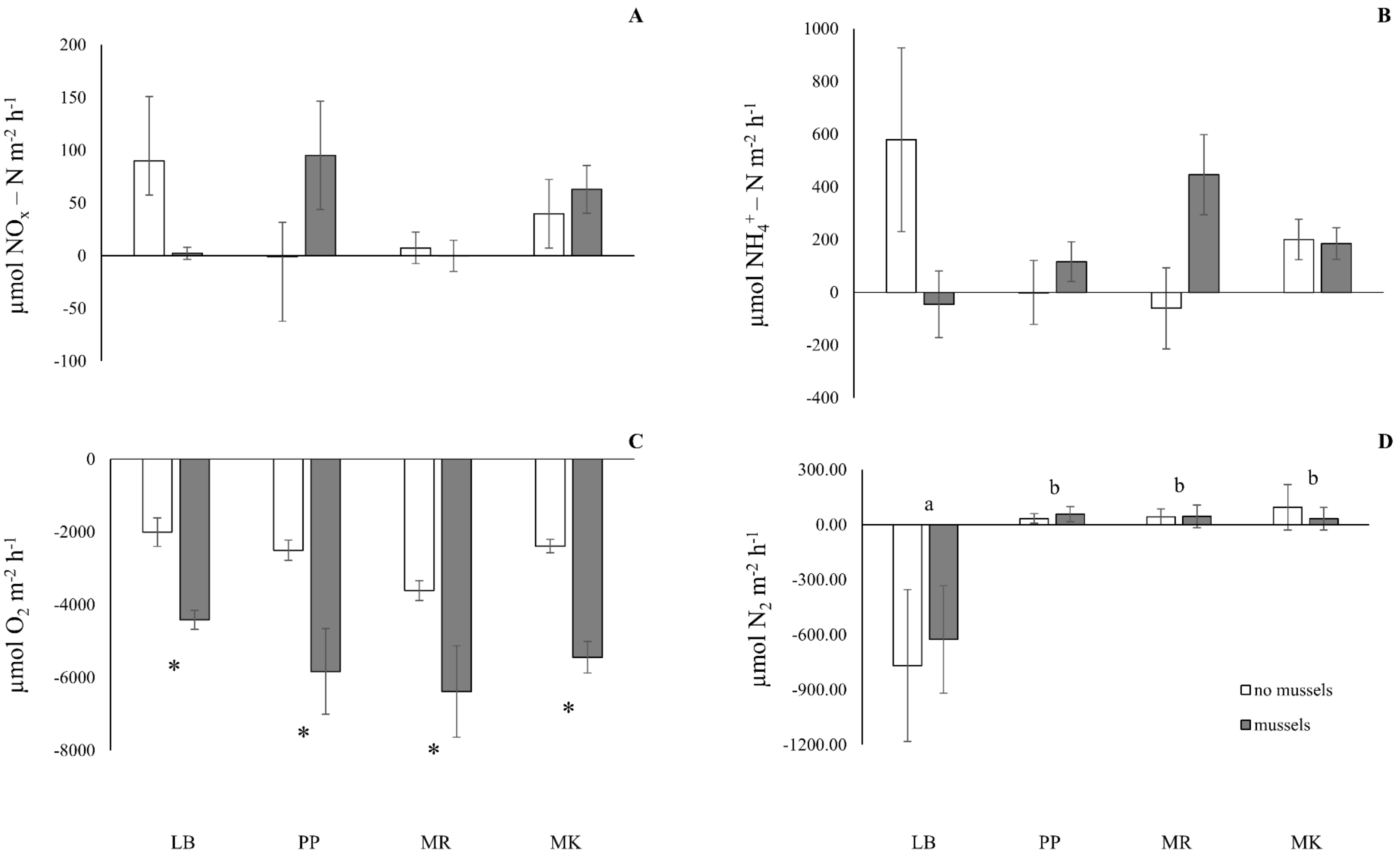

Figure 3.2 Net fluxes measured in chambers containing no mussels (white) and mussels (dark) across 4 field sites. (A) nitrate + nitrite (NO_x^-) fluxes; (B) NH_4^+ fluxes; (C) benthic oxygen consumption; (D) denitrification (positive) and nitrogen fixation (negative) rates. Note scale differences between graphs. Asterisks indicate significant differences ($p < 0.05$) between chambers with and without mussels while letters signify differences in mean flux rates between sites. Sites are arranged across a decreasing mud gradient. Lagoon Bay = LB, Pukapuka = PP, Motuora = MR, and Motoketekete = MK.

3.4.2 | Net DNF and Nutrient Fluxes

The magnitude of NO_x^- fluxes measured over the incubation period varied by roughly 100 µmol m^{-2} h^{-1} (Fig. 3.2A), with a net efflux measured in the majority of chambers. PO_4^{3-} fluxes (not shown) varied considerably across chambers, ranging from -2.26 ± 8.9 to 37.61 ± 16.2 µmol m^{-2} h^{-1} and no clear pattern related to the presence of mussels or location. 2-way ANOVA suggested that the effect of mussels on NH_4^+ fluxes was dependant on location ($F_{3,24} = 4.82$, $p < 0.01$), although post hoc pairwise comparisons were not powerful enough to detect significant pairs of means (Fig. 3.2B and Table AII.3). Sediment oxygen demand was significantly higher in chambers with mussels than chambers without mussels (2-way ANOVA; $F_{1,24} = 24.02$, $p < 0.001$), and this pattern was observed across sites (Fig. 3.2C).

Net N_2 fluxes reported in this study were highly variable, but observed on a scale similar to other coastal marine sediments globally (e.g. Seitzinger 1988, O'Meara et al. 2020 and references therein). Net N_2 fluxes in chambers without mussels were close to zero at PP, MR, and MK (33.86 ± 25.8, 42.66 ± 42.6, and 94.33 ± 124.1 µmol N m^{-2} h^{-1} respectively). Net nitrogen fixation was apparent in chambers with (-625.36 ± 292.8 µmol N m^{-2} h^{-1}) and without (-768.24 ± 414.3 µmol N m^{-2} h^{-1}) mussels at site LB (Fig. 3.2D). In chambers containing mussels, highest average sediment DNF rates were recorded at PP (56.82 ± 40.6 µmol N m^{-2} h^{-1}). Log-transformed N_2 fluxes at LB were significantly different from all other sites (2-way ANOVA; $F_{3,24} = 10.23$, $p < 0.001$), and the presence or absence of mussels did not significantly affect these rates (2-way ANOVA; $F_{1,24} = 0.02$, $p = 0.883$). While significant differences were not detected due to the high variance in measurements between chambers, DNF appeared higher in chambers without mussels at the sandiest site

(MK) and a net decrease in nitrogen fixation (potentially indicative of DNF) was seen in chambers with mussels at the muddiest site (LB; Fig. 3.2D).

Regression trees and the random forest algorithm utilised data from 32 benthic flux chambers that were deemed to have acceptable oxygen levels (non-hypoxic) and small changes in Ar concentration at the end of the incubation period. Regression tree analysis of N_2 fluxes produced a tree with 5 terminal nodes (Fig. 3.3A). The first branching point split relative to the percentage of SOM present, with chambers containing lower organic material (< 2.3%) having lower DNF rates (alternatively, higher net nitrogen fixation). From this split, the magnitude of predicted fluxes varied depending on mud content, with greater nitrogen fixation levels apparent in muddier (mud content ≥ 20%) sediments. In chambers with organic material > 2.3%, greatest DNF rates corresponded to higher NO_x^- effluxes. Chambers with lower NO_x^- values showed additional branching relative to the concentration of Chl *a*, with nitrogen fixation occurring in chambers with higher Chl *a* (≥ 7 µg g^{-1}), and DNF occurring in chambers with Chl *a* below this threshold. The random forest was able to explain 27.2% of the variance in observed nitrogen fluxes and ranked SOM as the most important environmental predictor of nitrogen fluxes, in alignment with the regression tree model. Oxygen demand and total macrofaunal abundance were also identified as influential explanatory variables (Fig. 3.3B).

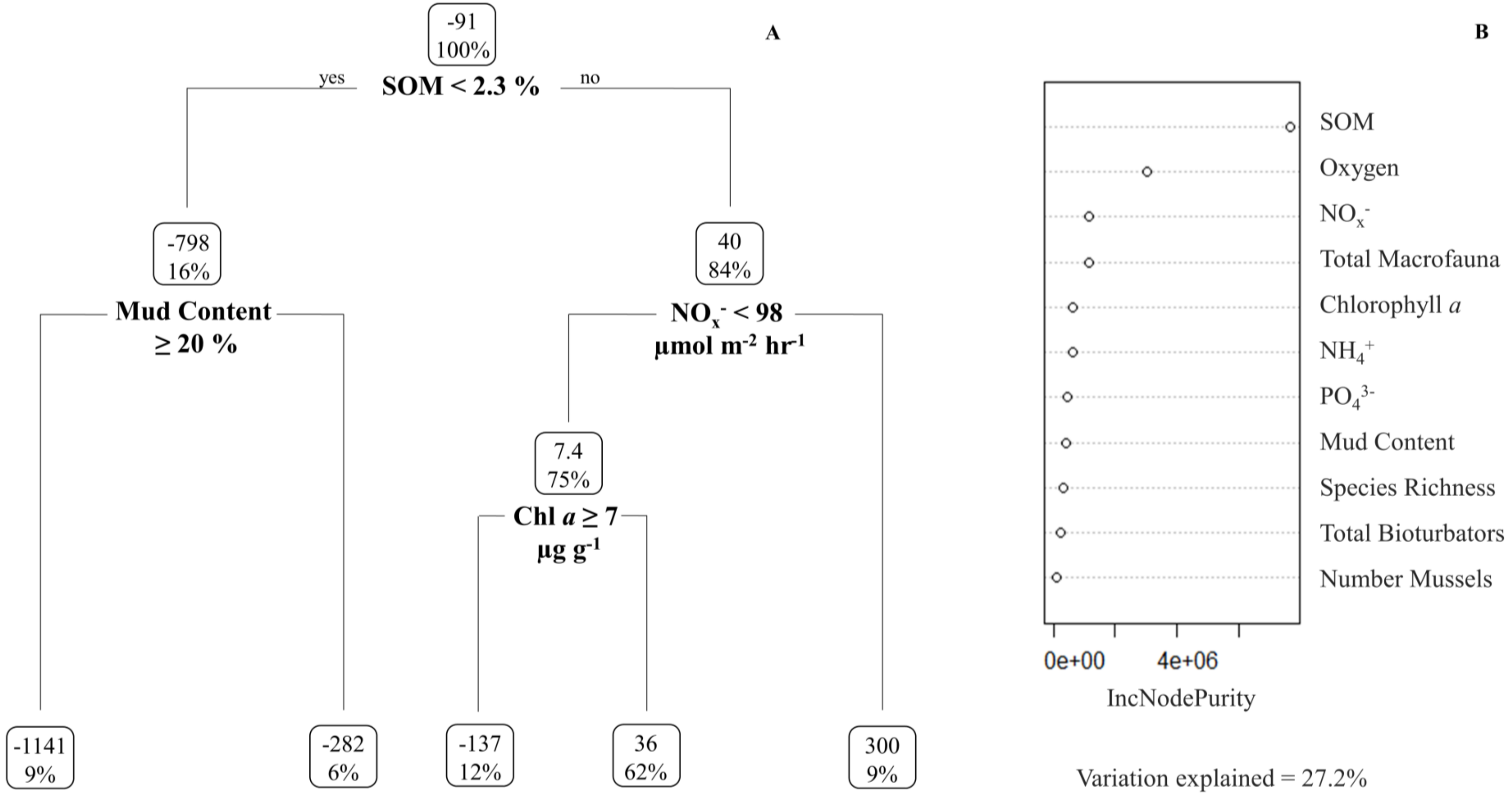

Figure 3.3 (A) Single best regression tree predicting denitrification rates in restored mussel beds. Each node highlights the selected environmental variable at its split point (bold), with positive responses separating left and negative responses separating right further down the tree. Terminal nodes show mean N_2 flux (μmol m^{-2} h^{-1}) and the percentage of total samples that contribute to the predicted rate. (B) List of decreasing variable importance (from top to bottom) assigned by the random forest, as determined by increase in node purity (IncNodePurity).

3.5 | Discussion

In this study we utilised benthic flux chambers to show that DNF occurs within restored mussel beds, and that the magnitude and extent of nitrogen removal is dependent on a variety of environmental and biogeochemical factors. As coastal systems are highly heterogeneous in nature, ecosystem services are often generated by complex and interrelated ecosystem processes that vary over space and time; this makes the quantification of nitrogen cycling and other ecosystem services provided by restored mussel beds very challenging. However difficult, it is important to recognise and assign value to these services, as more traditional management schemes have commonly failed to do so and have consequently permitted the degradation of ecosystems in ways that greatly reduce the value of services they provide (Costanza et al. 1997). The historic collapse of New Zealand's mussel beds (Paul 2012) is one such example of where services other than food provision did not influence the regulation of the fishery.

Multiple studies (e.g. Kellogg et al. 2013, Humphries et al. 2016, Smyth et al. 2018) have shown that DNF rates are significantly enhanced in restored bivalve beds; this study is first in building on that knowledge to determine if DNF rates vary at the patch scale within individual beds, and which environmental factors are most significant in explaining variability in measured rates. This in-situ study was conducted entirely within restored mussel beds of an oligotrophic coastal ecosystem, and net nitrogen fluxes are similar between chambers with and without mussels (Fig. 3.2D); this suggests that mussels influence DNF on scales larger than individual clumps, implying a bed scale phenomena rather than more localised effects around smaller patches. Measured environmental factors associated with restoration (e.g. enhanced SOM) are better predictors of sediment DNF rates in these systems.

This study highlights the importance of sediment organic material on N_2 fluxes, with greater DNF associated with moderate organic matter loading in mussel beds. The importance of SOM in partitioning N_2 fluxes in our models, across both chambers with and without mussels, suggests that carbon rich biodeposits generated by the enhanced benthic-pelagic coupling of restored mussel populations can provide a source of organic material necessary for remineralisation, the products of which (NH_4^+, NO_2^- and NO_3^-) go on to fuel DNF in localised regions of hypoxic sediment (Herbert 1999, Nizzoli et al. 2006). Stimulation of DNF as a result of bivalve biodeposits and increased sediment organic material has been reported elsewhere (e.g. Newell et al. 2002, Newell 2004, Kellogg et al. 2013) and likely contributed to increased nitrogen removal observed in chambers with higher SOM in this study. While we did not detect statistical differences in percentage SOM between chambers with and without mussels, studies utilising other bivalves in this harbour have documented increased sedimentation rates and organic carbon inputs closer to individuals of the large pinnid bivalve *Atrina zelandica* (Norkko et al. 2001), reaffirming the idea that bivalve beds retain organic material enriched in carbon and nitrogen. It is possible that the biodeposits created by the smaller green-lipped mussels are not trapped locally but are instead spread over the entire bed. This increased SOM dispersion would account for potential effects at the scale of mussel beds rather than individual clumps and could help explain the similarities in DNF observed between chambers with and without mussels in this study.

Nitrification and DNF are tightly coupled processes at low nitrate concentrations (e.g. Gongol & Savage 2016, Vieillard et al. 2020), suggesting that the enhanced nitrogen removal capacity resulting from increased SOM observed here may have an upper limit. However, this was not observed at organic matter loads present in our beds. In our oligotrophic system we conclude that highest DNF rates will be achieved with moderate organic matter enrichment, as insufficient SOM will limit ammonification and nitrate production, and excessive SOM will result in higher oxygen demand and the potential production of sulfides (e.g. Christensen et al. 2003) which would further inhibit these processes (Joye & Hollibaugh 1995). These relationships are mirrored in the conceptual models of others (Hoellein & Zarnoch 2014) who have predicted the role of bivalve biodeposition on DNF will have the greatest effect in oligotrophic systems, as bivalves likely stimulate coupled nitrification-denitrification (as opposed to ammonium immobilization in eutrophic systems high in sediment organic carbon), and competition for nitrate (shown to be important in regression tree outputs) is reduced in low-nutrient environments. Even if competition is reduced in these systems, both regression tree and random forest models indicate the importance of NO_x^- effluxes from chamber incubations on DNF rates, with higher NO_x^- availability corresponding to maximum nitrogen removal. It is likely that the nitrogenous products of organic matter mineralisation (resulting from biodeposits and mussels themselves) supply the NO_x^- necessary to fuel eventual nitrogen removal, and that the coupling of nitrification and denitrification is important in these epifaunal mussel beds.

When NO_x^- levels measured during incubations were low, Chl *a* content influenced whether nitrogen fixation or DNF took place, with higher chlorophyll levels associated with nitrogen fixation and lower Chl *a* levels associated with DNF. This suggests a weak yet negative influence of local microphytobenthic communities on sediment DNF possibly due to competition for nitrogen (also noted by O'Meara et al. 2020). Microphytobenthos (MPB) are known to reduce nutrient fluxes across the sediment-water interface as a result of nutrient uptake necessary for their growth and survival, and the sticky polymers produced by MPB can alter sediment cohesion (Newell et al. 2002, Hope et al. 2020); together, these actions result in the reduction of solute transport and could help explain lower predicted DNF rates in regression tree outputs. The influence of MPB was not expected as experiments were carried out under dark conditions; however, others have shown that nutrient uptake by MPB continues in the dark (Rysgaard et al. 1993, Evrard et al. 2008, Longphuirt et al. 2009), and this dark ammonium uptake was observed at 3 of our field sites. It is known that MPB are capable of outcompeting nitrifiers and denitrifiers for dissolved inorganic nitrogen (Newell et al. 2002, Sundbäck & Miles 2002, Cook et al. 2004), and appear to do so when found at higher concentrations in these systems. As all fluxes in this study were measured under dark conditions, it should be noted that these results reflect an approximation of maximum DNF potential under no-light conditions. The presence of light would likely enhance nutrient uptake by MPB, but would also increase oxygen availability as a result of photosynthesis (potentially available for further nitrification which is then coupled to DNF); it would be useful in the future to measure and balance these fluxes over a diel cycle in order to upscale nitrogen removal rates on restored mussel beds. In addition, active epibenthos such as bivalves can respond rapidly to changes in their environment (e.g. fluctuations in suspended sediment and phytoplankton concentrations; Higgins 1980, Ward & MacDonald 1996). Future studies linking changes in environmental conditions to *P. canaliculus* behaviour could further our understanding of how bivalves affect nitrogen dynamics at larger spatial and temporal scales.

Our flux measurement incubation period represents one point in time. While ambient inorganic nitrogen concentrations and measured fluxes have not shown substantial seasonality in northern New Zealand (Jones et al. 2011, Tay et al. 2012), temporal variation was not addressed in the current study. These scale constraints are common with field-based research and suggest that any information used from this study to inform the placement of future beds be nested in an adaptive management framework to re-evaluate the success of mussel restoration projects on nitrogen removal over longer timescales.

In the present study, net DNF increased with decreasing mud content, and these results are mirrored by others who have carried out benthic chamber experiments in the same harbour (O'Meara et al. 2020). The regression tree suggests that percentage mud content becomes most important at low levels of SOM (< 2.3%). It should be noted that the terminal nodes associated with this split both result in apparent nitrogen fixation, yet this rate decreases with decreasing mud content. Grain size affects the transport of nutrients into and out of marine sediments, and larger particle size typically corresponds to higher porosity associated with increased porewater advection and the transport of nutrients (Santos et al. 2012). At low organic matter loads, mussel biodeposits make a greater contribution to DNF in more permeable, nutrient-poor sediments, increasing the availability of bioreactive nitrogen while creating anoxic microsites required for DNF (Seitzinger et al. 2006). It is also well documented that macrofaunal abundance and species richness increase with decreasing mud content (e.g. Mannino & Montagna 1997, Thrush et al. 2003, Thrush et al. 2004, Pratt et al. 2014) and that macrofauna stimulate increased nitrogen removal (reviewed by Stief 2013). Following these relationships it follows that DNF increases with decreasing mud content, as the abundance of these sediment dwelling species also increases under these circumstances. While not emphasised by regression tree analysis, the significance of macrofaunal abundance as an important variable was reflected in random forest outputs.

Of notable interest are the net fluxes at site LB, where net nitrogen fixation was apparent in all chambers. Nitrogen fixation (breaking dinitrogen's triple bond) is an energetically costly process. Even in the presence of bivalves that emit nitrogenous compounds, it is clear that a demand for bioavailable nitrogen exists at LB; this nitrogen demand is perhaps not surprising for a low-nutrient system, and nitrogen fixation has previously been recorded in other bivalve studies (Humphries et al. 2016) and under dark conditions (Fulweiler et al. 2007). Cyanobacteria known to produce nitrogenase (the enzyme responsible for reducing N_2 to ammonia) have been found in local estuarine sediments of this region (Hicks & Silvester 1985) and could account for observed nitrogen fixation. LB's NO_x^- and NH_4^+ emissions in chambers without mussels possibly reflect the products of microbial nitrogen fixation; lower NO_x^- and NH_4^+ fluxes observed in chambers with mussels suggest tight recycling/high turnover of nitrogen in these places. In many oligotrophic environments, a larger percentage of total bioreactive nitrogen is assimilated rather than removed as N_2 gas; this increased nitrogen retention, however, can support increased secondary and tertiary production (Cook et al. 2004, Vieillard et al. 2020).

Understanding nitrogen dynamics in oligotrophic environments is crucial as these systems are highly underrepresented in the literature (Vieillard et al. 2020) and restoration projects in these places likely differ in their capacity to support enhanced nitrogen removal services (Hoellein & Zarnoch 2014). In such a system we conclude that the effects of mussel restoration on sediment DNF are not restricted to the patch-scale, and that dispersed SOM is an important predictor of net N_2 fluxes at restoration sites. This study contributes to the small but growing literature-base on nitrogen cycling in oligotrophic estuaries of the southern hemisphere, with project insights helping us better understand ecosystem functionality in restored bivalve systems.

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Chapter 4

The influence of mussel restoration on coastal carbon cycling[*]

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4.1 | Abstract

Increasing responsiveness to anthropogenic climate change and the loss of global shellfish ecosystems has heightened interest in the carbon storage and sequestration potential of bivalve-dominated systems. While coastal ecosystems are dynamic zones of carbon transformation and change, current uncertainties and notable heterogeneity in the benthic environment make it difficult to ascertain the climate change mitigation capacity of ongoing coastal restoration projects aimed at revitalising benthic bivalve populations. In this study we sought to distinguish between direct and indirect effects of subtidal green-lipped mussels (*Perna canaliculus*) on carbon cycling, and combined published literature with in-situ experiments from restored beds to create a carbon budget for New Zealand's shellfish restoration efforts. A direct summation of biogenic calcification, community respiration, and sediment processes suggests a moderate carbon efflux (+100.1 to 179.6 g C m^{-2} yr^{-1}) occurs as a result of recent restoration efforts, largely reflective of the heterotrophic nature of bivalves. However, an examination of indirect effects of restoration on benthic community metabolism and sediment dynamics suggests that beds achieve greater carbon fixation rates and support enhanced carbon burial compared to nearby sediments devoid of mussels. We discuss limitations to our first-order approximation and postulate how the significance of mussel restoration to carbon-related outcomes likely increases over longer time scales. Coastal restoration is often conducted to support the provisioning of many ecosystem services, and we propose here that shellfish restoration not be used as a single measure to offset carbon dioxide emissions, but rather used in tandem with other initiatives to recover a bundle of valued ecosystem services.

4.2 | Introduction

Humankind has altered the global carbon cycle with concerning pace (Falkowski et al. 2000, Hansen et al. 2006), the physical and biological consequences of which are currently being experienced in both terrestrial and marine realms (Rosenzweig et al. 2008, Hoegh-Guldberg & Bruno 2010, Doney et al. 2012). Rising concerns over global environmental change have been met with a rapid increase in research outputs related to climate change mitigation and adaptation strategies (Einecker & Kirby 2020) and the potential carbon storage/uptake capacity of natural systems, with scientific efforts largely concentrated on the ability of vegetated coastal habitats to fix and store carbon (e.g. 'blue carbon' habitats such as mangroves, salt marshes, sea grasses; Duarte et al. 2010, Mcleod et al. 2011, Alongi 2012, Duarte et al. 2013, Macreadie et al. 2021).

Located at the land-sea interface, coastal ecosystems are dynamic zones of carbon transformation and change (Chen & Borges 2009, Najjar et al. 2018), and their sediments have been identified as major sites of carbon burial, with coastal oceans contributing to as much as 80% of total organic carbon burial (Berner 1982, Rabouille et al. 2001, Burdige 2007, Keil 2017). Storage in these sediment systems is complex and variable over space and time (Bianchi et al. 2018), involving the rate of settlement, particle mixing, and subduction of carbon deep into sediments. Large, reef-building bivalves such as oysters and mussels are seen as important in their contributions to benthic-pelagic coupling and generating microhabitats conducive to the mediation of biogeochemical cycles (e.g. Ray et al. 2019). As carbon is also utilised in the shell formation process, there has been recent interest in understanding how globally-threatened, bivalve systems influence coastal carbon cycling at ecologically relevant scales (Filgueira et al. 2015, Fodrie et al. 2017).

Conventionally, the influence of bivalves on coastal carbon cycling has been reduced to the summation of an individual's carbon dioxide (CO_2) emissions (resulting from respiration and calcification) versus carbon stored in the individual's shell and tissue (see Filgueira et al. 2019). This however neglects to address the engineering role (Gutiérrez et al. 2003, Meadows et al. 2012) and upscaling effects (Sea et al. 2021) that bivalves demonstrate at the ecosystem level. For example, populations of bivalve filter feeders play a significant role in benthic-pelagic coupling (the exchange of energy and nutrients between the water column and seafloor; Griffiths et al. 2017), exerting top-down control on carbon-fixing phytoplankton populations and influencing nutrient availability and energy transfer in these systems (Newell 2004). Furthermore, the physical protrusion of bivalve beds above the seafloor alters local hydrodynamics, traps sediment particles, and stabilises the seafloor (Butman et al. 1994, Widdows et al. 2002, Gutiérrez et al. 2003, Riisgård et al. 2011), encouraging sedimentation and carbon storage in areas rich with organic material resulting from filter-feeding. The effects of bivalves on carbon cycling are thus more complex than simple shell formation and dissolution equations at the individual scale, and questions regarding their implications on coastal carbon must be resolved using an ecosystem approach (e.g. Filgueira et al. 2015) which encompasses direct and indirect feedbacks (physical, biological, and biogeochemical) of bivalves on their environment. Using a more holistic standpoint, some bivalve systems have been deemed carbon sinks (e.g. Fodrie et al. 2017), although the magnitude and extent of this sink capacity across species and geographic locations is currently unknown.

There is further uncertainty surrounding the appraisal of biogenic calcification to coastal carbon budgets (Macreadie et al. 2017, Saderne et al. 2019), as this process increases

$p\mathrm{CO}_2$ of seawater and (to a certain extent) results in atmospheric carbon emissions (Zeebe & Wolf-Gladrow 2001). Those focused on culturing bivalves for human consumption have suggested carbon accounting systems which split carbon fluxes attributed to shell formation from carbon fluxes associated with tissue (Filgueira et al. 2015, Filgueira et al. 2019). Others have chosen to omit shell formation from evaluations completely, acknowledging “a lack of consensus on whether calcification represents a source or sink of CO_2” (van der Schatte Olivier et al. 2020). Uncertainties associated with shell production must be addressed if we hope to accurately assess carbon cycling in systems dominated by these calcium-carbonate secreting organisms.

To understand the consequences of coastal bivalve beds in a climate context, the effects of filtration and organic matter deposition need to quantified and balanced against shell production and metabolic processes, as heterotrophic bivalves respire and produce CO_2. When considered at the ecosystem scale, diverse benthic communities supported by biogenic reef habitat also need to be included in calculations, as they consume locally trapped organic matter before burial and respire, producing further CO_2. Bivalve reefs will be net carbon sinks if their capacity to store organic carbon in local sediments and inorganic carbon in shell material outweighs carbon dioxide emissions resulting from ecosystem metabolism and biogenic calcification. This capacity almost certainly varies between systems and species, making it important to resolve if we are to advance shellfish restoration or conservation as a viable climate mitigation strategy.

Extensive shellfish beds with the potential to affect carbon cycling at ecologically relevant scales have largely been decimated worldwide (e.g. Lotze et al. 2006, Airoldi & Beck 2007, Gillies et al. 2018), and restoration projects are currently underway to recover the ecosystem services they provide (zu Ermgassen et al. 2020). With increased interest in restoring coastal habitats and mitigating anthropogenic carbon emissions, there is a significant need to determine how large-scale bivalve restoration projects affect carbon budgets of coastal systems. In light of current uncertainties, our overall objective was to document and quantify carbon transformations following recent restoration efforts (focusing solely on carbon transformations after addition of adult mussels to soft-sediment systems), utilising a holistic, ecosystem-based approach to approximate a first-order carbon budget in restored beds. We address carbon cycling concepts by combining in-situ and laboratory experiments with published and unpublished data obtained from subtidal mussel restoration efforts in the Hauraki Gulf of northern New Zealand, and report ranges of potential carbon uptake (-) and/or emissions (+) resulting from calcium carbonate production, ecosystem metabolism, and dynamic sediment processes. Our examination of New Zealand's restored mussel beds highlights how heterogeneity affects carbon outcomes at the bed-scale and is important in addressing current knowledge gaps related to coastal carbon cycling and sequestration potential in restored bivalve systems.

4.3 | Methods

4.3.1 | Study Area and Budget Overview

Where possible, our carbon budget utilises data collected from studies of shallow subtidal, green-lipped mussels in the Hauraki Gulf of New Zealand. Over the past five years, more than 200 tonnes of adult mussels have been relocated from regional longline mussel farms to soft-sediment locations around the Gulf, generating multiple beds near Mahurangi Harbour and Kawau Bay (Fig. 4.1). While these mussel beds are of similar depth (5-15 m) and size (~ 10-25 m^2), the locations they were restored in were chosen to encompass a range of environmental conditions (namely variations in exposure, sediment grain size, porosity, organic matter, and macrofaunal assemblages) capable of affecting biogeochemical cycling at ecologically relevant scales (e.g. Hillman et al. 2021). Combining field collections with laboratory experiments and published literature, we utilised data from as many of these sites as possible (Table AIII.1) to capture environmental variation and expand the generality of our results. If data necessary for the carbon budget were not available from these beds, we expanded our search to publications using green-lipped mussels throughout New Zealand, or, if unavailable, to other bivalves in similar temperate climates.

We conceptually divided the effects of mussel restoration on carbon cycling into three parts: A. mussel shell formation/dissolution; B. benthic metabolism of mussel bed communities (local respiration of the benthic communities versus carbon fixation); and C. carbon storage in sediments beneath mussel beds (Fig. 4.2). Our calculations are based on an idealised 15 adult (95 mm-115 mm) mussels per m^2, an estimate designed to express the patchy spatial aggregation patterns observed on these beds (Sea et al. 2021). The following sections summarise what is known about the role of green-lipped mussels in carbon cycling to justify selected values and calculations used to create our budget.

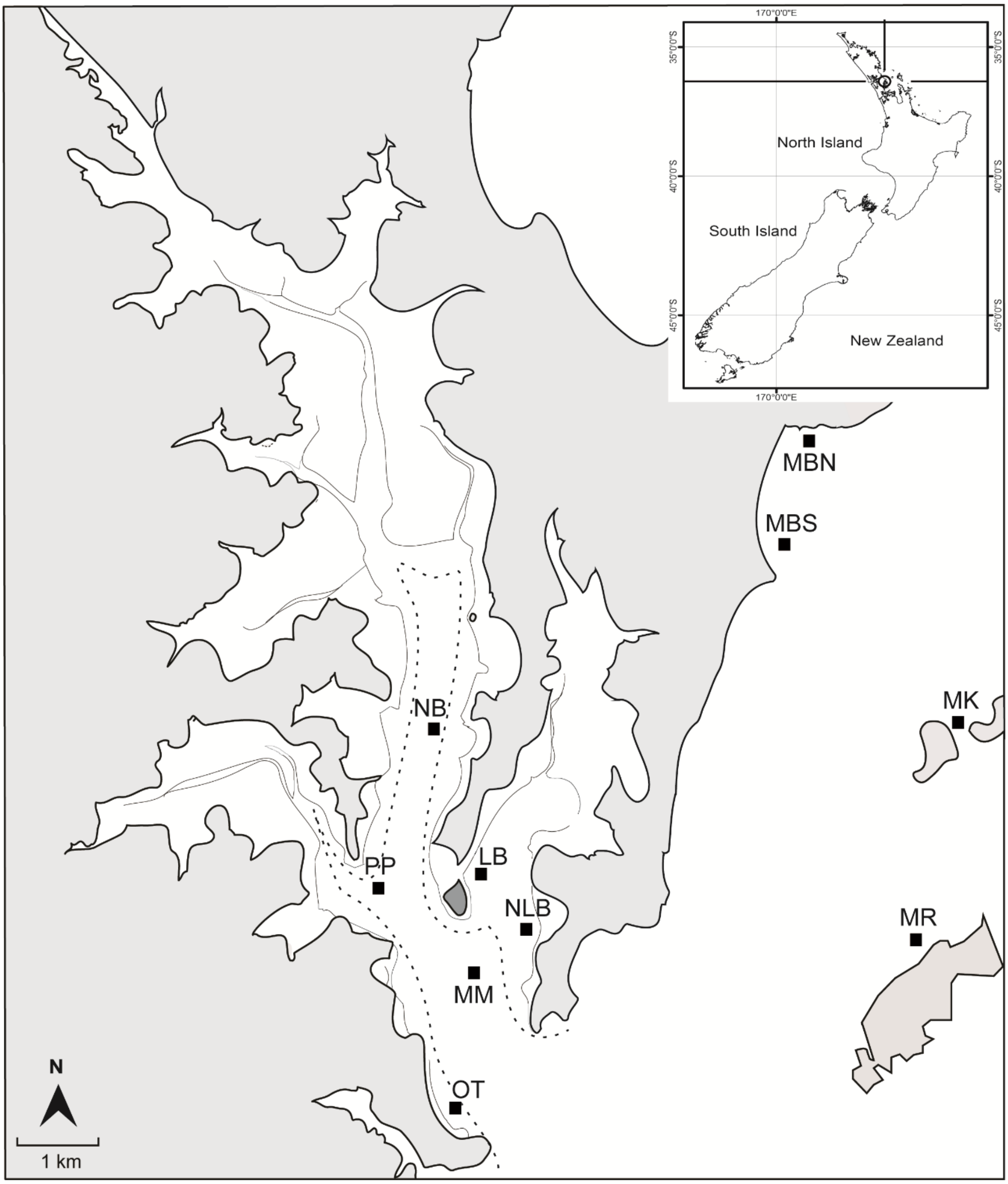

Figure 4.1 Location of mussel restoration sites in Mahurangi Harbour and Kawau Bay, New Zealand. Site labels: MM = Mahurangi Mid, MBN = Martins Bay North, MBS = Martins Bay South, MR = Motuora, MK = Motoketekete, NB = Ngaio Bay, NLB = New Lagoon Bay, LB = Lagoon Bay, OT = Otarawao Bay, and PP = Pukapuka.

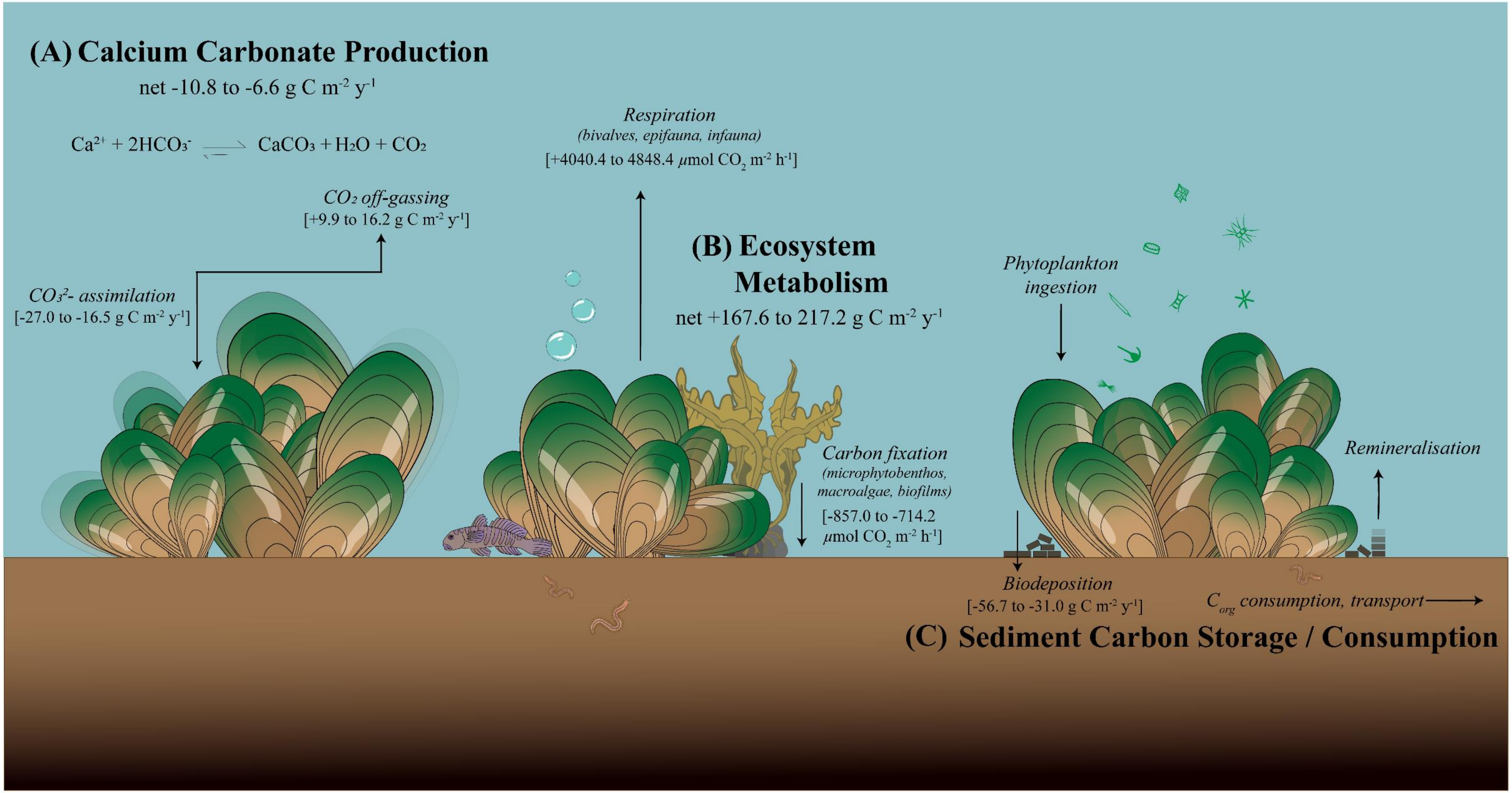

Figure 4.2 Infographic illustrating carbon cycling and transformations in restored green-lipped mussel beds. Negative values (-) are indicative of processes that promote carbon storage, drawdown, or fixation while positive values (+) denote carbon effluxes. Information is conceptually divided into three parts: (A) calcium carbonate production, (B) ecosystem metabolism, and (C) sediment consumption/storage. Note that part (C) has no associated total net flux, as biodeposit remineralisation processes are mechanistically linked to experimental methods utilised in part (B) of the budget.

4.3.2 | Shell Formation and Dissolution

Bivalves utilise bicarbonate ions (HCO_3^-) in seawater to synthesise a calcium carbonate shell ($CaCO_3$), a process which is generally summarised using the below equation (4.1):

$$Ca^{2+} + 2HCO_3^- \rightleftharpoons CaCO_3 + H_2O + CO_2 \qquad \text{Eq. 4.1}$$

This equation for the precipitation of calcium carbonate, however, is a rather "simplistic representation" which can lead to the mistaken conclusion that 1 mol of carbon dioxide is generated per mol of calcium carbonate produced; in reality, roughly 0.6 mol CO_2 is produced for every mol of precipitated $CaCO_3$ (Ware et al. 1992).

This effect can be attributed to the chemical nature of carbon dioxide, which dissociates in seawater and takes on four different inorganic forms which readily switch between one another and collectively regulate seawater pH (referred to as the bicarbonate buffer system or the carbonate system; Zeebe & Wolf-Gladrow 2001). A portion of CO_2 produced from biogenic calcification forms HCO_3^- while the remaining CO_2 is either released to the atmosphere or inhibits additional carbon uptake by the oceans (Ray et al. 2018). While the amount of CO_2 emitted as a result of shell formation has been shown to vary with temperature, salinity, and local carbonate chemistry (Morris & Humphreys 2019), it is generally accepted that ~0.6 mol CO_2 is produced for every mol of precipitated $CaCO_3$ (Macreadie et al. 2017, Saderne et al. 2019). Here, we credit carbon temporarily stored in mussel shell over an average individual's lifetime, or roughly 30 years (a time scale comparable to the rotation of timber stands recognised as carbon stores in the terrestrial realm; Brockerhoff et al. 2008, Zhang et al. 2012, Xie et al. 2020). To quantify carbon stored in shell, we used the following equation (eq. 4.2):

$$C_s = W * \ C_f \qquad \text{Eq. 4.2}$$

Where C_s is the amount of carbon stored (g m^{-2} y^{-1}), W is the annual change in dry shell weight, and C_f is the carbon fraction of mussel shell's dry weight. Using molar ratios outlined by Ware (1992), we then multiplied calculated C_s values by 0.6 to estimate CO_2 production resulting from the precipitation of calcium carbonate. Other studies (Tang et al. 2011, Filgueira et al. 2019) report carbon constitutes 11.1-12.7% of scallop, mussel, oyster, and clam shells' dry weight (a C_f value of 0.111-0.127). For our calculations we used a slightly wider range (10-15% or a C_f value of 0.1-0.15) which has previously been reported for green-lipped mussel shells (Stenton-Dozey & Broekhuizen 2019).

In order to determine annual change in shell weight (W), it was necessary to first establish shell growth rates for green-lipped mussels. McLeod et al. (2012) showed average adult green-lipped mussel shell length increased 0.04 mm per day on the seafloor of the Hauraki Gulf, which we extrapolated to 14.6 mm per year (a growth rate similar to reports from unpublished theses, ranging from 11.7 to 15.3 mm per year; Van Kampen 2017, Wilcox 2017). We used linear regression to create a model that predicts adult green-lipped mussel shell weight from a given shell length (Fig. AIII.1), and, assuming an adult growth rate of 14.6 mm per year, we estimated an average annual increase in shell weight (g) given known shell lengths. We multiplied this value by 15 to estimate the weight increase of our hypothetical mussel clump (m^2) on an annual basis.

Note that equation 4.2 does not quantify total carbon stored in mussel shells (only carbon captured as a result of adult growth), as we are concerned with carbon effects post-restoration. The majority of growth (and therefore carbon capture in shells) occurs in the first few years of development and decreases significantly with mussel size (Hickman 1979), and is therefore not accounted for here.

4.3.3 | Ecosystem Metabolism

Mussel bed ecosystem metabolism was determined through flux measurements capturing the net effects of respiration (CO_2 effluxes from both bivalves and the benthic communities they support) and carbon fixation (CO_2 captured by photosynthetic macroalgae, microphytobenthos, and biofilms on shell surfaces). Less time consuming and costly than measuring carbon fluxes directly, oxygen demand was converted to a carbon mineralisation or uptake rate (e.g. Oviatt et al. 1986, Clavier 1994) using respiratory and photosynthetic quotients respectively.

In February of 2017 and March-April of 2019, in-situ benthic flux chambers (0.25 x 0.25 m; volume = 41 L) were placed over mussel clumps and control sediments devoid of mussels (~ 5 m away) at a total of nine different restoration sites in Mahurangi Harbour and Kawau Bay (Table AIII.1) to determine net oxygen fluxes. This was done both under dark and light conditions achieved by covering and uncovering chambers with black polyethylene (methods adapted from Lohrer et al. 2004). Briefly, water samples were taken at the beginning and end of the incubation period (~ 4 h) and analysed for O_2 concentration using a quadrupole membrane inlet mass spectrometer (with Pfeiffer Vacuum Prisma Plus QMG220 M1 QMS, Bay Instruments); these oxygen measurements were checked for congruence with values obtained from miniDOT optical oxygen loggers (Precision Measurement Engineering) placed within the chamber space. With known incubation times for each chamber, fluxes were calculated as the difference between initial and final concentrations and corrected for volume and surface area of the chamber to obtain a flux in μmol m^{-2} h^{-1} (e.g. O'Meara et al. 2020). Oxygen flux data were combined from two published studies (total chambers inside beds = 49, total chambers outside beds = 39; Hillman et al. 2021, Sea et al. 2021) and converted to a carbon mineralisation rate using respiratory quotients of 1 and 1.2 under dark conditions, and photosynthetic quotients of 1 and 1.2 under light conditions (deemed acceptable for photic, oligotrophic marine sediments; Clavier 1994, Carlsson et al. 2010, Attard et al. 2020). Finally we assumed a 12:12 hour light cycle and multiplied carbon fluxes from light and dark chambers accordingly to obtain the net carbon ecosystem metabolism over a diel cycle (μmol m^{-2} d^{-1}), achieved by subtracting carbon uptake during daylight hours from carbon effluxes during darkness. This net value was converted to grams of carbon per year to achieve units comparable to the rest of the carbon budget.

4.3.4 | Sediment Carbon Storage and Consumption

The amount of carbon stored in mussel bed sediments is dependent on local sedimentation rates, biodeposition rates (which also vary with the quality and quantity of organic material filtered and eventually digested/egested), and the rate of carbon degradation at various sediment depths. Each of these ideas is explored below.

4.3.4.1. Carbon from biodeposition. Using a laboratory experiment, we determined the amount of carbon available to surficial sediments resulting from mussel biodeposition. Our experiment used adult green-lipped mussels from five restoration sites collected in winter 2017 (Table AIII.2). Mussels were allowed to acclimatise to laboratory conditions for three days in 10 L aerated tanks (seawater flow-through system, filtered on 200 μm mesh; average water temperature 17° C; 10:14 hour light cycle). On the day of the experiment, sets of three mussels each were placed in a smaller tank (3 L) and allowed a

1-hour filtration period in seawater obtained from one of two Mahurangi water sources with contrasting suspended sediment concentrations (either “high” or “low”; 66.67 ± 1.5 or 13.83 ± 1.9 FNU respectively), ensuring that trials encompassed a range of natural suspended sediment concentrations typically experienced within the Mahurangi Harbour and Kawau Bay. Biodeposits were collected at the end of the filtration period, dried at 60° C for 48 hours and weighed. Biodeposition values obtained from mussels filtering each water source (high: $n = 15$; low: $n = 15$) were multiplied by 5 (to upscale to our 15 mussel scenario), and then converted to a biodeposition rate in g m^{-2} yr^{-1}. Finally, this value was multiplied by the average carbon fraction (6.4 %) of mussel biodeposits (Giles & Pilditch 2004, 2006, Filgueira et al. 2019) to obtain an estimated carbon production rate in g C m^{-2} yr^{-1}.

4.3.4.2 Biodeposit degradation and subduction rates. Only a portion of carbon in biodeposits is subducted into marine sediments. Others have shown that mussel biodeposits rapidly degrade, and a portion of resultant organic carbon is remineralised (Giles & Pilditch 2006, Carlsson et al. 2010). Note that the biodeposits of green-lipped mussels have been shown to exhibit a half-life of 4.3 days under similar temperature conditions (Giles & Pilditch 2006), but we do not directly calculate remineralisation rates here, as increases in sediment oxygen demand (and subsequent CO_2 effluxes) resulting from the degradation of biodeposits are included in net fluxes derived from chamber experiments outlined in section 4.3.3 above.

4.3.4.3. Carbon consumption in mussel bed sediments. Finally, not all carbon drawn down to the seafloor is sequestered, as a portion of this carbon fuels microbial and macrofaunal activity in local sediments; resultant bioturbation can further influence sediment organic matter degradation rates and carbon remineralisation efficiency (Burdige 2007, Arndt et al. 2013). We trialled the application of a rapid organic matter assay (ROMA, methods described in O'Meara et al. 2018) to measure carbon consumption rates at different sediment depths. Briefly, two assay plates (with a series of machined wells corresponding to sediment depths of 1, 3, 7, 10, and 15 cm) were filled with a carbon/agar solution, allowed to solidify, and carefully inserted into mussel bed sediments using SCUBA. Plates were left out for a total of two weeks at five restoration sites (Table AIII.1) and carbon degradation at various depths determined by calculating the net difference in agar volume over the experiment duration. These methods were repeated in soft-sediment control locations ~ 5 m away from restoration sites. ROMA plates were not recovered from the Ngaio Bay control, leaving a total of four control sites used for comparison purposes. Carbon degradation rates generally decrease with depth (O'Meara et al. 2018), and our goal was to determine how relative rates vary with increasing depth across sites and treatments (mussel beds vs. control sediments), and to establish if a ‘sequestration depth’ (consumption rate < 1 g C m^{-2} d^{-1}) was apparent under the given experimental conditions.

4.3.4.4. Visualising carbon storage at various depths. To visualise long-term changes in carbon storage as a result of mussel restoration, triplicate sediment cores (5 cm diameter, 30 cm length) were taken inside and outside (> 5 m away) of four restored beds in late January and early February of 2021 (Table AIII.1). Samples were split into depth segments which concentrated sampling efforts on the uppermost sediment layers (every centimetre for the first 5 cm, then aliquots taken from 5-10 cm and 10-20 cm) because restored beds had only been in place for a few years. Samples were dried at 60° C, ground with mortar and pestle, and stored in a desiccator prior to analysis. Percent total carbon content of dried sediment samples was determined using a CHSN elemental analyser

(Vario EL Cube, Elementar, Langenselbold, Germany), with results converted to g carbon using known sample weights and later scaled to 1 m^2. 2-way ANOVA was used to determine if total carbon content varied by restoration site and status (inside bed or outside bed). Values were log-transformed to meet assumptions of normality and homogeneity of variance. We adjusted *p*-values using Holm's Sequential Bonferroni Procedure to control the familywise error rate for multiple hypothesis tests. Significance levels were set to $\alpha < 0.05$, and standard errors (SE) were used to assess the precision of mean values. All analyses were conducted using the R statistical package (version 4.1.0).

4.4 | Results

4.4.1 | Shell Formation and Dissolution

Our regression model accurately predicted adult green-lipped mussel shell weight from shell length ($R^2 = 0.782$, $p < 0.0001$; Fig. AIII.1), and, assuming a growth rate of 14.6 mm per year, we estimated the weight of an adult mussel shell increases by 11 to 12 g annually. Using our 15 mussel m^2 model system, this becomes an additional 165-180 grams per year (term W in eq. 4.2). Given the accepted carbon fraction range for green-lipped mussels (C_f = .1-.15), we estimated carbon storage between 16.5 and 27.0 g C m^{-2} yr^{-1} (Fig. 4.2). This degree of calcium carbonate precipitation results in CO_2 emissions totaling ~ 9.9 to 16.2 g C m^{-2} yr^{-1}, or total net storage of -10.8 to -6.6 g C m^{-2} $year^{-1}$ as a combined result of shell formation and dissolution processes.

4.4.2 | Ecosystem Metabolism

We recorded net oxygen consumption in all benthic chambers under dark conditions within mussel beds, with an average uptake rate of -4040.4 ± 284.0 (SE) µmol O_2 m^{-2} h^{-1} (Fig. 4.3). Even with large heterotrophic bivalves in the chamber space, an average net oxygen efflux was observed under light conditions (857.0 ± 636.8 µmol O_2 m^{-2} h^{-1}), although these chambers exhibited roughly twice as much variation as their dark counterparts. Using the accepted range of respiratory and photosynthetic quotients resulted in a carbon mineralisation rate within mussel beds of 4040.4 ± 284.0 to 4848. ± 340.8 µmol CO_2 m^{-2} h^{-1} in the dark, and carbon uptake between -714.2 ± 530.6 to -857.0 ± 636.8 µmol CO_2 m^{-2} h^{-1} in the light. Over a diel cycle this becomes a net carbon efflux of 0.459 to 0.595 g C m^{-2} d^{-1}, or an annual efflux of 167.6 to 217.2 g C m^{-2} yr^{-1}.

In contrast, dark control sediments outside mussel beds had an average oxygen consumption rate roughly four times less than that of dark sediments within mussel beds (-940.6 ± 115.7 µmol O_2 m^{-2} h^{-1}). Average net oxygen production was again observed in light chambers outside mussel beds (1087.0 ± 191.8 µmol O_2 m^{-2} h^{-1}), a rate comparable to (but slightly greater than) those reported within beds (Fig. 4.3). These oxygen consumption and production rates in control sediments result in carbon mineralisation and fixation rates of 940.6 ± 115.7 to 1128.7 ± 138.9 µmol CO_2 m^{-2} h^{-1} and -905.9 ± 159.9 to -1087.0 ± 191.8 µmol CO_2 m^{-2} h^{-1} respectively. Corresponding net diel ecosystem metabolism in control sediments overlaps with zero (from carbon uptake of -0.021 g C m^2 d^{-1} to a carbon efflux of 0.032 g C m^2 d^{-1}), equating to an annual carbon emission/uptake range of -7.7 to +11.7 g C m^{-2} yr^{-1} in sediments devoid of mussels.

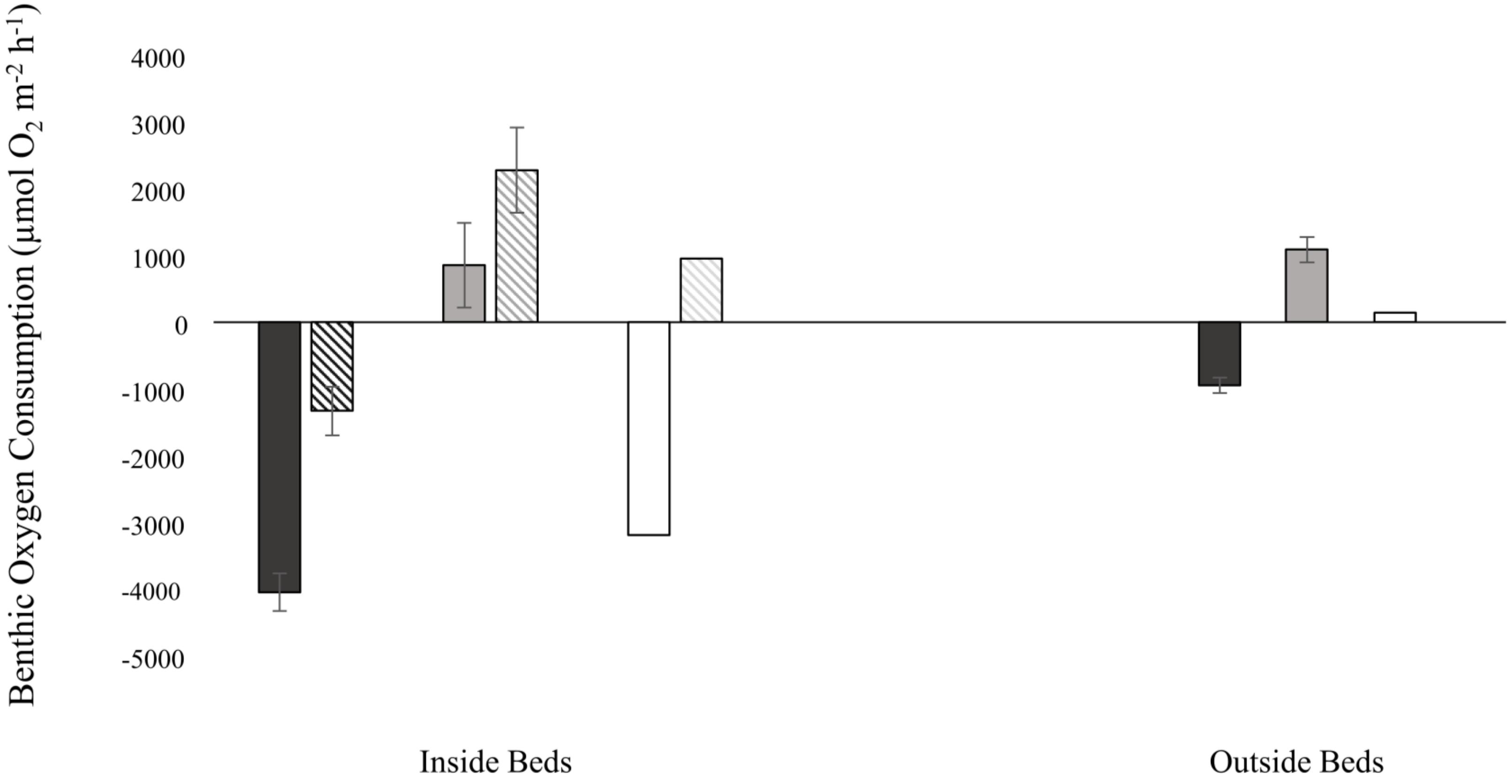

Figure 4.3 Results of benthic chamber experiments in nine restored mussel beds, comparing average benthic oxygen consumption inside and outside (> 5 m away) of mussel beds, under light and dark conditions (grey and black bars respectively). For visual reference, white bars show the calculated net oxygen flux (difference between light and dark chambers). Diagonal bars inside beds represent sediment oxygen demand after removing the direct effect of mussel respiration on measured O_2 fluxes from Hillman et al., 2021 (oxygen demand of 15 mussels per chamber determined using regression equation in Fig. AIII.3).

4.4.3 | Sediment Carbon Storage and Consumption

Nearly twice as many biodeposits were produced at higher concentrations of suspended solids (2-way ANOVA; $F_{1,24} = 27.8$; $p < 0.0001$; Fig. AIII.2). We averaged the biodeposit production rates across all five sites for each water source (high vs. low suspended solid concentration) to estimate that each clump of three mussels produces between 0.0110 ± 0.0007 and 0.0202 ± 0.0017 g biodeposits h^{-1}. Extrapolated to the idealised mussel clump scale on an annual basis, biodeposition rates were estimated between 483.0 and 883.9 g biodeposits m^{-2} yr^{-1}. Utilising the average carbon content of mussel biodeposits (6.41 %) results in additional carbon drawn-down (-) due to benthic-pelagic coupling of 31.0 to 56.7 g C m^{-2} yr^{-1} to the seafloor.

Carbon degradation rates calculated from rapid organic matter assays decreased with depth at all five restoration sites, but both the rate and magnitude of change in degradation varied by site (Fig. 4.4). Mussel beds within Mahurangi Harbour (MM, NB, PP) had surface degradation rates approximately three times lower than beds within (or near the entrance of) Kawau Bay. At control sites, greater carbon degradation rates were similarly observed in sandier Kawau Bay sediments, although notable increases in calculated degradation rates were observed in muddier, Mahurangi Harbour control sediments at 10 cm depth. With the exception of site OT, all mussel beds converged on carbon degradation rates between ~ 1 and 3 g C m^{-2} d^{-1} at the deepest sediment depth tested (15 cm). At this same depth, carbon degradation rates were roughly two to three-fold higher in control sediments. Comparing each mussel bed to its control sediments revealed a general pattern of increased degradation activity in soft-sediment controls, a pattern which continued down to depths of 15 cm at all but one site (OT; Fig. 4.4).

While carbon degradation rates were typically highest at the sandier, Kawau Bay sites, total carbon storage determined from sediment cores was also noticeably greater (roughly two times that of muddier beds; Fig. 4.5). Increased total carbon content was also observed in sediment cores taken outside the mussel bed at Motuora, and significant site differences in carbon content were detected at all sediment depths tested (Table AIII.2). Mussel beds did not have a significant effect on the amount of carbon recorded in surficial sediments (0-1 cm; 2-way ANOVA; $F_{1,20} = 1.8$; $p = 0.785$); but appeared to make notable contributions just below the sediment surface (depths of 1-2 and 2-3 cm; 2-way ANOVA; $F_{1,19} = 7.0$; $p = 0.093$ and 2-way ANOVA; $F_{1,19} = 9.6$; $p = 0.041$ respectively). With the exception of Lagoon Bay, total carbon measured in mussel beds increased for the first few centimetres of sediment, and then gradually decreased with increasing sediment depth, while carbon content at all non-restored sites initially decreased relative to surface sediments at these depths (Fig. 4.5).

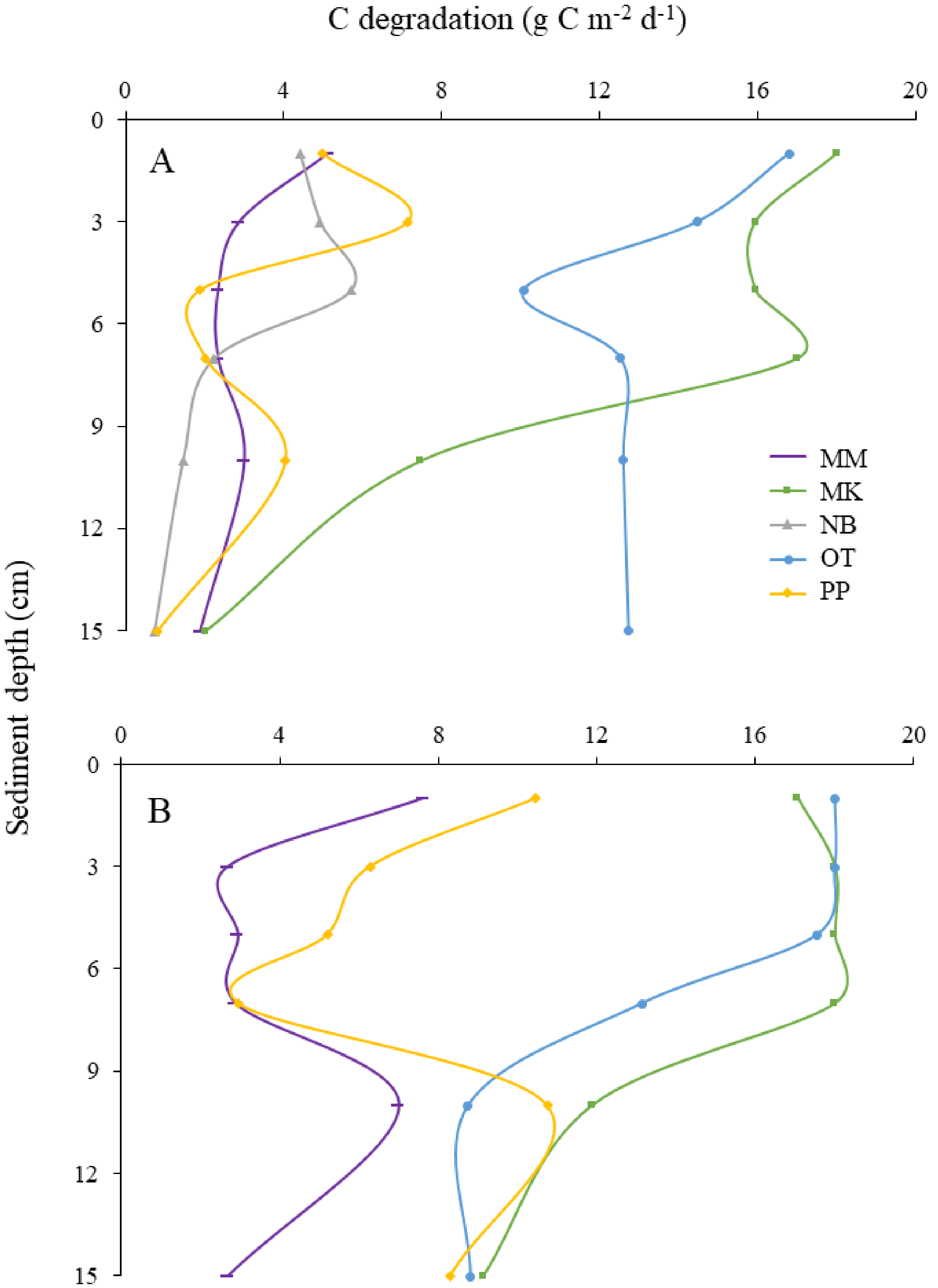

Figure 4.4 Calculated carbon degradation rates at various sediment depths using rapid organic matter assay (ROMA) plates in A. subtidal restored mussel beds, and B. control sediments devoid of mussels (~ 5 m away from restoration sites). Site labels: Mahu Mid = MM, Motoketekete = MO, Ngaio Bay = NB, Otarawao Bay = OT, and Pukapuka = PP.

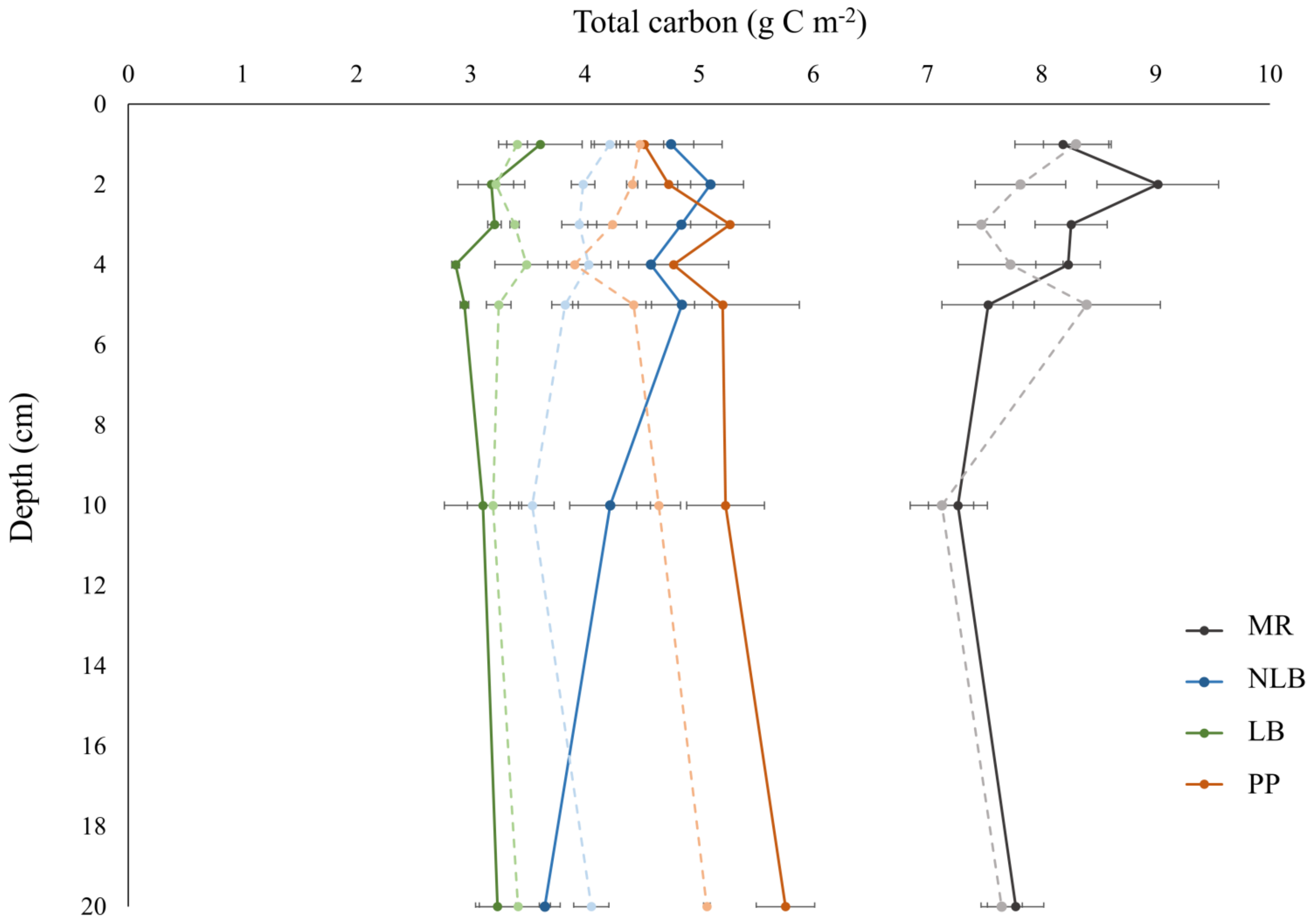

Figure 4.5 Total carbon from sediment cores, separated by depth at mussel restoration sites (solid lines) and nearby sediments (> 5 m away) devoid of mussels (dashed lines). Site labels: MR = Motuora, NLB = New Lagoon Bay, LB = Lagoon Bay, and PP = Pukapuka.

Figure 4.2 summarises the overall budget estimated for restored mussel bed sites. A direct summation of biogenic calcification, benthic ecosystem metabolism, and biodeposition processes suggests a moderate carbon efflux (+100.1 to 179.6 g C m^{-2} yr^{-1}) occurs within restored mussel beds, with bed sediments exhibiting lower carbon degradation rates and higher carbon content than controls. Control sites are considered relatively neutral in terms of their carbon contributions (benthic oxygen consumption resulting in -7.7 to +11.7 g C m^{-2} yr^{-1}), with sediments characterised by higher carbon degradation rates and lower carbon content than restored beds.

4.5 | Discussion

Here we combined published literature with experiments to illustrate that shallow, coastal environments dominated by dense bivalve beds are significant zones of carbon transformation contributing to ecosystem processes and community metabolism. Notable differences in carbon cycling occur at restoration sites compared to controls, with mussel beds characterised by greater carbon mineralisation rates but significantly higher carbon content and lower degradation rates in underlying sediments. We provide a carbon budget for New Zealand's shellfish restoration efforts and report a range of potential carbon effluxes, importantly demonstrating that carbon-related services/outcomes are affected by the highly heterogeneous nature of coastal ecosystems and selected restoration locations. Similar to other budgets, our work necessarily simplifies complexities inherent in dynamic, coastal systems, but our first-order approximation represents a current best-estimate given limited data availability and considers a number of ecological nuances often overlooked in comparable budgets of terrestrial forests (Aalde et al. 2006). Our reported carbon effluxes fit well within the accepted range of fluxes published in other studies balancing biogenic calcification and respiration against carbon sequestration in other mussel species (from a net sink of 7.6 g C m^{-2} yr^{-1} to a net source of 1656 g C m^{-2} yr^{-1}; Hily et al. 2013, Mistri & Munari 2013, Munari et al. 2013, Filgueira et al. 2019) and utilises a holistic approach to create ecologically meaningful conclusions regarding the role of bivalves in carbon cycling.

4.5.1 | Shell Formation/Dissolution

Biogenic calcification provides a modest form of carbon storage in restored mussel bed systems (Fig. 4.2). However our estimates are highly conservative owing to the wide range of possible C_f values for green-lipped mussels; the carbon fraction of mussel shell's dry weight is reported to be above 12% for other mussel species (van der Schatte Olivier et al. 2020), and refining this value for green-lipped mussels would better reflect (and likely increase) the carbon sink capacity of restored beds. In addition our methods do not consider carbon stored in mussels before utilisation in restoration projects; the majority of growth occurs in the first two years of life, with average juvenile growth rates three times higher than what we report for adult mussels in this study (Hickman 1979). If restored beds begin to self-recruit in the future, we could credit additional carbon storage occurring over the first few years of rapid development, potentially doubling our estimates of carbon in shell material. In the current absence of recruitment, however, restoration projects solely utilising juvenile mussels (< 30 mm shell length) have been trialled with limited success as a likely consequence of local predation pressures (Alder et al. 2021). Restoration in New Zealand will likely move forward using adult mussels, and until recruitment occurs there is little additional capacity for carbon storage through the process of shell-formation.

It is also important to note that carbon is only temporarily stored in a living mussel’s shell; the fate of this carbon depends on local environmental conditions (hydrodynamic forces, sediment conditions, local sedimentation rates, etc.), the combination of which ultimately determines if shell is buried and stored deep within the sediment before it is allowed to dissolve (e.g. DeAlteris 1988, Fodrie et al. 2017). Green-lipped mussel shells are thin and brittle, and previous studies (even preventing physical abrasion and mechanical breakdown of shell) within the Hauraki Gulf report dissolution rates of 5-8% of mussel shell weight within 300-500 days (Smith 1992). Furthermore, calcium carbonate actively breaks down in the uppermost layers of bioturbated sediments (Waldbusser et al. 2011), and, as pore water acidity rapidly increases within the first few centimetres of sediment in the Mahurangi Harbour, it is unlikely significant amounts of carbon captured in green-lipped mussel shells are stored below surficial sediment depths at present. We note however that the bioengineering capabilities of mussels might eventually allow beds to alter sediment conditions over longer time frames, encouraging the storage of shell material if sediments become well-oxygenated and exhibit higher porosity in the future. In light of current sediment conditions, those interested in tracing CO_2 emissions resulting from biogenic calcification might also note that if shell material is allowed to dissolve in seawater, ensuing alkalinity changes to the carbonate system will actually result in equivalent atmospheric CO_2 concentrations prior to shell formation. To simply these dynamics we have chosen to examine carbon storage on a similar timescale used in terrestrial forest budgets and report a downwards flux (-) of carbon as a result of biogenic calcification, but acknowledge that the long-term, inorganic carbon storage capacity of these systems is subject to a multitude of processes (local aerobic respiration, feedbacks with calcifiers, freshwater discharge, and anthropogenic CO_2 emissions) that ultimately influence the stability of carbonates in estuarine environments (Feely et al. 2002, Abril et al. 2003, Salisbury et al. 2008, Miller et al. 2009). We currently suggest that restoration practitioners interested in improving the carbon burial potential of restoration projects utilise calcium carbonate producing organisms with thicker shells less prone to dissolution (ideally with high C_f values), and consider restoration sites exhibiting less acidic sediment conditions (e.g., lower concentrations of organic material and well oxygenated, high porosity sediments). Initial assessments which verify the compatibility of environmental conditions with pre-determined restoration objectives will prove to be invaluable and will inform both the location and species most suitable for achieving desired ecosystem service outcomes.

Additionally, greenhouse gas projections indicate that the role of bivalve restoration projects in coastal carbon cycling will likely change under future climate change scenarios. Further oceanic uptake of anthropogenic carbon will alter seawater carbonate chemistry and reduce the ability of mussels to calcify, while changes in sea surface temperature will impact physiological processes outlined in our budget (e.g. Resgalla Jr et al. 2007, Talmage & Gobler 2010, Parker et al. 2013). Together these combined effects will influence biogeochemical cycles, ecosystem functioning, and the magnitude of services provided by bivalve-dominated systems. Restoration practitioners must be proactive in selecting species and sites which maximise the probability of ecosystem service delivery under future climate conditions.

4.5.2 | Ecosystem Metabolism

Results of net ecosystem metabolism experiments in mussel beds mirror those of others (Chauvaud et al. 2003, Lejart et al. 2012, Munari et al. 2013, Attard et al. 2020) who report bivalve respiration rates exceed rates of primary production and/or storage in shell material and consistently result in net carbon effluxes from the sediment (Fig. 4.2 and 4.3). In contrast to blue carbon habitats which support numerous faunal species and still contribute to net carbon draw-down, it seems unlikely that the additional contribution of large, heterotrophic bivalves at our restoration sites (also known to support abundant benthic communities which consequently respire further CO_2), could be offset by local autotrophic communities (oxygen-producing biofilms, macroalgae, microphytobenthos; Heisterkamp et al. 2013, Rodil et al. 2019). Despite their heterotrophic status, bivalve beds—by enhancing suitable substrate and nutrient availability—are increasingly acknowledged for their ability to support notable communities of carbon fixing primary producers (Norling & Kautsky 2007, Volaric et al. 2018, Attard et al. 2019) and can achieve gross primary production rates comparable to nearby vegetated coastal habitats (Attard et al. 2019, Attard et al. 2020). The additional role of bivalves in lowering turbidity through sediment stabilisation and filtration processes has also been shown to promote primary productivity (Newell & Koch 2004, Newell et al. 2005). Previous studies at green-lipped mussel restoration sites similarly indicate local enhancement of gross primary production through increased abundance of primary producing communities relative to nearby control sediments (Sea et al. 2022). Physical and biological alterations in the restoration environment are favourable to carbon-fixing autotrophs and can therefore result in additional sources of autochthonous, labile carbon significant to the overall influence of mussel restoration in mediating biogeochemical cycles.

It is noteworthy that other studies (e.g. Attard et al. 2020) attribute as much as half of reported carbon turnover in mussel beds to microbial species supported by beds. As some carbon accounting studies related to shellfish aquaculture call for separate carbon budgets regarding shell formation and tissue growth (a by-product vs. the portion valued for human consumption, e.g. Filgueira et al. 2015), this motivates theoretical questions regarding the appropriateness of dissociating the respiratory needs of heterotrophic bivalves from their effects on overall ecosystem metabolism (as our current report largely showcases an efflux inevitable of any primary consumer used in restoration efforts). Here we estimate the average oxygen demand of an adult, green-lipped mussel to be 94.8 µmol O_2 h^{-1} (Fig. AIII.3). Mussel respiration therefore accounts for a significant portion (an additional 1422 µmol O_2 h^{-1}) of the calculated carbon mineralisation rate in benthic chambers under dark conditions; after removing the direct effect of mussel respiration on measured fluxes, base-line oxygen demand is comparable to nearby bare sediments (Fig. 4.3). As we determined the oxygen requirements of 15 mussels m^{-2} to be substantial, it is notable that net fluxes observed under light conditions were similar inside and outside of beds (Fig. 4.3). Achieving a similar net SOD under light conditions is indicative of enhanced primary productivity within mussel beds.

4.5.3 | Sediment Carbon Storage/Consumption

To the best of our knowledge this work curates all previous attempts to document carbon-related changes in the sediment environment as a result of green-lipped mussel introductions, but we recognise the sediment carbon storage and consumption portion of our budget (part C in Fig. 4.2) is less well defined/constrained than other processes outlined in this text, attributable to the timescale of our studies and the complex, heterogeneous nature of coastal soft sediments. We utilised data from mussel beds under varying hydrodynamic and edaphic conditions to help scale our findings while encompassing natural variability, and can conclude at present that carbon storage is not comparable to typical blue carbon habitats (e.g. Duarte et al. 2005). Research efforts which further resolve related spatial and temporal heterogeneity in the sediment environment will make notable contributions in refining the carbon budget reported here.

Adult green-lipped mussels are known to alter feeding behaviour (filtration and rejection rates) in response to seston quality and quantity, although these rates generally increase at higher concentrations of organic matter in the water column (Hatton et al. 2005). By sourcing water of contrasting quality this additional source of variation was incorporated into our study design, and resulting biodeposition rates are in general agreement with other mussel studies (Hawkins et al. 1999, Hatton et al. 2005, Attard et al. 2020). While pairing increased filtration rates with increased rejection rates of lower-quality particles proves to be energy-efficient for mussels experiencing higher turbidity systems (Bayne et al. 1993, Hatton et al. 2005), it should be noted that, as restored green-lipped mussel beds continue to filter out large quantities of suspended material from the Hauraki Gulf's water column, the carbon content of biodeposits will likely increase over time (as the proportion of filtered seston including carbon-fixing phytoplankton increases). However, it is also possible that the ability of mussels to alter feeding behaviour may partially negate carbon-related benefits to the sediment environment as local sedimentation rates may decrease if filtration rates decrease.

Perhaps a subject which warrants further exploration is the quality and fate of locally produced biodeposits. Note that hydrodynamic forces and mussel spatial arrangement vary with restoration location and can result in the removal/transport of available, carbon-rich biodeposits from local restoration sites (Sea et al. 2022). A current lack of particle tracer studies also precludes the determination of how biodeposits interact with local sediments, as well as what proportion of produced biodeposits are subducted to a depth at which available carbon can be considered sequestered. Additionally, studies analysing biodeposit degradation from other mussel species suggest that, while the labile fraction of deposits is rapidly remineralised, a larger fraction of refractory carbon (as much as 80% of total POC in biodeposits) remains over longer time scales (days to weeks) and is available for potential storage in sediments (Carlsson et al. 2010). Further temporal resolution of biodeposit-associated carbon degradation and subduction in marine sediments is necessary and will have significant capacity to alter conclusions regarding the ability of restoration projects to sequester carbon.

Rapid organic matter assay trials suggest that the rate of organic carbon degradation varies with location, sediment depth, and restoration status. We note here that others have importantly shown biotic factors (specifically macrofaunal and microbial activity not linked to scale of individual ROMA plates in this study) are the most important drivers of observed carbon degradation rates in coastal marine sediments (O'Meara et al. 2018), and that significant carbon enrichment and alterations to macrofaunal biomass and community composition can occur at the bivalve-patch scale (Norkko et al. 2001). Global studies illustrate that the average maximum depth at which surface sediments are turned over through bioturbation activities is roughly 5-10 cm (Boudreau 1998, Teal et al. 2008), and we generally observed a trend in decreased carbon degradation activity below these depths. Although the observed variability in ROMA plate trials precludes our ability to confidently determine a ‘carbon sequestration depth’ here, it appears such a value would likely be realised at shallower sediment depths (perhaps near 15 cm) within restored mussel beds than nearby bare sediments (Fig. 4.4). The bioturbating activities of infauna are typically considered a source of additional oxygen to deeper sediment layers, which generally increases organic matter mineralisation in marine sediments (Burdige 2007).

This, in addition to the direct consumption of organic material by infauna, can lead to an overall decrease in sediment organic matter preservation. However, some infaunal species have been shown to play a significant role in the draw-down of refractory carbon at depth (Levin et al. 1997). We show elsewhere that infaunal community composition changes with mussel restoration site (Sea et al. 2022), and, coupled with the results of carbon cores, suggest that benthic infauna play a more complex role in the preservation of organic matter. Current estimates of carbon degradation and rates of burial within marine sediments vary significantly with space and time (Arndt et al. 2013 and references within), warranting more extensive analysis to resolve the heterogeneity observed within mussel beds. Determining both the decay rates of sediment carbon pools and the depth at which organic carbon is considered ‘sequestered’ are identified as major uncertainties in current carbon accounting efforts (Macreadie et al. 2017) which must be resolved to accurately inform future coastal carbon budgets.

Results from carbon cores (Fig. 4.5) show no significant difference in total carbon content at the approaching ‘sequestration depth’ of 15 cm in mussel bed sediments (established from Fig. 4.4); however, we estimate that sediments collected below this depth were at least 30 years old at the time of study (local sedimentation rate of ~ 4 mm yr^{-1}; Oldman et al. 2009) and were therefore incapable of being affected by recent restoration efforts. It is reasonable that significant differences in carbon content were only observed at depths in which restoration was able to influence the sediment environment (1to 3 cm in depth, or sediments estimated to be from the past 2-7 years at current sedimentation rates). While it is possible that large, bioturbating macrofauna introduce additional non-refractile carbon to sediment depths greater than what would be predicted through passive sedimentation alone (e.g. Levin et al. 1997, Schenone et al. 2019) such organisms were not observed in partitioned sediment samples. As we measured total carbon content here, forthcoming analyses separating the proportion of inorganic carbon (a fraction which can be differentiated into geogenic and biogenic sources) from organic carbon (which can be further differentiated into autochthonous and allochthonous sources of significance to New Zealand estuaries; Bulmer et al. 2020) will make worthwhile contributions to our understanding of sediment dynamics in restored mussel bed systems.

Our results document how green-lipped mussel restoration projects affect carbon cycling but also importantly infer that the loss of extensive regional beds (~1500 km^2) has resulted in major changes in carbon transport and transformation in the Hauraki Gulf. Disturbance of carbon stocks (dredging, deforestation, etc.) can transform systems of net carbon storage into net sources of atmospheric carbon (Donato et al. 2011, Mcleod et al. 2011, Luisetti et al. 2019). And while not directly examined here, mussel beds are known to significantly increase sediment stabilisation and cohesion (Widdows & Brinsley 2002), important in the modern ocean where anthropogenic disturbance of sediments and resulting resuspension reduces organic carbon content by as much as 50% (Pusceddu et al. 2014) and decreases overall carbon turnover and preservation in marine sediments (Keil 2017). Therefore, conservation and restoration solutions which avoid further degradation, protect natural carbon sinks, and actively create further potential for storage should be given high management priority. Quantification of organic carbon stocks will likely provide justification for the expansion of regional mussel restoration projects and make a case for their protection, as global reef dredging was estimated to have reintroduced over 400,000,000 Mg of carbon into coastal waters (Fodrie et al. 2017) and New Zealand's former shellfisheries (e.g. Paul 2012) partially responsible for emissions.

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Chapter 5

General Discussion & Conclusions

5.1 | Synthesis

New Zealand's subtidal mussel restoration projects began over seven years ago, yet little work has focused on evaluating the many ecosystem services generated from these efforts. In order to assess the value of key coastal ecosystems, Barbier et al. (2011) suggested a multi-step approach which first involves the identification of key services provided by the system of interest (which I've outlined in section 1.3 for green-lipped mussels). The second step involves gaining an understanding of the "ecological production functions" which support ecosystem service generation, a step which is admittedly more complex owing to biotic and abiotic interactions and feedbacks unique to each system and location. The body of this thesis serves to address the second step in the valuation process by advancing our understanding of such functions underpinning three distinctly different yet interconnected services identified in restored mussel habitats.

Using a combination of in-situ and laboratory techniques, I showed that restored mussel beds are multifunctional and influence the delivery of ecosystem services at a variety of spatial scales considered here. In addition each data chapter provides unique insights on the role of mussel beds in influencing services realised at varying soft-sediments locations. Chapter 2 illustrates that relationships between biodiversity and ecosystem services are complex and context dependent. By separating communities by scales of mobility I was able to demonstrate how different mechanisms drive community changes at different organisational levels. The magnitude and effect of restoration on resulting communities were also influenced by bed location and environmental conditions (especially apparent in macrofaunal communities), and this context dependency has significant implications for the delivery of other ecosystem services measured.

As it is known that biodiversity losses negatively affect ecosystem service provision (Worm et al. 2006), it then follows that increases in diversity associated with restoration efforts would exhibit a generally positive relationship with the recovery of multiple services. Chapter 3 importantly demonstrates that the delivery of ecosystem services is not driven by singular mussels acting independently; rather, restored mussel beds behave as a cohesive unit, affecting ecosystem functionality at the larger bed scale. Therefore increasing our understanding of the biology or physiology of mussels alone, for example, would not effectively translate into better predictions of ecosystem service delivery at ecologically relevant spatial scales. This insight on the scale of responses and emergent properties is mirrored in the work of others (Coco et al. 2006) who established that patchy bivalve beds interact with their environment in ways that are fundamentally different than what could be experienced by individuals. Emergent properties we observed are a result of complex, nonlinear feedbacks between mussel beds and their environment, and the strength of these feedbacks and resulting biological responses (which then influence ecosystem service delivery) varies with environmental setting. The implied bed-scale phenomena observed in my denitrification chapter suggests that, in this case, it is reasonable to upscale service valuations from multiple benthic chambers to entire beds as done so by Hillman et al. (2021).

The oceanic carbon cycle is complex and not well understood, yet it is largely mediating the global response to climate change (Heinze et al. 2015, Keil 2017). Discontinuities became generally apparent regarding the role of coastal systems as sources or sinks of carbon (e.g. Chen & Borges 2009), and available studies disproportionately examined vegetated habitats as natural climate change solutions (e.g. Duarte et al. 2005, Duarte et al. 2013, Macreadie et al. 2021). To the best of my knowledge, a comprehensive assessment of the ecological production function of restored mussel beds to coastal carbon cycling had not been conducted to date, precluding valuations of this ecosystem service. Chapter 4 therefore serves as a first attempt to collate all knowledge relevant to carbon cycling in these systems. Results suggest that mussels recycle notable quantities of carbon and that the carbon storage capacity of restored, mussel bed sediments may increase over time. Our increasing desire to engineer climate solutions can result in hasty or ineffective actions which precede scientific evidence; this body of work is an important first step in setting realistic expectations regarding the contribution of mussel restoration to climate change mitigation efforts relative to other strategies (e.g. Griscom et al. 2017).

Having advanced our understanding of the underlying ecology of restored mussel bed systems, we can now apply generated knowledge of ecosystem service delivery to practical restoration activities. A clear theme emerges across all data chapters, involving how the magnitude and extent of service delivery is dependent on restoration location. This leads to questions regarding where restored mussel beds should be placed in the future to maximise these services. In terms of biodiversity enhancement, large changes were observed in community structure at Motuora, and abundance of mobile species increased ~21 fold (note I chose to focus on mobile communities here as substantial but generally similar epifaunal changes were seen across all restoration sites regardless of location). However, if restoration practitioners are interested in enhancing habitat structure for specific species with cultural or commercial value, inner harbour sites might be better suited (as is the case with juvenile snapper in this example). Denitrification results from Hillman et al. (2021) comparing rates inside and outside of restored beds showed the biggest increases in nitrogen removal at a mid-harbour site (Mahurangi Mid), although similarly large increases in nitrogen removal were realised in a restored bed further out in

Kawau Bay (Martins Bay South). Multiple factors affect site selection (including logistics and the viability of beds), so it is perhaps important to specify here that improving upon a site’s baseline conditions (increasing functionality relative to nearby, nonrestored sediments) is not equivalent to placing beds in areas where the maximum service value can be achieved. If this were the case we might choose to establish all mussel beds in areas that already exhibit high denitrification rates pre-restoration, but overall this would not prove as beneficial to ecosystem revitalisation, especially if restoring more heavily degraded areas results in a larger net change in service delivery. This appeared to be the case in my study, where the highest overall DNF rates were achieved at Motoketekete, but the greatest net increase in nitrogen removal potential was observed at an inner harbour site (Lagoon Bay). Finally, while a greater number of carbon-containing biodeposits can be produced at mussel beds located in turbid, inner harbour sites, sediment cores taken from the Motuora bed had the highest overall carbon content (significantly increased compared to nearby control sediments).

When considering the combined results of all biodiversity, denitrification, and carbon related studies, the most substantial changes in functionality (associated with the greatest increases in service value) generally occur at Kawau Bay restoration sites (Fig. 5.1). This conclusion however slightly undervalues nitrogen removal services realised at inner harbour locations, a trade-off which is arguably acceptable given the general oligotrophic status of New Zealand estuaries at present (Vieillard et al. 2020). Kawau Bay sites are characterised by lower mud content and organic matter, and higher sediment porosity and ambient oxygen concentrations. The addition of mussels to such locations would feasibly result in greater environmental changes than would be seen in Mahurangi Harbour (e.g. introducing fine particles of high organic content through biodeposition would not substantially alter sediment conditions already experienced at inner harbour sites), resulting in greater changes to ecosystem functionality and service delivery in Kawau Bay. Additionally the proximity of Kawau Bay sites to anthropogenic stressors such as sedimentation and runoff is likely much less than that of inner harbour sites (although the direct effects of such stressors on mussel condition and ecosystem service delivery were not studied here). If we anticipate further degradation of near-shore systems, creating restored beds further off shore could prove advantageous to mussel functioning and survival (by limiting cumulative effects of multiple stressors). Mussel deployments to suggested locations will undoubtedly be influenced by logistics; is it likely more expensive to create beds further off shore, and they could be prone to other stressors not considered in this thesis. Additionally, the study of other ecosystem services will likely alter the suggested best locations for restoration (for example, future studies of sediment stabilisation and coastal protection could prove inner harbour restoration sites more effective in their potential service delivery). While sites similar in condition to Kawau Bay might maximise the specific services studied here, this thesis more broadly demonstrates that, overall, mussel restoration can and should be considered a tool to recover otherwise-diminished services in coastal ecosystems.

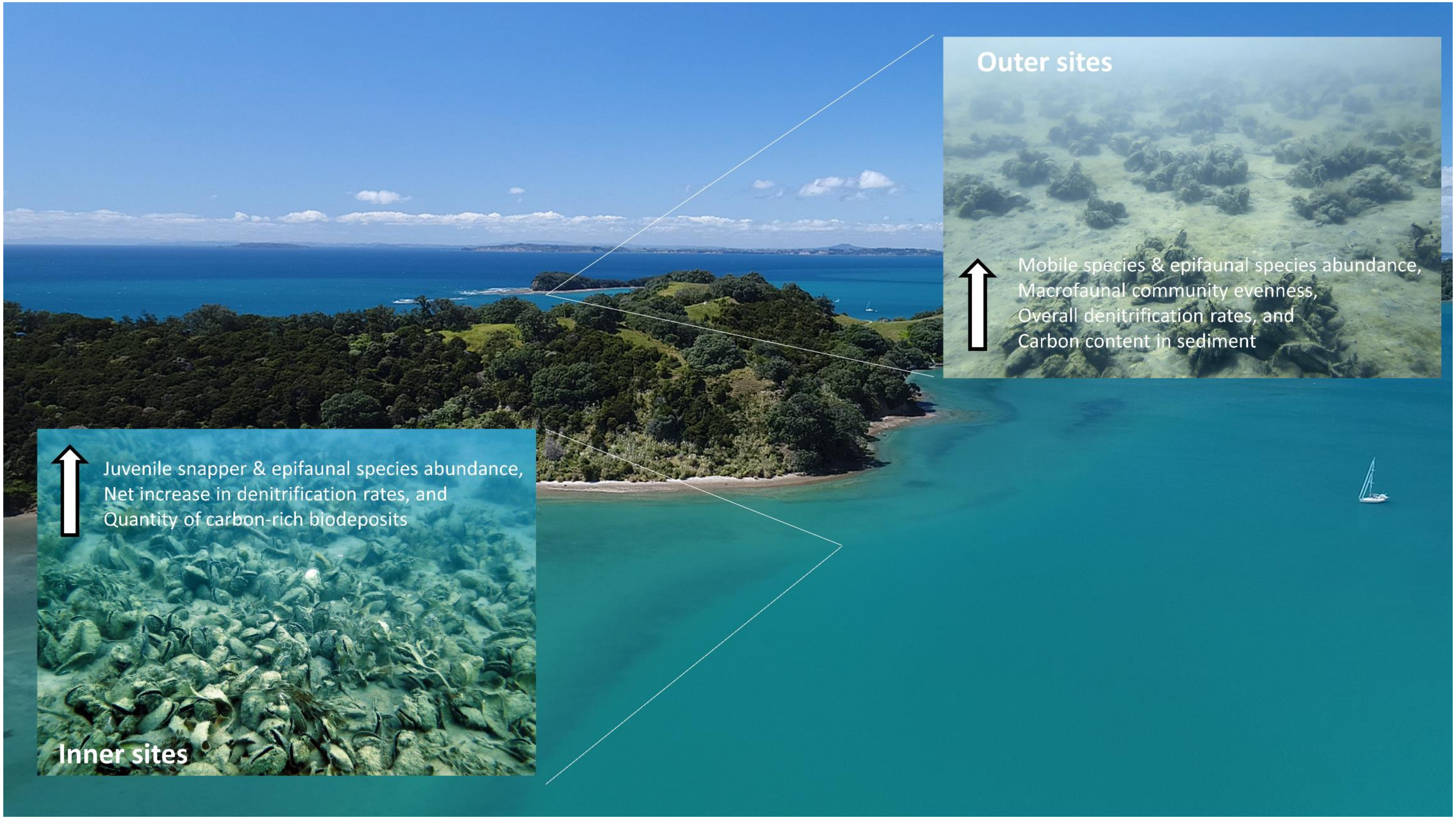


Figure 5.1 A comparison of ecosystem services realised in Mahurangi Harbour (inner sites) and Kawau Bay (outer sites), highlighting that restoration location influences service delivery.

5.2 | Limitations and Future Directions

What I’ve done here—rather than comparing restoration sites to natural beds—is to show that improvement to ecosystem functionality occurs (and to what extent) through restoration efforts in various locations. We do not understand how changes to ecosystem functionality track to natural subtidal beds, an idea which has been identified as important for reefs in Australia (Gillies et al. 2017). This limitation is uniquely the result of a lack of natural, subtidal green-lipped mussel beds in the region. If natural beds are found and studied (under reasonably similar conditions), we could begin to evaluate how ‘successful’ our restoration projects are in recovering ecosystem services in reference to natural baselines (note current baselines are not meant to replace historical baselines but are the best we could hope to achieve in this instance). This could address further spatial and temporal knowledge gaps related to mussel restoration (for example, how other environmental conditions might influence sediment denitrification, or establishing carbon content of mature mussel reefs at depths which date back decades or more). By using information acquired from natural mussel beds, we could begin to develop specific and measurable goals that compare ecosystem service delivery from restoration projects to natural reference systems.

While I chose to examine ecosystem services relevant to three environmental crises of today, it should be noted that ‘service value’ is a human construct which can change with context and time. The suggested best places for restoration will ultimately depend on what is determined to be most valuable to mankind under given social and environmental circumstances. For example, it is likely that carbon cycling/sequestration potential would have been deemed a less-important service when natural green-lipped mussel beds collapsed roughly 60 years ago, and within the New Zealand context I explicitly chose to value other ecosystem services over nitrogen removal in my above synthesis. Shifting environmental valuation discourse towards the ‘Nature’s Contributions to People’ framework (NCP) could be beneficial, as it reflects the idea that the perceived value of a contribution (be it beneficial or detrimental) will vary with spatial and temporal context (Díaz et al. 2018). With the ability to analyse ecosystems and functions from either generalisable or contextual perspectives, the NCP framework is generally seen as more inclusive of a wider range of knowledge systems (Díaz et al. 2018) and could prove useful for New Zealand’s mussel restoration efforts which have seen high levels of indigenous and local stakeholder involvement. Such conceptual changes should be encouraged if they result in higher inclusivity and trust, which typically enhances scientific and political legitimacy and the likelihood of achieving sustainable ocean outcomes (e.g. Turner et al. 2016).

Many ecological functions which underpin service delivery are carried out simultaneously and by a variety of species (Slade et al. 2017, Siwicka et al. 2020); this ecosystem multifunctionality is relevant to coastal restoration, as the species we utilise or enhance through our efforts will ultimately influence the services we recover. A major point of consideration then is that additional mussel deployments alone will not reduce all stressors currently experienced in coastal ecosystems, and importantly, these projects should not be substituted for land-based carbon mitigation and nitrogen reduction plans. Rather than focusing solely on mussels, perhaps in the future we should consider deploying multiple strategies which best reflect desired service combinations and site suitability for restoration. Projects which enhance positive biological interactions between species can potentially result in a wider range of recovered ecosystem services; bivalve-seagrass interactions, for example, are generally quite positive owing to alterations in wave energy, nutrient availability, and water clarity (Tan et al. 2020 and references within), but more research is needed to confidently determine how restoration might influence ecosystem functionality and service delivery in restored systems. And while I showed in chapter 4 that green-lipped mussels can act as a carbon source on their own, other carbon-limited species have been shown to effectively absorb CO_2 produced from bivalves, resulting in overall negative air-sea fluxes when considered in tandem (Han et al. 2017). These species interactions affect ecosystem-level processes and future studies should necessarily address the relevance of restoration to service recovery at appropriate spatial and temporal scales. The successful revitalisation of coastal ecosystems will depend on a variety of factors but should ultimately move away from single-fix action plans and instead utilise ecosystem-based management approaches which are interdisciplinary, holistic, and necessarily adaptive to dynamic changes in coastal environments. By determining what services are valued and what trade-offs we are willing to accept, we can begin to create strategies that will better reflect the environment we wish to create for future generations.

I believe there are still pressing and unresolved questions regarding the role of mussel restoration in affecting carbon degradation and sequestration in coastal soft sediments. We generally documented larger decreases in carbon degradation rates (which should allow for further carbon storage) at inner harbour sites; however this was not consistent across sediment depths, and Kawau Bay sites supported higher carbon storage overall. Similar questions regarding the decay rates as well as the depth at which accumulated carbon can be considered ‘sequestered’ have also been identified for blue carbon systems (Macreadie et al. 2017) and should be considered high priority for future research efforts. A better understanding of the relationship between carbon degradation and storage in these sediments could have significant implications for how we frame our justifications for restoration efforts.

As mentioned previously, future studies resolving additional ecosystem services will influence the suggested ‘best locations’ for future mussel deployments. One area of staggeringly underdeveloped research involves cultural service value derived from mussel restoration. Cultural services are often underrepresented in the ecosystem service literature due to complications involved with valuing largely intangible assets (Chan et al. 2012, Hernández-Morcillo et al. 2013), and no published examples of such services were identified in a recent review of bivalve restoration benefits (zu Ermgassen et al. 2020). I believe there is much room for improvement regarding the integration of diverse social perspectives both into the framework and in general regards to shellfish restoration and management in New Zealand. While mussel restoration efforts are progressing in a rather unified manner between all stakeholder groups at present, I don’t believe that the end goals (or the answer to the question ‘why restore?’) are the same for all groups involved. For example, some iwi have expressed their desire to eventually harvest mussels while others seek their protection to enhance non-extractive values. Studies in other parts of the world have shown that bivalve removal will diminish service value and greatly lengthen the amount of time it takes to receive a return on restoration investment (if at all; Grabowski et al. 2012). However, cultural considerations and community buy-in are arguably just as relevant to the long-term sustainability and success of restoration projects (e.g. Fox & Cundill 2018). As cultural services regarding traditional mahinga kai have not been explicitly valued and the relevant ecological trade-offs with such practices currently undefined, future social-ecological studies resolving these considerations are relevant and essential to the success of mussel restoration efforts in New Zealand.

Perhaps a final step in the valuation process of mussel restoration would involve assigning economic values to identified ecosystem services. Undertaking such a step would first necessitate careful consideration of theoretical and operational concerns involved with putting a dollar value on the natural realm (e.g. valuation of non-material services, the creation of winners and losers, human-centric ideologies; Robertson 2004, Redford & Adams 2009, Jax et al. 2018). Even if we chose to move forward with economic valuations, this process (and subsequent applications such as international credit based initiatives or markets for services) has proved challenging, specifically for regulating services that are considered public goods (e.g. Alexander et al. 2016). In the case of carbon-related services, for example, oceanic carbon emission reductions are not recognised by the UN Framework Convention on Climate Change and can therefore not be traded in global carbon markets, nor are they typically considered in voluntary markets (Zarate-Barrera & Maldonado 2015). Both the advantages and disadvantages of potential economic applications need to be carefully considered, as these markets for services can be volatile and can even promote further environmental degradation (Palmer & Filoso 2009). Such economic valuations and market assessments of related ecosystem services are not undertaken here, but, as others move forward with monetising ecosystem services (Cordier et al. 2014, Temel et al. 2018), we should assess both the appropriateness and implications of such valuations in delivering desired conservation outcomes in New Zealand, as well as considering our timeline and possible alternatives (e.g. Baveye et al. 2013) to achieve similar goals.

5.3 | Concluding Remarks

A unified message of anthropogenically-induced degradation of natural habitats has been received across nearly all land and seascapes. Coastal ecosystems are considered among the most threatened by the actions of mankind, and this deterioration is both severe and increasing (Lotze et al. 2006, Worm et al. 2006, Halpern et al. 2008, Barbier et al. 2011). However, the general persistence of alarmist content (academic or otherwise) outlining the dire consequences of human actions and 'impending environmental doom' greatly increases levels of eco-anxiety among youth and vulnerable communities. Given the current social climate, it is perhaps more necessary now than ever that our science instead gives hope of a better future for those willing to make positive changes. Coastal restoration projects such as those examined in this thesis importantly demonstrate that intentional, impactful interventions can reverse negative environmental trends in ways which support both mankind and the ecosystems we rely upon. Restoration efforts return a sense of empowerment to involved communities while transforming a dismal ocean narrative to a story of generating solutions, measuring successes, and defining further opportunities to support progress and positive change into the future.

Large-scale restoration efforts necessitate cooperation and support by multiple players (e.g. local community groups, regional stakeholders, and national governments), and New Zealand is building capacity to implement meaningful changes to its coastal zone. I take the stance here that New Zealand stakeholders should maintain momentum in the green-lipped mussel restoration space while investigating additional restoration works to provide a wide range of services to mankind and ultimately revitalise the historic ecosystem functionality of the Hauraki Gulf.

Appendix I

Supplemental Materials for Chapter 2

Table AI.1 Summary of SIMPER results for mussel reefs and control sediments for all three methodologies, highlighting average abundance of discriminating species from each group, their percent contribution to the dissimilarity between groups, and the total cumulative percent contributions (cut off at 70%).

Mobile Species

Un-baited Remote Underwater Videos

Average Dissimilarity = 62.94 %

	Abundance		Contribution %	Cumulative %
Species	**Mussel Bed**	**Control**		
Triplefins (Tripterygiidae)	9.89	2.48	28.51	28.51
Snapper (*Chrysophrys auratus*)	5.29	4.67	13.84	42.35
Parore (*Girella tricuspidata*)	2.70	1.82	10.87	53.22
Mackerel (*Trachurus* spp.)	2.52	1.53	8.31	61.53
Mullet (Mugilidae)	1.82	0.00	6.26	67.79
Trevally (*Pseudocaranx dentex*)	1.25	0.47	6.21	74.00

Epifauna/ Benthic Invertebrates

Video Transects

Average Dissimilarity = 88.76 %

	Abundance		Contribution %	Cumulative %
Species	**Mussel Bed**	**Control**		

Barnacles	13.37	0.00	39.53	39.53
Seaweeds	2.34	0.38	8.01	47.53
Ascidians	2.13	0.35	7.70	55.23
Egg mass (likely gastropod)	3.13	0.00	7.60	62.83
Gastropods	2.40	0.40	5.72	68.54
Sea Cucumbers	1.62	0.22	5.62	74.17
Macrofauna Sediment Cores *Average Dissimilarity = 67.68 %*				
	Abundance		Contribution %	Cumulative %
Species	**Mussel Bed**	**Control**		
Theora lubrica	2.88	1.61	7.69	7.69
Ostracod	1.87	2.44	6.06	13.75
Pseudopolydora	1.13	1.48	5.50	19.24
Prionospio aucklandica	1.21	0.46	4.03	23.27
Phoxocephalidae	1.35	1.46	4.00	27.27
Arthritica bifurca	0.45	1.01	3.54	30.81
Schistomeringos	0.56	0.79	3.17	33.98
Armandia maculata	0.97	0.50	3.14	37.12
Boccardia	0.29	0.76	2.91	40.03
Linucula hartvigiana	0.37	0.80	2.82	42.85
Cossura consimilis	0.72	0.71	2.61	45.45
Prionospio spp. (other)	0.62	0.40	2.55	48.00
Oligochaeta	0.53	0.57	2.45	50.45
Lysianassidae	0.31	0.66	2.29	52.74

Nereidae	0.45	0.52	2.26	55.00
Labiosthenolepis	0.33	0.54	2.24	57.24
Capitella spp.	0.48	0.45	2.18	59.42
Colurostylis lemurum	0.40	0.40	2.04	61.46
Exogoninae	0.35	0.60	1.96	63.42
Hemiplax hirtipes	0.16	0.42	1.83	65.25
Lumbrineridae	0.24	0.35	1.79	67.04
Gastropod spp. (other)	0.25	0.34	1.72	68.76
Aricidea	0.38	0.09	1.54	70.29

Table AI.2 Summary of SIMPER results for mussel beds and control sediments for all three methodologies, highlighting average abundance of species that contribute to within-group similarity, their percent contribution to the similarity, and the total cumulative percent contributions (cut off at 70%).

Mobile Species Un-baited Remote Underwater Videos			
	Abundance	Contribution %	Cumulative %
Mussel Beds *Average similarity = 44.29 %*			
Triplefin (Tripterygiidae)	9.89	48.03	48.03
Snapper (*Chrysophrys auratus*)	5.29	24.39	72.42
Soft-sediment Controls *Average similarity = 41.12 %*			
Snapper (*Chrysophrys auratus*)	4.67	49.08	49.08
Triplefin (Tripterygiidae)	2.48	31.36	80.43
Epifauna/ Benthic Invertebrates Video Transects			
	Abundance	Contribution %	Cumulative %
Mussel Beds *Average similarity = 47.23 %*			
Barnacle	13.37	53.51	53.51
Seaweed	2.34	12.77	66.28
Sponge	1.70	8.58	74.86
Soft-sediment Controls *Average similarity = 13.28 %*			

Sponge	0.50	33.36	33.36
Fan worm spp. (other)	0.83	32.12	65.48
Sabella spallanzanii	0.50	16.77	82.25
Macrofauna			
Sediment Cores			
	Abundance	Contribution %	Cumulative %
Mussel Beds			
Average similarity = 34.89 %			
Theora lubrica	2.88	33.04	33.04
Phoxocephalidae	1.35	12.71	45.74
Ostracod	1.87	12.34	58.09
Cossura consimilis	0.72	6.19	64.28
Prionospio aucklandica	1.21	5.71	69.99
Prionospio spp. (other)	0.62	3.06	73.05
Soft-sediment Controls			
Average similarity = 35.11 %			
Ostracod	2.44	19.26	19.26
Theora lubrica	1.61	15.37	34.63
Phoxocephalidae	1.46	9.09	43.73
Arthritica bifurca	1.01	8.22	51.95
Cossura consimilis	0.71	6.24	58.18
Linucula hartvigiana	0.80	5.21	63.40
Pseudopolydora	1.48	4.08	67.48
Lysianassidae	0.66	3.60	71.07

Table AI.3 Results of 2-way ANOVA showing the effects of Site and Status separately on measured diversity indices from un-baited remote underwater videos. Significant results (p ≤ 0.05) are indicated in bold. Analyses performed on untransformed data. Site labels: Pukapuka = PP, Lagoon Bay = LB, New Lagoon Bay = NLB, and Motuora = MR.

Factor	df	SS	MS	F value	p-value
Species Richness					
Site	4	5.33	1.33	5.33	0.164
Status	1	15.69	15.69	62.75	**0.016**
Site x status	4	10.15	2.54	10.15	0.092
Residuals	2	0.50	0.25		

Factor	df	SS	MS	F value	p-value
Total Abundance					
Site	4	55776.00	13944.00	3.94	0.213
Status	1	79186.00	79186.00	22.38	**0.042**
Site x status	4	22462.00	5616.00	1.59	0.422
Residuals	2	7077.00	3538.00		

Table AI.4 Results of 2-way ANOVA showing the effects of Site and Status (mussel reef or control) on measured diversity indices from video transects capturing epifauna and benthic invertebrate species. Significant results (p ≤ 0.05) are indicated in bold. Abundance data was log-transformed prior to analysis. Site labels: Pukapuka = PP, Lagoon Bay = LB, New Lagoon Bay = NLB, and Motuora = MR.

Factor	df	SS	MS	F value	p-value
Species Richness					
Site	3	68.19	22.73	21.39	**<0.001**
Status	1	126.56	126.56	119.12	**<0.001**
Site x status	3	8.19	2.73	2.57	0.127
Residuals	8	8.50	1.06		

Factor	df	SS	MS	F value	p-value
Total Abundance					
Site	3	2.15	0.72	5.07	**0.029**
Status	1	4.56	4.56	32.24	**<0.001**
Site x status	3	0.22	0.07	0.51	0.687
Residuals	8	1.13	0.14		

Table AI.5 Results of 2-way ANOVA showing the effects of Site and Status (mussel reef or control) on measured diversity indices and sediment characteristics from macrofaunal cores. Significant results ($p \leq 0.05$) are indicated in bold. Abundance, SOM, Porosity, Mud, and Chlorophyll a data were log-transformed prior to analysis. Site labels: Pukapuka = PP, Lagoon Bay = LB, New Lagoon Bay = NLB, and Motuora = MR. SOM = sediment organic matter.

Factor	df	SS	MS	F value	p-value
Species Richness					
Site	3	2692.00	897.30	53.52	**<0.001**
Status	1	28.90	28.90	1.72	0.195
Site x status	3	83.90	28.00	1.67	0.184
Residuals	56	938.90	16.80		

Factor	df	SS	MS	F value	p-value
Total Abundance					
Site	3	20.90	6.97	29.28	**<0.001**
Status	1	0.17	0.17	0.71	0.403
Site x status	3	0.95	0.32	1.33	0.274
Residuals	56	13.32	0.24		

Factor	df	SS	MS	F value	p-value
SOM					
Site	3	1.13	0.38	26.18	**<0.001**
Status	1	1.23	1.23	85.11	**<0.001**
Site x status	3	0.51	0.17	11.82	**<0.001**
Residuals	56	0.81	0.01		

Factor	df	SS	MS	F value	p-value
Porosity					
Site	3	0.90	0.30	9.91	**<0.001**
Status	1	0.07	0.068	2.25	0.140
Site x status	3	0.36	0.12	3.92	**0.013**
Residuals	56	1.69	0.03		

Factor	df	SS	MS	F value	p-value
% Mud Content					
Site	3	35.07	11.69	148.05	**<0.001**
Status	1	0.26	0.255	3.234	0.078
Site x status	3	0.23	0.077	0.978	0.410
Residuals	56	4.42	0.079		

Factor	df	SS	MS	F value	p-value
% Coarse Sand					
Site	3	129.92	43.31	26.50	**<0.001**
Status	1	3.19	3.19	1.95	0.168
Site x status	3	7.43	2.48	1.52	0.221
Residuals	56	91.51	1.63		

Factor	df	SS	MS	F value	p-value
Chl *a*					
Site	3	1.59	0.53	7.66	**<0.001**
Status	1	3.83	3.83	55.45	**<0.001**
Site x status	3	4.58	1.53	22.11	**<0.001**
Residuals	56	3.86	0.07		

Appendix II

Supplemental Materials for Chapter 3

Text AII.2 Description of mussel aggregation patterns at various sites.

Capable of local movement, the mussels self-arranged to create beds that differ in spatial composition. The bed at Motuora appeared the most complex and varied in terms of arrangements seen, with large, densely packed sections and small to large clumps separated by sporadic areas with no mussels. A greater number of clumps and individual mussels appeared near the edges of the bed. In contrast, Motoketekete was the most patchy bed, with small clumps and individuals spread out over greater distances. This bed was largest in terms of physical distance, but population density was quite low (mussels predominately found in clumps of 5-10 individuals). No dense patches of mussels were observed at this site. Lagoon Bay was also characterized by many clumps, but these mussels congregated in larger assemblages (rather than the smaller clumps observed at Motoketekete) with many sizeable, densely-packed patches (>100 mussels) and some smaller aggregations (roughly 10-30 mussels per clump) as well. No mussels were seen living individually at this site. Like Lagoon Bay, no individuals were found at Pukapuka; uniformity was greatest at this long, narrow bed, with densely-packed mussels throughout. Some larger clumps (of around 50-75 congregated individuals) were also found at this site, but overall very few gaps were observed at the time of sampling.

Table AII.3 Summary of chamber contents and incubation times for all benthic chambers used in this experiment.

Site	Number of Mussels	Incubation Start Time	Incubation End Time	Total Incubation Time (min)
Pukapuka				
	0	10:40	13:45	185
	0	10:45	13:55	190
	0	10:52	14:07	195
	40	10:41	13:47	186
	40	10:46	13:57	191
	50	10:48	14:01	193
Lagoon Bay				
	0	11:32	14:32	180
	0	11:30	14:42	192
	0	11:34	14:46	192
	11	11:30	14:30	180
	11	11:43	14:40	177
	16	11:37	14:36	179
Motuora				
	0	10:53	13:53	180
	0	10:55	13:55	180
	3	11:01	14:01	180
	4	11:07	13:59	172
	6	10:57	13:57	180
	10	11:01	13:53	172
	12	11:03	14:03	180
	20	11:05	13:57	172
Motoketekete				
	0	10:49	14:13	204
	0	10:54	14:18	204
	0	10:55	14:19	204
	0	10:59	14:21	202
	4	10:53	14:17	204
	4	11:01	14:22	201
	5	10:48	14:12	204
	7	10:50	14:14	204
	9	10:52	14:16	204
	11	10:57	14:20	203
	13	10:51	14:15	204
	14	11:03	14:22	199

Table AII.2 Environmental characteristics measured at each site. PAR = photosynthetically active radiation. Where relevant, data represent the mean ± SE.

Site	Salinity (ppt)	Temperature (ºC)	Depth (m)	PAR (counts)	Ambient NO_x (µmol L^{-1})	Ambient NH_4^+ (µmol L^{-1})	Ambient O_2 (µmol L^{-1})
Lagoon Bay	32.6	21	5.0	3135 ± 310	1.18 ± 0.12	10.33 ± 0.84	358.58 ± 4.34
Pukapuka	33.3	21	5.5	2169 ± 118	1.26 ± 0.59	7.46 ± 1.50	370.85 ± 7.13
Motuora	31.5	21	8.0	865 ± 52	1.12 ± 0.15	11.14 ± 1.10	393.84 ± 7.61
Motoketekete	32.8	21	8.5	2665 ± 165	1.56 ± 0.17	6.54 ± 0.82	399.07 ± 2.78

Table AII.3 Results of 2-way ANOVA showing the effects of site and chamber contents (presence or absence of mussels) on measured sediment characteristics and nutrient fluxes. Significant results ($p \leq 0.05$) are indicated in bold. N2 and NH4+ flux data were log-transformed prior to analysis. Site labels: Lagoon Bay = LB, Pukapuka = PP, Motuora = MR, and Motoketekete = MK. SOM = sediment organic matter.

Factor	df	SS	MS	F value	p-value
N$_2$ flux					
Site	3	0.030	0.010	10.232	**<0.001**
Chamber contents	1	0.000	0.000	0.022	0.883
Site x chamber contents	3	0.000	0.000	0.167	0.918
Residuals	24	0.024	0.001		
Pairwise comparisons for sites (Tukey)		Diff in Means	Lower confidence interval	Upper confidence interval	Adjusted p-value
LB-MR		-0.078	-0.125	-0.032	**<0.001**
PP-MR		-0.000	-0.047	0.047	0.999
MK-MR		0.001	-0.039	0.040	0.999
PP-LB		0.078	0.028	0.128	**0.001**
MK-LB		0.079	0.036	0.122	**<0.001**
MK-PP		0.001	-0.043	0.044	0.999
Factor	df	SS	MS	F value	p-value
% Mud					
Site	3	2443.6	814.5	333.426	**<0.001**
Chamber contents	1	5.4	5.4	2.215	0.150
Site x chamber contents	3	20.4	6.8	2.783	0.063
Residuals	24	58.6	2.4		
Pairwise comparisons for sites (Tukey)		Diff in Means	Lower confidence interval	Upper confidence interval	Adjusted p-value
LB-MR		15.045	12.716	17.374	**<0.001**

PP-MR	16.802	14.473	19.130	**<0.001**
MK-MR	-3.186	-5.154	-1.218	**<0.001**
PP-LB	1.757	-0.733	4.246	0.236
MK-LB	-18.231	-20.387	-16.075	**<0.001**
MK-PP	-19.987	-22.143	-17.832	**<0.001**

Factor	df	SS	MS	F value	p-value
SOM					
Site	3	4.921	1.640	8.045	**<0.001**
Chamber contents	1	0.001	0.001	0.004	0.953
Site x chamber contents	3	0.580	0.193	0.949	0.433
Residuals	24	4.893	0.204		

Pairwise comparisons for sites (Tukey)	Diff in Means	Lower confidence interval	Upper confidence interval	Adjusted p-value
LB-MR	-0.985	-1.658	-0.313	**0.003**
PP-MR	0.037	-0.636	0.710	0.999
MK-MR	-0.591	-1.160	-0.023	**0.039**
PP-LB	1.022	0.303	1.742	**0.003**
MK-LB	0.394	-0.229	1.017	0.323
MK-PP	-0.628	-1.251	-0.005	**0.047**

Factor	df	SS	MS	F value	p-value
Chl *a*					
Site	3	45.320	15.105	5.310	**0.006**
Chamber contents	1	1.590	1.591	0.559	0.462
Site x chamber contents	3	17.490	5.828	2.049	0.134
Residuals	24	68.270	2.844		

Pairwise comparisons for sites (Tukey)	Diff in Means	Lower confidence interval	Upper confidence interval	Adjusted p-value
LB-MR	2.573	0.060	5.085	**0.043**
PP-MR	-0.510	-3.023	2.002	0.943

MK-MR		1.867	-0.256	3.991	0.099
PP-LB		-3.083	-5.769	-0.397	**0.020**
MK-LB		-0.705	-3.032	1.621	0.837
MK-PP		2.378	0.051	4.704	**0.044**

Factor	df	SS	MS	F value	p-value
Macrofaunal abundance					
Site	3	27074	9025	8.350	**<0.001**
Chamber contents	1	295	295	0.273	0.606
Site x chamber contents	3	3139	1046	0.968	0.424
Residuals	24	25940	1081		

Pairwise comparisons for sites (Tukey)		Diff in Means	Lower confidence interval	Upper confidence interval	Adjusted p-value
LB-MR		-69.500	-118.479	-20.521	**0.003**
PP-MR		-66.167	-115.146	-17.188	**0.005**
MK-MR		-15.000	-56.395	26.395	0.751
PP-LB		3.333	-49.027	55.694	0.998
MK-LB		54.500	9.154	99.846	**0.014**
MK-PP		51.167	5.821	96.512	**0.023**

Factor	df	SS	MS	F value	p-value
NO$_x^-$ flux					
Site	3	14930	4977	1.283	0.303
Chamber contents	1	556	556	0.143	0.708
Site x chamber contents	3	26414	8805	2.269	0.106
Residuals	24	93118	3880		

Factor	df	SS	MS	F value	p-value
PO$_4^{3-}$ flux					
Site	3	1166	388.6	1.339	0.285
Chamber contents	1	315	314.5	1.084	0.308
Site x chamber contents	3	2278	759.5	2.618	0.074
Residuals	24	6964	290.2		

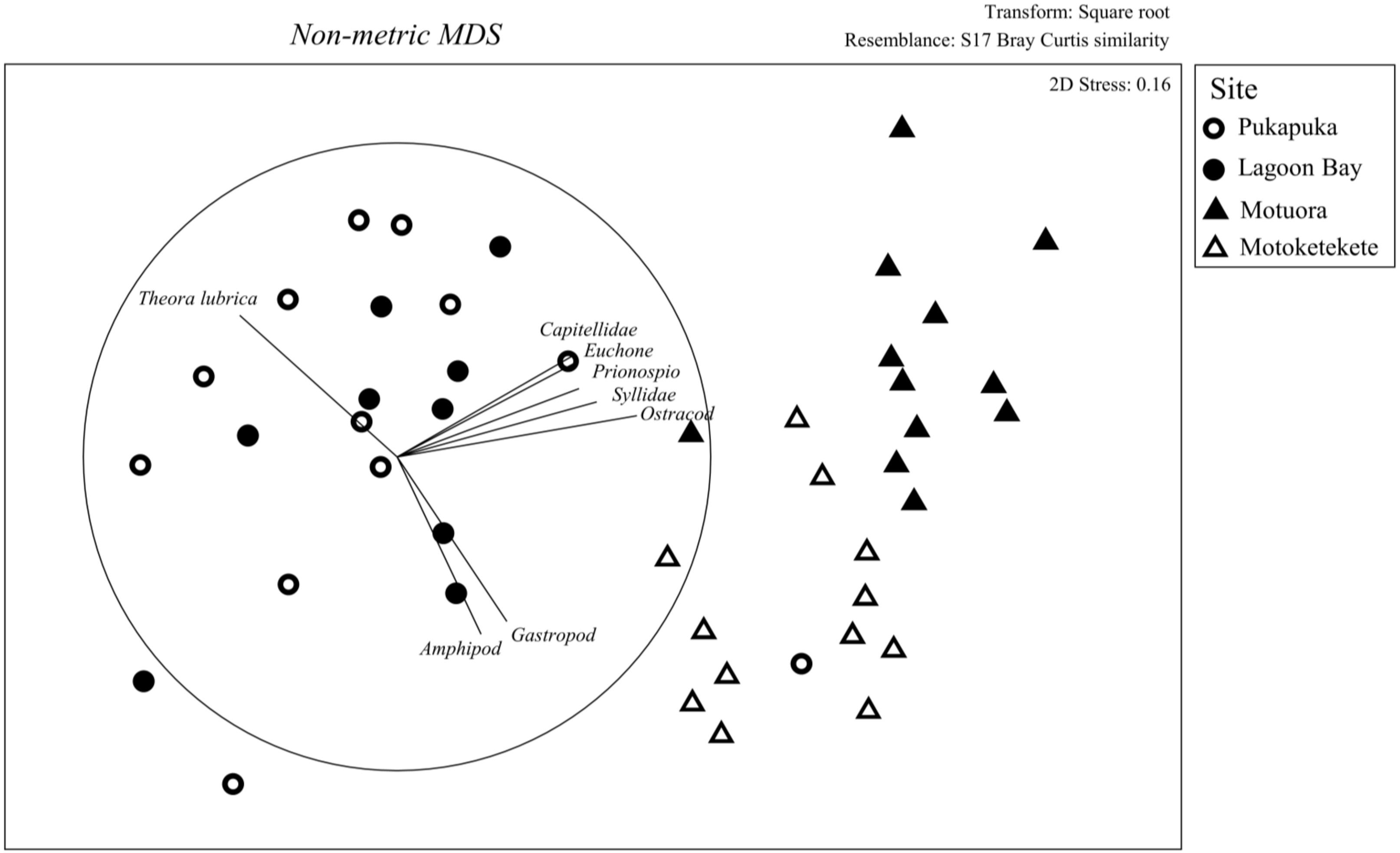
Factor	df	SS	MS	F value	p-value
NH_4^+ flux					
Site	3	0.918	0.306	0.840	0.486
Chamber contents	1	0.056	0.056	0.154	0.670
Site x chamber contents	3	5.267	1.756	4.816	**0.009**†
Residuals	24	8.750	0.365		

Factor	df	SS	MS	F value	p-value
O_2 flux					
Site	3	16241621	5413874	1.883	0.159
Chamber contents	1	69036931	69036931	24.018	**<0.001**
Site x chamber contents	3	300960	100320	0.035	0.991
Residuals	24	68985297	2874387		

Pairwise comparison for chamber contents (Tukey)	Diff in Means	Lower confidence interval	Upper confidence interval	Adjusted p-value
No mussels-Mussels	2965.770	1688.070	4243.480	**<0.001**

† Pairwise comparisons for NH_4^+ fluxes (site x chamber contents interaction) not shown, as Tukey tests were insignificant for all pairs of means.

Figure AII.4 Two-dimensional non-metric multidimensional scaling plot for visualisation of differences in macrofaunal community structure observed between sites. Plot overlaid with species that significantly contribute (Pearson correlation coefficient of > 0.6) to the resulting ordination. Ordination created using PRIMER v. 7 (Clarke & Gorley 2015; available at http://www.primer-e.com/) on square root transformed data.



Appendix III

Supplemental Materials for Chapter 4

Table AIII.1 List of mussel restoration sites and local sediment characteristics (mean and range) as determined by Sea et al. (2021), Hillman et al. (2021), and Sea et al. (2022). Black squares indicate where data from a particular mussel bed were included in the carbon budget. Mud is comprised of silt + clay (< 63 µm). SOM: sediment organic material.

Site	Date established	Deployment (tonnes)	Mud content (%)	SOM (%)	Chl a content (µg g-1)	Shell weight vs. length regression	Benthic chambers	Biodeposit experiment	ROMA plates	Carbon cores
Mahurangi Mid	October 2016	1	26.1 (23.3-30.3)	3.1 (2.7-3.5)	21.1 (20.4-21.8)	■	■	■	■	
Martins Bay N	October 2016	1	14.2 (10.6-19.5)	2.8 (2.3-3.4)	6.7 (6.3-7.2)		■			
Martins Bay S	October 2016	1	6.7 (5.3-9.7)	2.0 (1.7-2.5)	5.4 (4.4-6.4)		■			
Motuora	November 2017	10	7.0 (4.2-10.4)	3.4 (2.6-4.8)	4.9 (2.9-6.2)	■	■	■		■
Motoketekete	October 2016	1	7.5 (6.4-8.7)	2.0 (1.8-2.1)	8.6 (8.2-8.9)		■		■	
Ngaio Bay	October 2016	1	50.2 (39.9-59.1)	4.9 (4.6-5.2)	9.1 (8.8-9.5)	■	■	■	■	
New Lagoon Bay	July 2019	20	24.2 (14.6-44.1)	5.9 (4.1-8.0)	10.9 (5.2-14.0)					■
Lagoon Bay	November 2018	10	22.9 (20.2-25.8)	2.7 (2.0-3.4)	5.6 (1.6-7.9)		■			■
Otarawao Bay	October 2016	1	24.5 (20.2-28.1)	3.2 (2.6-3.9)	13.0 (12.7-13.3)	■	■	■	■	
Pukapuka	November 2018	10	32.2 (28.3-35.8)	3.9 (3.3-4.3)	9.4 (7.6-11.3)	■	■	■	■	■

Table AIII.2 Results of 2-way ANOVA showing the effects of Site and Status (mussel bed vs. bare sediment) on total carbon content at different sediment depths. Analyses performed on transformed data to meet normality assumptions. P-values were adjusted using Holm's sequential Bonferroni procedure. Site labels: Pukapuka = PP, Lagoon Bay = LB, New Lagoon Bay = NLB, and Motuora = MR.
* = p < 0.1, ** = p < 0.5, *** = p <0.01

Factor	df	SS	MS	F value	p-value
Depth: 0-1 cm					
Site	3	3.063	1.021	62.865	< 0.001***
Status	1	0.030	0.030	1.821	0.785
Residuals	20	0.325	0.016		
Pairwise comparison for site (Tukey)		Diff in Means	Lower confidence interval	Upper confidence interval	Adjusted p-value
NLB-MR		-0.668	-0.866	-0.469	< 0.001***
LB-MR		-0.913	-1.111	-0.714	< 0.001***
PP-MR		-0.663	-0.861	-0.464	< 0.001***
LB-NLB		-0.245	-0.451	-0.039	0.016**
PP-NLB		0.005	-0.201	0.211	0.999
PP-LB		0.250	0.044	0.456	0.014**
Factor	df	SS	MS	F value	p-value
Depth: 1-2 cm					
Site	3	2.908	0.970	95.849	< 0.001***
Status	1	0.071	0.071	7.049	0.093*
Residuals	19	0.192	0.010		

Pairwise comparison for site (Tukey)	Diff in Means	Lower confidence interval	Upper confidence interval	Adjusted p-value
NLB-MR	-0.621	-0.784	-0.458	< 0.001***
LB-MR	-0.967	-1.130	-0.804	< 0.001***
PP-MR	-0.606	-0.769	-0.443	< 0.001***
LB-NLB	-0.346	-0.509	-0.183	< 0.001***
PP-NLB	0.015	-0.148	0.178	0.993
PP-LB	0.361	0.198	0.524	< 0.001***

Factor	df	SS	MS	F value	p-value
Depth: 2-3 cm					
Site	3	2.358	0.786	93.745	< 0.001***
Status	1	0.081	0.081	9.612	0.041**
Residuals	19	0.159	0.008		

Pairwise comparison for site (Tukey)	Diff in Means	Lower confidence interval	Upper confidence interval	Adjusted p-value
NLB-MR	-0.587	-0.736	-0.438	< 0.001***
LB-MR	-0.868	-1.017	-0.720	< 0.001***
PP-MR	-0.510	-0.659	-0.362	< 0.001***
LB-NLB	-0.281	-0.430	-0.132	< 0.001***
PP-NLB	0.077	-0.072	0.225	0.483
PP-LB	0.358	0.209	0.507	< 0.001***

Factor	df	SS	MS	F value	p-value
Depth: 3-4 cm					
Site	3	2.715	0.905	57.818	< 0.001***
Status	1	0.014	0.015	0.926	0.999
Residuals	19	0.297	0.016		

Pairwise comparison for site (Tukey)	Diff in Means	Lower confidence interval	Upper confidence interval	Adjusted p-value
NLB-MR	-0.618	-0.821	-0.415	< 0.001***
LB-MR	-0.926	-1.129	-0.723	< 0.001***
PP-MR	-0.617	-0.821	-0.414	< 0.001***
LB-NLB	-0.308	-0.511	-0.104	0.002***
PP-NLB	0.001	-0.202	0.204	0.999
PP-LB	0.308	0.105	0.511	0.002***

Factor	df	SS	MS	F value	p-value
Depth: 4-5 cm					
Site	3	2.737	0.912	44.189	< 0.001***
Status	1	0.014	0.014	0.681	0.999
Residuals	19	0.392	0.021		

Pairwise comparison for site (Tukey)	Diff in Means	Lower confidence interval	Upper confidence interval	Adjusted p-value
NLB-MR	-0.611	-0.844	-0.377	< 0.001***
LB-MR	-0.941	-1.175	-0.708	< 0.001***
PP-MR	-0.515	-0.748	-0.282	< 0.001***
LB-NLB	-0.331	-0.564	-0.097	0.004***
PP-NLB	0.096	-0.138	0.329	0.663
PP-LB	0.426	0.193	0.659	< 0.001***

Factor	df	SS	MS	F value	p-value
Depth: 5-10 cm					
Site	3	2.308	0.769	59.528	< 0.001***
Status	1	0.028	0.028	2.171	0.785

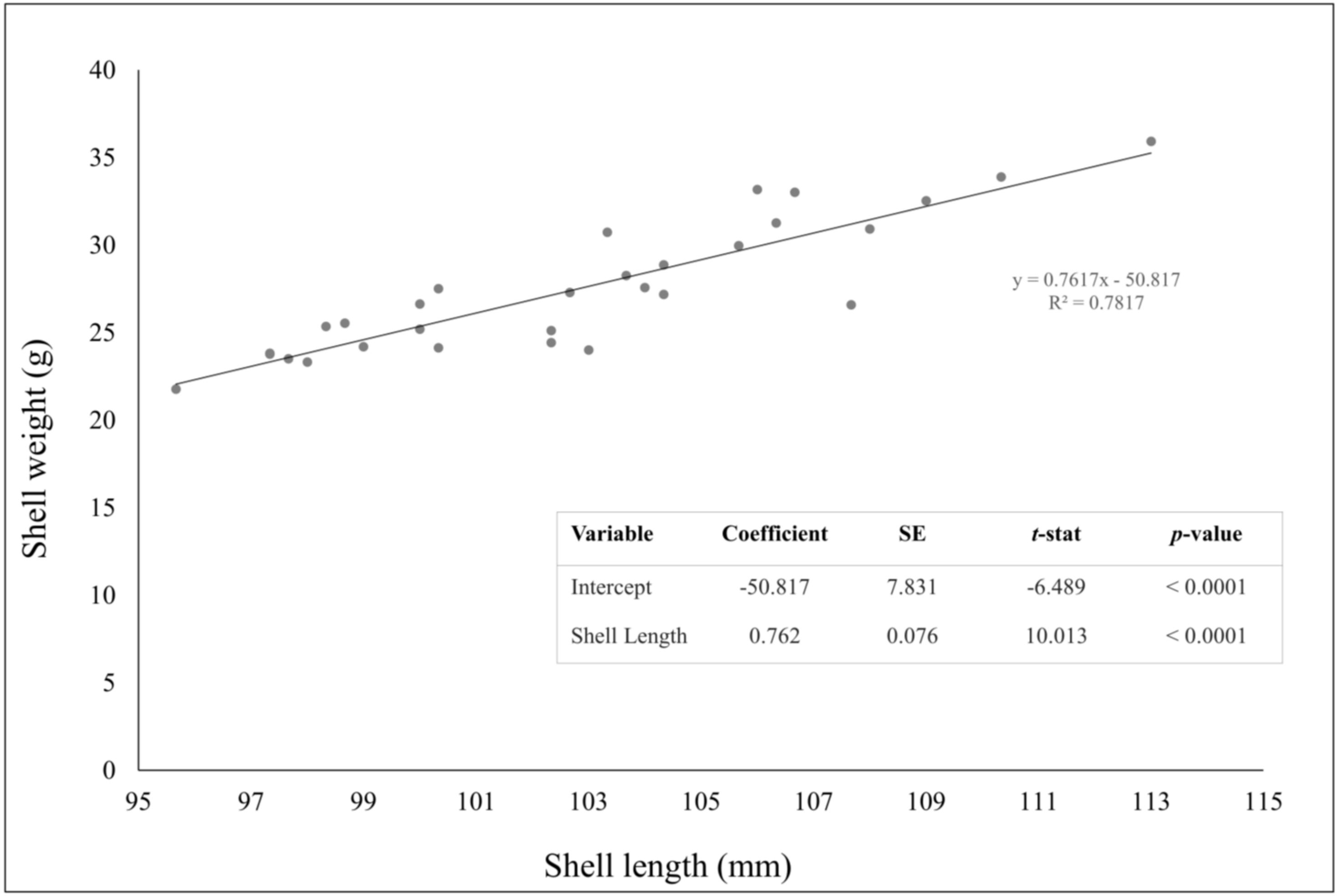
Residuals	19	0.246	0.013		

Pairwise comparison for site (Tukey)	Diff in Means	Lower confidence interval	Upper confidence interval	Adjusted p-value
NLB-MR	-0.625	-0.810	-0.441	< 0.001***
LB-MR	-0.833	-1.018	-0.649	< 0.001***
PP-MR	-0.380	-0.564	-0.195	< 0.001***
LB-NLB	-0.208	-0.392	-0.023	0.024**
PP-NLB	0.245	0.061	0.430	0.007***
PP-LB	0.453	0.269	0.638	< 0.001***

Factor	df	SS	MS	F value	p-value
Depth: 10-20 cm					
Site	3	2.566	0.855	106.265	< 0.001***
Status	1	0.000	0.000	0.004	0.999
Residuals	19	0.153	0.008		

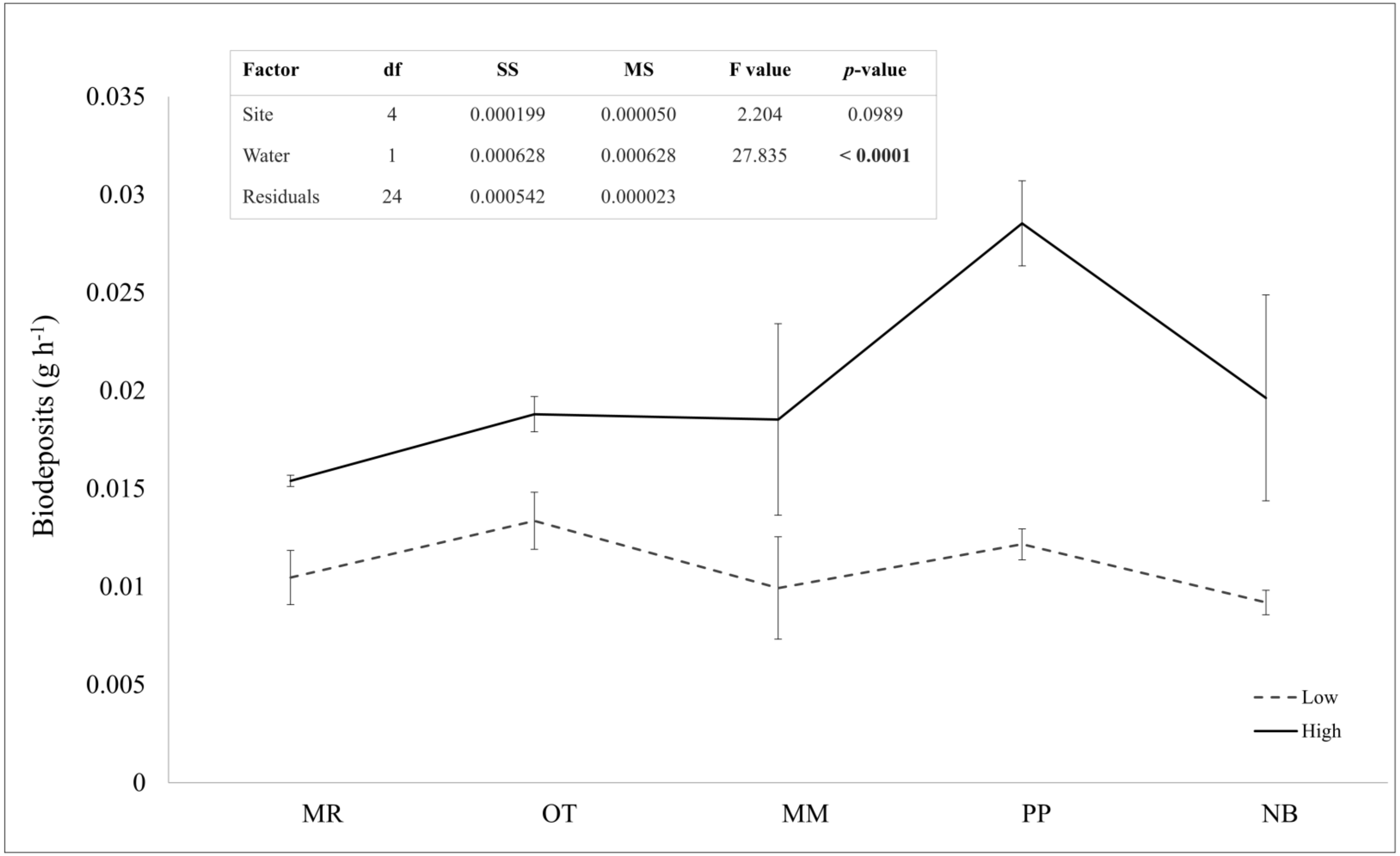
Pairwise comparison for site (Tukey)	Diff in Means	Lower confidence interval	Upper confidence interval	Adjusted p-value
NLB-MR	-0.696	-0.841	-0.550	< 0.001***
LB-MR	-0.848	-0.994	-0.702	< 0.001***
PP-MR	-0.356	-0.502	-0.211	< 0.001***
LB-NLB	-0.152	-0.298	-0.007	0.038**
PP-NLB	0.339	0.193	0.485	< 0.001***
PP-LB	0.492	0.346	0.637	< 0.001***

Figure AIII.1 Linear regression predicting green-lipped mussel shell weight from given shell length. Regression statistics summarised in the inset for n = 30 observations.



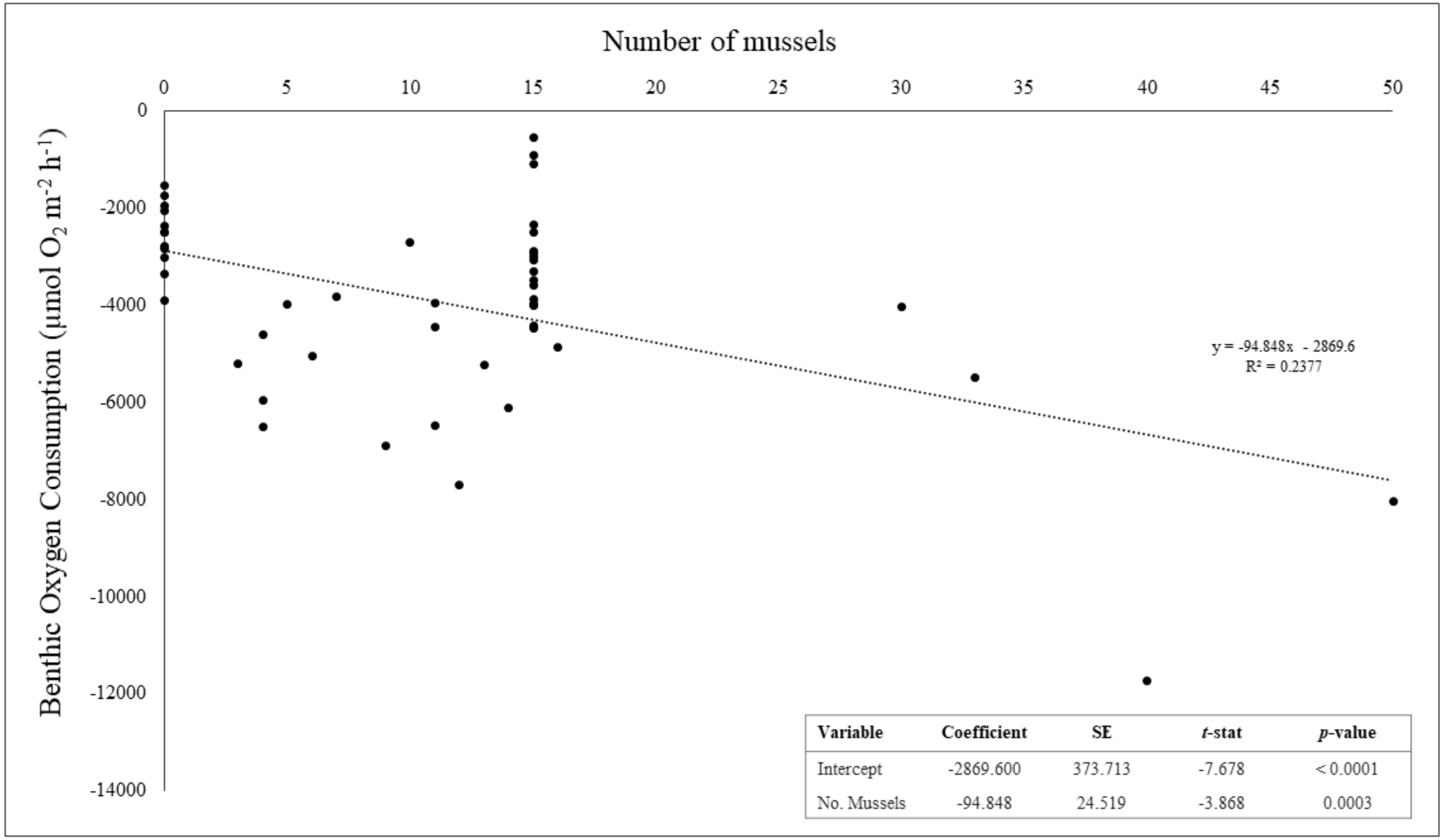
Variable	**Coefficient**	**SE**	***t*-stat**	***p*-value**
Intercept	-50.817	7.831	-6.489	< 0.0001
Shell Length	0.762	0.076	10.013	< 0.0001

Figure AIII.2 Results of 2-way ANOVA showing the effects of water quality (high vs. low suspended solids conc.) and location that mussels were collected from (site) on biodeposition rates. Analyses performed on untransformed data. Data represent the mean ± SE. Sites arranged over an increasing mud gradient, from outer bay to upper harbour. Site labels: Motuora = MR, Otarawao Bay = OT, Mahu Mid = MM, Pukapuka = PP, and Ngaio Bay = NB.



Factor	**df**	**SS**	**MS**	**F value**	***p*-value**
Site	4	0.000199	0.000050	2.204	0.0989
Water	1	0.000628	0.000628	27.835	**< 0.0001**
Residuals	24	0.000542	0.000023		

Figure AIII.3 Linear regression plotting sediment oxygen demand vs. number of mussels in darkened, benthic flux chambers (0.25 x 0.25 m; volume = 41 L). Data obtained from Hillman et al., 2021 and Sea et al., 2021. A singular, adult green-lipped mussel is predicted to increase oxygen demand by 94.8 µmol O_2 h^{-1}. Regression statistics summarised in the inset for n = 50 observations.



Variable	Coefficient	SE	*t*-stat	*p*-value
Intercept	-2869.600	373.713	-7.678	< 0.0001
No. Mussels	-94.848	24.519	-3.868	0.0003

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