# THE IMPACT OF MACROPHYTE MATS ON BIODIVERSITY AND ECOSYSTEM FUNCTIONING OF BENTHIC COMMUNITIES

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***~ To my parents,***

***Isaac and Elmarie,***

***who fostered in me a love and***

***respect for the ocean ~***

# ABSTRACT

Macroalgal blooms, such as *Ulva* spp., are a common disturbance to estuarine benthic fauna worldwide. As large quantities of macroalgae break free from growing substrates, drifting mats are formed that eventually deposit in low energy environments, including intertidal sandflats. The mats will typically settle on the sediment surface as large sheets. Once these sheets start to decompose, detritus is formed, which is eventually incorporated into the benthic food web; however, the ability to process detritus is dependent on the species present. This thesis examined the impacts of *Ulva* on the benthic macrofaunal communities and ecosystem functions, at different phases of decomposition; firstly, as large sheets (Chapter 2), then as detritus (Chapter 3), and finally as the *Ulva* detritus is incorporated and reworked into the sediment and foodweb (Chapters 4 and 5).

The impacts of intact macroalgal mats on the sediment characteristics, community composition and ecosystem functions (i.e. benthic primary production, metabolism, and nutrient cycling) associated with an intertidal macrofaunal community in Tauranga Harbour were measured in a manipulative field experiment. Temporal changes and recovery of the community and the ecosystem functions they provide were measured twice over a 14-day period. Subtle treatment effects were observed in the macrofaunal community and sediment characteristics, which in turn resulted in subtle shifts in chlorophyll *a* (chl *a*) corrected gross primary production. However, there were no significant impacts on the key benthic species (the suspension-feeder *Austrovenus stutchburyi* and the deposit-feeder *Macomona liliana*) at this site, which is likely the reason more significant treatment effects were not observed in the measures of ecosystem function. Significant temporal variation was measured in most sediment properties (all except for phaeophytin), and also in benthic primary production. This study emphasized the importance of temporal variability when measuring ecosystem functions in shallow intertidal environments.

A second manipulative field experiment examined the impact of different quantities of *Ulva* detritus (low [60 g dw $m^{-2}$], medium [120 g dw $m^{-2}$] and high [240 g dw $m^{-2}$]) on the sediment properties, benthic community composition and ecosystem functions of an estuarine benthic community. Plots were sampled on three occasions over an 8-week period, to examine changes in macrofauna and/or ecosystem functions over time. The only significant treatment effect was less gross ammonium flux from the sediment in procedural controls compared to low density treatments. Although no further significant treatment effects were detected, important temporal variation was observed. The macrofaunal community varied significantly between all three the sampling dates, while gross primary production, sediment oxygen consumption, and nutrient efflux from the light and dark chambers varied between at least two of the sampling dates. The results from this study again highlighted the important temporal changes that can be observed over relatively short time scales, and the importance of measuring temporal variation, particularly when measuring ecosystem functions such as primary production and benthic metabolism.

Finally, in a laboratory study, I examined the density dependent effects of two key intertidal bivalve species (*A. stutchburyi* and *M. liliana*) and their associated communities on the breakdown, loss, burial and uptake of *Ulva* detritus. Results showed that the site dominated by *A. stutchburyi* (AS) had higher overall chl *a* biomass and chl *a* was distributed evenly throughout the sediment profile, whilst less labelled *Ulva* was retained in the sediment. Both chl *a* biomass and the amount of *Ulva* recovered were correlated with the density of *M. liliana*, but only in cores collected from AS sites. Cores collected from the *M. liliana* dominated site (ML) showed an exponential decline in chl *a* with depth, and chl *a* biomass was negatively correlated with species richness. In ML cores (which had very low *A. stutchburyi* abundances), *M. liliana* densities showed no correlation with chl *a* biomass or the amount of *Ulva* that was recovered, which suggested that the community present in cores from AS is important in facilitating the impact by *M. liliana* on the mixing and the reworking of organic matter. These results emphasize the value of considering whole communities, and not just key species, when trying to understand sediment mixing and organic matter processing.

Although the addition of *Ulva* did not result in large and obvious shifts in community composition or ecosystem function, small subtle shifts were observed. These results underpinned the complex nature of biotic and abiotic interactions in dynamic systems like estuaries, but show that for the most part, ecosystem functions are robust. Temporal variations in ecosystem functions appeared to be largely driven by environmental conditions such as light availability, and were a more prominent driver in the differences observed in ecosystem function compared to treatment effects of *Ulva* addition. The results also highlighted the importance of community composition in the processing and reworking of macroalgal detritus, which further emphasized the complexity of estuarine systems. The results from this research suggest that on intertidal sandflats, providing the abundances of key species remain intact, the benthic community composition can shift, and species can be lost, without a significant loss or shift in overall ecosystem function.

# PREFACE

This thesis has been written as three standalone papers, which comprise the main research chapters (Chapters 2 – 4), and will be prepared for publication upon the successful submission of the thesis. As a result, some of the methods described in the separate chapters overlap. I was responsible for the fieldwork, the laboratory and data analysis, and the writing. Furthermore, Chapter 5 comprises a published paper, where I was the second author. My contribution to this paper is outlined below. The information contained within this thesis was produced from my own ideas, unless otherwise referenced. The work was carried out under the supervision of Prof. Conrad A. Pilditch and Assoc. Prof. Ian D. Hogg from the University of Waikato, and Prof. Ingrid Kröncke, University of Bremen.

Chapter 2 is currently in preparation for publication, under the title "The effects of *Ulva* mats on inter-tidal benthic biodiversity and ecosystem function" by C. Niemand, R.J. Harris, and C.A. Pilditch.

Chapter 3 is currently in preparation for publication, under the title "Effects of detrital enrichment on intertidal benthic biodiversity and ecosystem function" by C. Niemand, C.A. Pilditch, A.M. Lohrer and I.D. Hogg.

Chapter 4 is currently in preparation for publication, under the title "The density dependent effects of two key bivalve species on the distribution and processing of macroalgal detritus in intertidal communities" by C. Niemand, A.M.L. Karlson, C.A. Pilditch, and C. Savage.

Chapter 5 is a companion paper to Chapter 4, where I contributed to the experimental design, the fieldwork and the laboratory analyses. This paper examined the processing and assimilation of *Ulva* by intertidal communities, and is referred to in Chapter 4. The paper has been published in PLoS ONE (2016) volume 11(7), under the title "Density of key-species determines efficiency of macroalgae detritus uptake by intertidal benthic communities" by A.M.L. Karlson, C. Niemand, C. Savage, and C.A. Pilditch.

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# 1.0 CHAPTER ONE: INTRODUCTION

## 1.1 Estuaries and their ecosystem services and functions

Estuaries constitute vast areas of coastal systems and provide a primary habitat for many species including invertebrates, vertebrates and birds. Estuarine systems support a network of complex and delicate food webs, and have been suggested to be some of the most productive ecosystems in the world (McLusky & Elliott, 2004), providing a basis for many ecosystem services (Costanza et al., 1997). Ecosystem services refer to the service any particular ecosystem function provides to human populations. Ecosystem functions refer to the biological or system properties or processes of ecosystems. In estuaries, these include measures such as fluxes of oxygen and nutrients at the sediment water interface (Lohrer et al., 2004; Hewitt et al., 2006; Thrush et al., 2006; Norling et al., 2007). Ecosystem services can be direct (e.g. pollination or erosion prevention) or indirect (e.g. nutrient cycling or climate regulation). Estuaries provide vital ecosystem services globally, including nutrient cycling and food production (Costanza et al., 1997). As estuaries are vital to ecosystem services, the functioning of estuarine ecosystems have become a focus for marine ecologists in recent times (Stachowicz et al., 2002; Baird et al., 2004; Solan et al., 2004; Srivastava & Vellend, 2005).

Large areas of New Zealand estuaries are comprised of intertidal flats. Intertidal areas are low energy environments, and have highly variable environmental conditions (e.g. salinity, temperature, light, etc.) due to anthropogenic (e.g. runoff) and natural (e.g. rainfall and tidal inundation) factors. These systems often contain a high biodiversity and species that are specifically adapted to these variable conditions. Understanding the dynamics and species interactions within intertidal food webs, as well as the importance of different species and functional groups in an ecosystem function and services framework, are vital to ensure key ecosystem services are adequately maintained.

## 1.2 Biodiversity and macroalgae

Increased natural and anthropogenic stresses on many ecosystems have resulted in changes in community structure (species diversity, biomass and composition), often reducing the overall biodiversity (Warwick & Clarke, 1995). The loss of biodiversity, and the impact such losses have on ecosystem functions and services in both terrestrial and marine habitats, have been a concern for ecologists for decades. However, it was not until the 1990's that researchers started to actively research the topic (Cardinale et al., 2006; Loreau, 2010). As more and more ecosystems and species are significantly impacted and face extinction due to anthropogenic stressors, there is a growing need and urgency to understand these interactions.

It has been widely suggested that reducing species richness or diversity, or excluding species that are important contributors to ecosystem processes, also referred to as key species (Mills et al., 1993), will usually result in a negative shift in ecosystem functioning (Loreau et al., 2001; Cardinale et al., 2006; Thrush et al., 2006), unless ecological redundancy exist. Redundancy suggests that individual species can be lost from an ecosystem without any real consequence on the functioning, as long as the major functional groups are still present (Walker, 1992). Functional groups can be defined as groups of species that share common traits, e.g. feeding modes (Naeem, 1998). Key species are often directly involved in important ecosystem functions; however, the concept of redundancy assumes that more than one species can perform any given function in an ecosystem, and that following the removal of a species, the remaining species will compensate for the loss (Walker, 1992). As a result, it is possible to maintain ecosystem functioning even when biodiversity is low, providing a degree of functional diversity exists (Lawton, 1994). Consequently, many of the species present in ecosystems are considered redundant and are only necessary to ensure ecosystem resilience to stresses, but not for the daily function of the system. Very few studies have directly examined the diversity-functioning relationship following shifts in overall biodiversity and during subsequent ecosystem recovery (Lohrer et al., 2010).

Disturbance events in an ecosystem framework result in shifts in population structure, and can lead to the reduction or extinction of one or more species. Disturbances in intertidal systems can be both natural (e.g. stingray pits) or anthropogenic (e.g. shellfish harvesting). One such disturbance is yearly spring macroalgal blooms, which result in the formation of extensive macroalgal mats (wrack). As large quantities of macroalgae break free from the growing substrate, drifting mats are formed which are transported by the wind and tides. These mats are generally deposited in regions of low flow, such as intertidal estuarine environments, in patches which vary in density and biomass (Olabarria et al., 2010), and persist until they decompose or are removed by storm events. As a result, the mats can remain in an estuary for weeks to months, creating a significant disturbance (Bolam et al., 2000).

Algal mats have been shown to have significant effects on the community composition of soft sediment systems (Raffaelli et al., 1991; Everett, 1994; Norkko & Bonsdorff, 1996a, b, c; Cardoso et al., 2004), although the nature of the effects (positive or negative) remains unclear. Some studies have reported significant declines in species diversity and richness under such mats (Perkins & Abbott, 1972; Norkko & Bonsdorff, 1996b), whilst others have reported either no change or at times an increase in the abundance of some species in the presence of macroalgal mats (Soulsby et al., 1982; Cardoso et al., 2004). One explanation for a decline in species diversity and richness is a significant reduction in oxygen ($O_2$) levels under dense and widespread mats. The reduced $O_2$ levels, which can create hypoxic conditions in the substrata, smothers many estuarine benthic organisms and have been well documented (Perkins & Abbott, 1972; Everett, 1994; Norkko & Bonsdorff, 1996a; Reise, 2012). The decomposing mats also results in increased concentrations of toxins such as hydrogen sulphide ($H_2S$) and ammonium ($NH_4^+$), which could further result in the death of underlying benthic fauna (Perkins & Abbott, 1972; Reise, 2012), and alters nutrient cycling within the sediment.

The resilience and subsequent recovery of different species to a particular disturbance will vary for many reasons. For example, some species may be more tolerant due to having been pre-exposed to similar disturbances (Amaral et al.,

2011); others may be adapted to living in low oxygen environments and so are less affected by hypoxic conditions (Dauer, 1984); whilst others may benefit from the mats by using them as a food source and shelter from predators (Soulsby et al., 1982; Norkko et al., 2000). These factors may explain the reason, in some studies, abundances of some species increased in association with macroalgal mats (Soulsby et al., 1982; Cardoso et al., 2004), and is discussed in more depth in Chapter 2. In terms of recovery, life histories (e.g. presence/absence of planktonic larval stages) and mobility become important attributes when considering larger scale disturbances (Dauer, 1984). It is generally agreed that most disturbances will, however, alter the community composition and biodiversity in some capacity (Soulsby et al., 1982; Thiel et al., 1998; Van Colen et al., 2008). Subtle changes in biodiversity can have real implications for ecosystem functions such as primary production, benthic respiration and nutrient regeneration, which in turn influence the ability of a system to adequately provide ecosystem services. This thesis will examine the linkages between biodiversity, ecosystem functions and estuarine benthic food webs.

Once the conditions for growth become less suitable, macroalgal mats (wrack) will begin to decompose and become detritus. Often the detritus will become covered and buried by sediment (Hull, 1987; Ford et al., 1999), adding organic matter and nutrients (particularly carbon, nitrogen and phosphorous) to the benthos (Rossi, 2007). Additional nutrients often result in an increase in the biomass of microphytobenthos (MPB) and other primary producers (Posey et al., 1999), as well as aerobic and anaerobic bacteria (Raffaelli et al., 1998). An increase in the detrital load and the biomass of MPB and primary producers can be beneficial to grazers and deposit feeders, increasing the abundance and richness of these trophic groups (Hull, 1987; Ford et al., 1999; Rossi & Underwood, 2002). However, high densities of decomposing detritus can also cause anoxic conditions and sulphide production in the sediments (Nedergaard et al., 2002), which can result in widespread mortality and subsequent reductions in species diversity. Detrital additions, can therefore alter species composition and diversity, which may have negative effects on ecosystem functioning. It is predicted that the impact of macroalgal detritus on a system is largely dependent on the amount of wrack (biomass) that is deposited and incorporated.

Estuarine systems are well suited to disturbance-recovery experiments as; (1) they are inhabited by copious species comprising a variety of trophic and taxonomic groups; (2) disturbances at relevant spatial scales are relatively easy to create; and (3) recovery can be observed over relatively short time periods (Lohrer et al., 2010). Solute fluxes, such as oxygen and nutrients, across the sediment-water interface have previously been used to quantify the functionality of soft sediment ecosystems (Lohrer et al., 2004, 2010; Hewitt et al., 2006; Thrush et al., 2006; Norling et al., 2007). This thesis will use benthic primary production, benthic metabolism (sediment oxygen consumption), and nutrient regeneration as functional measures of the impacts of macroalgal on benthic communities.

## 1.3 Macroalgal blooms in New Zealand

A common macroalgal species in New Zealand and overseas is *Ulva* sp. (hereafter referred to as *Ulva*) (Everett, 1994). *Ulva*, commonly referred to as sea lettuce, is a green leafy macroalgae which naturally occur in many littoral ecosystems worldwide. *Ulva* attaches to the substratum by a simple holdfast, and parts of, or entire blades (thalli), may easily become detached and has the ability to continue to grow (Fritsch, 1971), which can result in thick high-density drifting mats. During warmer spring/summer months, and under suitable conditions, *Ulva* growth is significantly enhanced, and can be as much as 20 – 30% per day (Parker, 1981). Globally, *Ulva* blooms have increased in both frequency and biomass, fuelled by excess nutrients entering the coastal systems causing increasingly eutrophic systems (Vermaat & Sand-Jensen, 1987).

Tauranga rate payers and regional Councillors have expressed concerns over the impact of periodic large-scale *Ulva* blooms in Tauranga Harbour. In addition to understanding the drivers responsible for *Ulva* blooms, managers need to know the impact on other components of the ecosystem. Changes in benthic species diversity and nutrient cycling can potentially have far-reaching effects on the functioning of estuarine ecosystems. For example, the demise of key benthic species (e.g. crabs, cockles, worms) may reduce food availability for higher trophic levels such as fish and birds (e.g. Breitburg et al., 1999; Jones et al.,

2016), and change decomposition rates of organic matter and nutrient fluxes at the sediment-water interface. In shallow water systems like Tauranga Harbour, benthic nutrient regeneration plays a key role in pelagic nutrient dynamics and thus primary production (Fisher et al., 1982; Sundbäck et al., 2003).

While the effects of macroalgal mats on benthic communities have been documented in systems across the globe (e.g. Thrush, 1986; Lavery & McComb, 1991; Norkko & Bonsdorff, 1996a; b; c) results are not directly applicable to Tauranga Harbour, as each estuary has a unique benthic community and environmental conditions. Furthermore, the impact of macroalgae to wider ecosystem functions have been largely overlooked. The impact of macroalgal mats will be a function of the spatial and temporal scale of the disturbance (i.e. size of the mats and their persistence), the resident benthic community, and how quickly sediments are re-colonised following the removal or breakdown of the mat. All these factors vary considerably from one system to the next. From a management perspective, knowing how *Ulva* mats impact benthic communities and their functioning, and how quickly the benthos can recover, will assist in predicting the harbour-wide effects of changes in bloom intensity and duration. Moreover, continued large-scale community changes documented worldwide, prompts an urgent need for a deeper mechanistic understanding of the interrelatedness of biodiversity and ecosystem functioning.

## 1.4 Research chapters and aims

The overall aim of my thesis was to examine the impact of macroalgal mats on the biodiversity and ecosystem functioning of estuarine intertidal habitats, and the importance of key species and species diversity, on the processing and reworking of macroalgae in estuarine ecosystems. I also wanted to monitor the changes in biodiversity and ecosystem function over time, to ascertain the temporal significance of these disturbances and the recovery time of ecosystems from a sudden pulse of macroalgae. I aimed to follow the ecological pathway of macroalgae, from when it first enters the benthic system as large mats, to when it is broken down into detritus, and finally, as it is reworked into the sediment and

enters the benthic food web (see Figure 1.1). This was achieved through a series of three experimental studies, which were written as individual chapters/papers. The experiments were all conducted at Tuapiro Point, which is an estuary situated in the northern part of Tauranga Harbour (see Figure 1.2). In order for each chapter to be published individually, information in some sections (e.g. methods) has been repeated across multiple chapters.

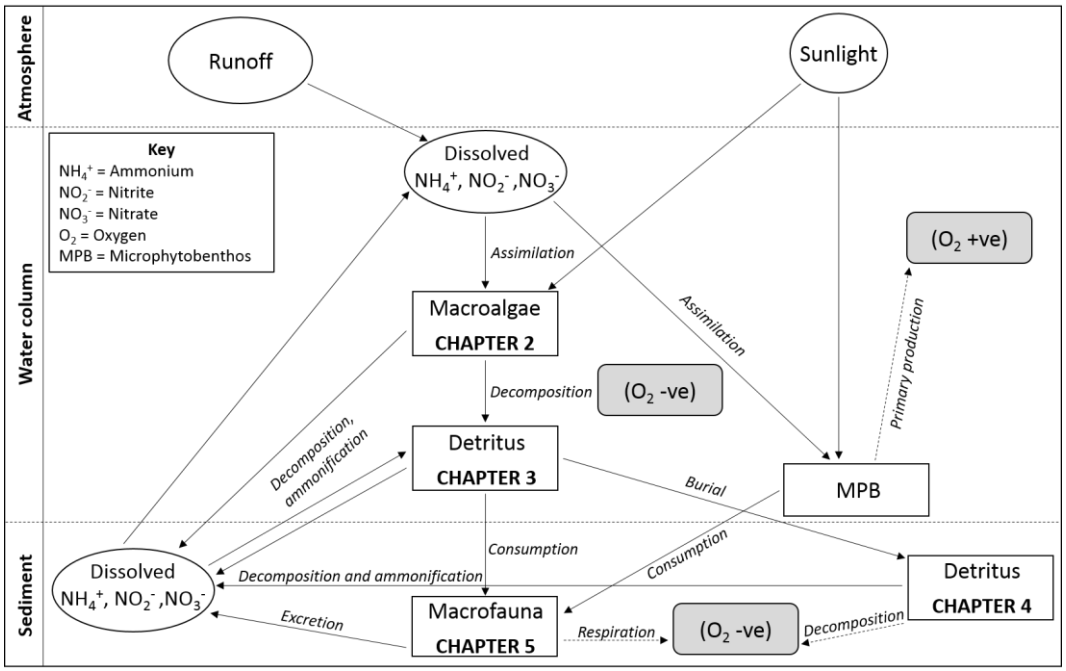


**Figure 1.1.** A conceptual diagram (adapted from Herbert, 1999; Tyler et al., 2003; Gruber, 2008) illustrating the pathways of macroalgae production and decomposition in estuaries, from the initial bloom (Chapter 2), through the decomposition phase as the larger sheets become detritus and settle on the sediment surface (Chapter 3), through to the detritus incorporation and reworking into the sediment (Chapter 4) and the food web (Chapter 5). Influential abiotic and biotic processes are given in italics. The four research chapters and how they relate to the macroalgal pathway are indicated in bold.

Firstly, I aimed to quantify the recovery of biodiversity and ecosystem function following a disturbance caused by macroalgal mats (Chapter 2). Next, I examined the impact of different quantities of macroalgal detritus on the biodiversity and ecosystem function of a sand flat habitat (Chapter 3). Finally, through a laboratory mesocosm experiment, I investigated the density dependent effect of two key estuarine species on the distribution and processing of macroalgae detritus in intertidal sediments (Chapter 4). The uptake of macroalgae by the benthic macrofauna was quantified in a separate study (see Karlson et al., 2016), and is included as Chapter 5.

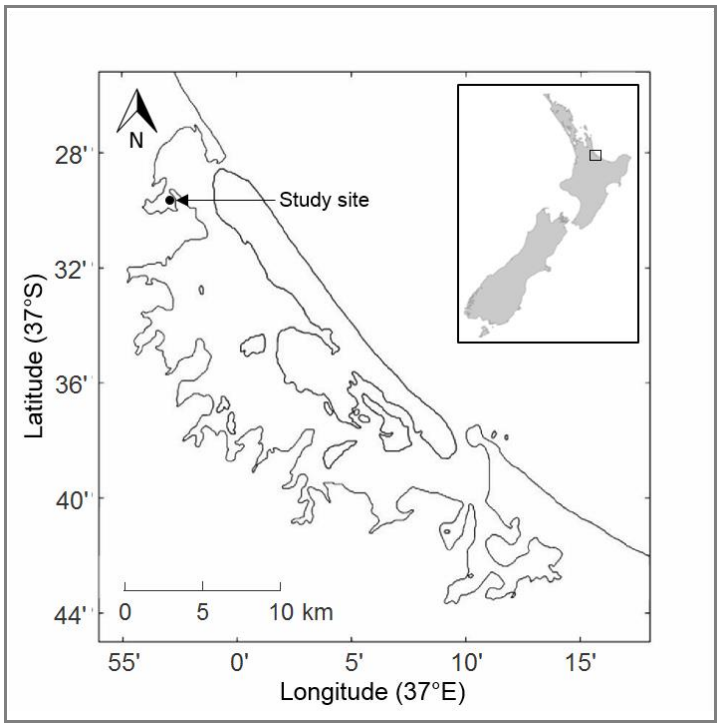


**Figure 1.2.** Site map indicating the study site at Tuapiro Point, in the northern part of Tauranga Harbour, New Zealand.

# 2.0 CHAPTER TWO: THE EFFECTS OF *ULVA* MATS ON INTER-TIDAL BENTHIC BIODIVERSITY AND ECOSYSTEM FUNCTION

## 2.1 Introduction

The loss of biodiversity and the impacts of such losses on ecosystem function and services in both terrestrial and marine habitats have been a concern for ecologists for decades. It was not until the 1990's, however, that researchers actively started to consider the way species diversity may impact the functioning of the larger ecosystem (Tilman et al., 1997; Cardinale et al., 2006; Loreau, 2010). It has been widely suggested that reducing species abundance, richness or diversity, or excluding key species from a system, will result in a shift in ecosystem functioning (Loreau et al., 2001; Cardinale et al., 2006; Thrush et al., 2006). In marine environments, however, very few studies have directly examined the diversity-functioning relationship following a shift in the overall biodiversity (e.g. as is evident following a disturbance event), and during the subsequent recovery period (Lohrer et al., 2010).

In estuaries all around the world, natural processes of eutrophication have become exacerbated due to anthropogenic nutrient inputs as a result of land use change and run-off (Anderson et al., 2002; Shaw et al., 2003), which in turn fuels macroalgal blooms (Valiela et al., 1997). Annual blooms of macroalgae, which result in large drifting algal mats, are a common disturbance in intertidal ecosystems worldwide (Everett, 1994). The presence of large macroalgal mats have increased globally, and can have significant effects on the community composition of soft sediment systems (Everett, 1994; Norkko & Bonsdorff,

1996a, b, c; Cardoso et al., 2004; Valença et al., 2016). Drifting mats will generally accumulate in regions of low flow, such as intertidal estuarine environments.

A common nuisance bloom forming species of macroalgal around the world is *Ulva* (Teichberg et al., 2010). Accumulating *Ulva* mats can have physical, biological and biogeochemical impacts on estuarine systems, and these impacts are often inter-dependent. For example, mats can create a physical barrier between primary producers, such as microphytobenthos (MPB), and sunlight which fuels the production of these microphyte communities (Sundbäck & McGlathery, 2005; Thrush et al., 2014). MPB, in turn, provides an important food source for many grazers and deposit feeding macrofauna (Thrush et al., 2006; Jones et al., 2016), and a reduction in this primary food source will have cascading bottom up effects on the overall community and ecosystem function. Furthermore, macroalgal mats may inhibit the feeding capabilities of suspension feeders that rely on the food particles from the water column.

Underneath decomposing mats, there can be a significant reduction in oxygen supply and increased concentrations of toxins such as hydrogen sulphide ($H_2S$), which can induce hypoxia within the sediment, and result in widespread mortality of the resident benthic fauna (Perkins & Abbott, 1972; Norkko & Bonsdorff, 1996a; Reise, 2012). The dead and decaying organic matter and macrofauna further fuels the release of ammonium ($NH_4^+$), as anaerobic bacteria begin the breakdown process (Anderson & Kristensen, 1991), which in turn fuels blooms by providing more available nutrients in the water column.

Estuarine macrofauna perform important ecosystem functions such as sediment reworking and nutrient regeneration, which in turns fuels primary production (Lohrer et al., 2004; Sandwell et al., 2009). Macrofauna further contribute to the remineralisation of organic matter through activities such as feeding, bioturbation and habitat construction (i.e. burrows and tubes). Thus, a reduction in the key species which contribute to these activities can be devastating for the overall function of an ecosystem (Norkko et al., 2013; Gammal et al., 2017).

The impact of macroalgal mats on benthic estuarine communities are dependent on the spatial and temporal scales on which they occur. Mats can remain in an estuary at high biomass for weeks to months, thus creating significant disturbances for extended periods of time (Bolam et al., 2000). Although the initial impact of macroalgal mats are dependent on the severity, frequency and the duration of the disturbance, the response of the macrofauna depend upon the resilience of individual species as well as the overall community composition (Norkko & Bonsdorff, 1996b, c). Small scale blooms are more common compared to large scale blooms, and communities that are frequently exposed to relatively moderate blooms may therefore become resilient. Ecosystem function may also remain unchanged if multiple species within the macrofaunal community share biological traits, as this provides functional redundancy even when some species are affected by the disturbance (Walker, 1992; Naeem, 1998). Therefore, there is a need to understand the response of benthic communities and the ecosystem functions they provide to small-scale macroalgal disturbances, and how these systems recover, in order to correctly interpret the role of these transient disturbances (Norkko & Bonsdorf, 1996b, c).

Estuarine intertidal habitats are ideal systems in which to examine the response of macrofaunal communities to macroalgal disturbances, as they are relatively easy to manipulate, and recovery is usually rapid and can be observed over short temporal scales (Lohrer et al., 2010). Intertidal communities in New Zealand estuaries are often dominated in biomass by two large bivalve species; *Austrovenus stutchburyi* and *Macomona liliana* (e.g. Thrush et al., 1996). Both species contribute to important ecosystem functions such as nutrient regeneration, benthic metabolism and primary production through feeding and bioturbating activities (Thrush et al., 2006, 2014; Jones et al., 2011; Pratt et al., 2015). Although studies have examined the role of these larger key species, the role of numerically abundant co-existing smaller macrofauna is often overlooked. In addition, studies often simplify complicated systems in an attempt to understand macrofaunal interactions and processes which govern large scale ecosystem functions, despite a need for field data from more complex systems (Naeem & Wright, 2003; Snelgrove et al., 2014). In this study, I aimed to bridge this knowledge gap by investigating the response of an intact, diverse macrofaunal

community to a macroalgal disturbance event, and document the response over time. Shifts in community composition have the potential to impact important benthic ecosystem functions such as primary production, benthic metabolism and nutrient regeneration (Snelgrove et al., 2014).

In New Zealand, periodic, extensive macroalgal blooms, which are likely fuelled by nutrients entering the system from land runoff and point source discharges, regularly occur in Tauranga Harbour (Park, 2011). In this study, I mimicked the disturbance caused by these frequently occurring, small-scale *Ulva* mats (Busing, 1999; Park, 2011), to determine the impacts on the intertidal benthic community and the community recovery over time. I also sought to understand the implications of changes in community composition to the wider ecosystem function. I predicted that macroalgal mats would induce hypoxia within the sediments resulting in a loss of species, and an efflux of $NH_4^+$ from the sediment as a result of the decay process. I further predicted that there would be a decrease in the key species and the overall species abundance due to the disturbance and that this would translate to a reduction in benthic metabolism. Lastly, I predicted that primary production would be negatively impacted immediately following the disturbance as a result of reduced light conditions, but that the recovery will be rapid due to $NH_4^+$ efflux from the sediment. This chapter will examine the initial impact and subsequent recovery of a typical New Zealand soft sediment community following a macroalgal disturbance, and build on the current scientific knowledge of the restoration of biodiversity and functioning following transient disturbances.

## 2.2 Methods

### *2.2.1 Study site and experimental design*

The study was conducted at Tuapiro Point, a sheltered intertidal estuary located in the northern part of Tauranga Harbour, on the east coast of New Zealand (S 37° 29.374, E 175° 57.04, see Figure 1.2). For a species list of macrofauna found at this site see Appendix 1. Between April – May 2013, a manipulative field

experiment was conducted in the mid-intertidal area, where 36 identical 1 $m^2$ plots were established. Each plot was randomly assigned to one of three treatments; ambient control, procedural control (PC) and an *Ulva* treatment (UT), with each treatment comprising 12 replicates. Each of the plots were at least 2 m apart. The size of the plots was representative of small macrofaunal mats typically found in Tauranga Harbour (Busing, 1999), and was comparable to other studies (e.g. Everett, 1994; Green & Fong, 2016).

### *2.2.2 Ulva collection and treatment preparation*

In the month preceding the experiment, fresh *Ulva* was collected from various inter- and sub-tidal locations within Tauranga Harbour. Care was taken to collect only *Ulva* which did not show any visible signs of decomposition (i.e. white, yellow or brown patches). *Ulva* was rinsed in seawater and any visible organisms were removed. The *Ulva* was subsequently air dried and frozen at -2°C for up to two weeks to prevent rapid decomposition, as I needed to collect *Ulva* on three separate occasions before I had collected the quantity needed for the experiment. The freezing process also killed the *Ulva*, which provided an accurate representation of *Ulva* at the onset of decomposition when it was added to the plots. Mesh bags were created by cutting mesh netting (approximately 1 × 1 cm spacing) into 1 $m^2$ squares, and sewing two squares together. The *Ulva* was defrosted, and an equal quantity of *Ulva* (3 kg fresh weight [2 kg dry weight]) was placed into the mesh bags. This biomass was chosen as 3 kg ww $m^{-2}$ is a realistic quantity for a naturally occurring moderate to intense bloom (Hull, 1987), and is representative of biomasses previously recorded in Tauranga Harbour (Busing, 1999).

### *2.2.3 Experimental setup*

At the beginning of April, the UT plots, which comprised the mesh bags each containing 3 kg ww $m^{-2}$ *Ulva*, and the PC plots, which consisted of mesh bags with no added *Ulva*, were established by pegging the bags onto the plots with galvanised steel pegs, using approximately 10 pegs per plot (see Figure 2.1).

Ambient control plots were left untouched. The timing for the experiment coincided with the end of the growing season for *Ulva* when natural blooms had subsided in order to limit the influence of any natural blooms on plots. This overlapped with the period when natural blooms would start to decay and break down, as was the state of the added *Ulva* after freezing. The PC and UT plots were left for a 30-d period, as previous studies have suggested that decomposition of algal mats on the surrounding benthos occurred within one month (Buchsbaum et al., 1991; Nedergaard et al., 2002), and decomposition effects can be observed within 2 – 10 weeks of deposition (Hull, 1987; Bolam et al., 2000).

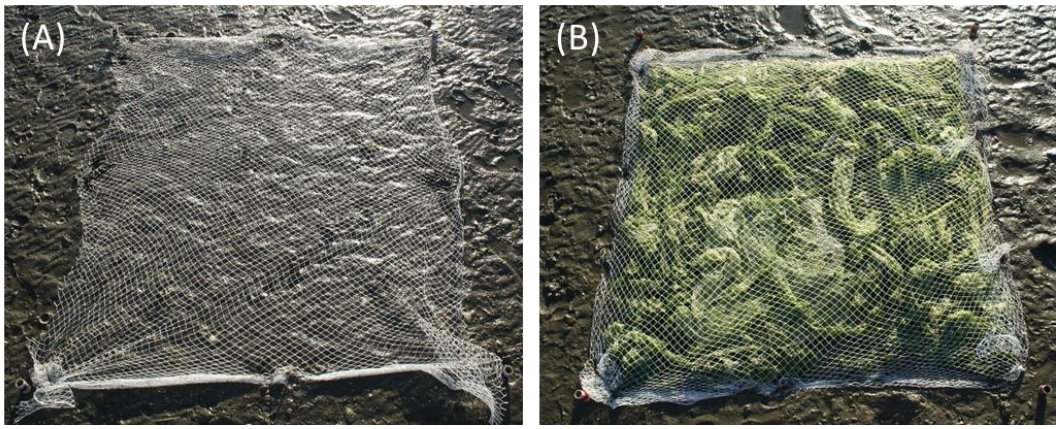


**Figure 2.1.** Experimental setup showing (A) the procedural control (PC) and (B) the treatment (UT) plots.

After 30 d, the mesh bags were removed from the PC and UT plots during an afternoon low tide. The following day (hereafter d1), after one tidal cycle, the first set of detailed samples and measurements were collected from each of the 36 experimental plots. Ambient control plots (i.e. those representative of un-manipulated, natural sediment) were only sampled on d1 to quantify the effect (if any) of the mesh bags irrespective of the *Ulva* treatment, and were not subsequently re-sampled. After 14 days (hereafter d14), the sampling process was repeated for the PC and UT plots in order to ascertain short-term recovery following the disturbance. The area of the plots that was sampled on d1 was noted to avoid re-sampling on d14, and clean, defaunated sand was used to replace any sediment that was removed as part of the sampling process to avoid infilling from the surrounding sediment (Lohrer et al., 2010). These sampling times were selected as they were comparable with previous studies which examined the impacts of *Ulva* on macrofaunal assemblages (e.g. Kelaher & Levinton, 2003;

Rossi et al., 2013), and also to coincide with mid-day high tides in order to optimise the measurements of ecosystem function variables (see below).

### *2.2.4 Field sampling*

Plots were sampled for macrofaunal community composition (abundance and richness), sediment properties (median grain size, organic matter, % mud content), microalgal biomass (chlorophyll *a*, phaeophytin), and solute fluxes (oxygen and nutrients), which were used to derive proxies of ecosystem function (i.e. primary production, community metabolism and nutrient regeneration).

Oxygen and nutrient fluxes were measured at the sediment-water interface in two paired benthic chambers (one light and one dark), according to the methods of Lohrer et al. (2010). One light and one dark chamber were placed in each plot on an incoming tide between 10:00 and 11:00 h, once the water was deep enough to completely cover each chamber (approx. 20 cm). The chambers were pushed 2 cm into the sediment to ensure that the water remained sealed within the chamber. Each benthic chamber covered an area of 0.016 $m^2$, and trapped 0.85 L of seawater above the sediment-water interface. The chambers had two ports, allowing water to re-enter (through the inlet port) as water was being sampled from the chamber (sampling port). I also assessed ambient water column oxygen and nutrient concentrations by placing pairs of light and dark bottles, each containing 1.5 L of ambient seawater, in the water column directly above the chamber heights at three random plots across the experimental site. Benthic chamber results were corrected for water column processes (usually < 5% of the measured sediment-water column fluxes). The light and dark bottles were sampled concurrently with the benthic chambers.

Once the chambers were placed, they were then left to incubate for approximately 4 h during peak sunlight hours over a mid-day high tide. Water samples (60 ml) were collected at the beginning and the end of the incubation period by attaching a screw top syringe to the end of the sampling port, and drawing water into the syringe. Prior to the initial sample being collected, 20 ml of seawater was drawn and discarded to flush any water that may have been contained in the tubing.

Immediately following the collection of water samples, levels of dissolved oxygen (DO) were measured using a calibrated optical probe (RDO, In-Situ Incorporated, Fort Collins, Colorado, USA). Water samples were then filtered (Whatman GF/C grade filter, 1.2 µm pore size), kept on ice in darkness and subsequently frozen until inorganic nutrient analyses could be completed. Dissolved oxygen fluxes provided quantitative measures of primary production (i.e. net primary production [NPP] and gross primary production [GPP]) and community metabolism (i.e. sediment oxygen consumption [SOC]), while nutrient fluxes provided quantitative measures of uptake or regeneration (influx or efflux from the sediment, respectively). HOBO data loggers were deployed at six randomly selected plots at the start of each sampling day to quantify ancillary bottom water temperature and light availability during the incubation period, as these factors can vary greatly and impact biological reactions and solute changes (Zlotnik & Dubinsky, 1989).

After the final water samples were collected, macrofauna were sampled from directly underneath the dark chamber in each plot using a large core (10 cm diameter) to a depth of 10 cm. The cores were sieved on a 500 µm mesh and the contents of the sieve were preserved in 70% isopropyl alcohol for subsequent macrofauna identification and analyses. Four smaller cores (2.7 cm diameter) were also taken within the plot to a depth of 5 cm using a 50 ml cut-off syringe and pooled for analysis. From these cores, chlorophyll *a* (chl *a*), phaeophytin (phaeo), organic matter (OM), median grain size (GS) and % mud were determined. The sediment samples were kept in the dark and on ice (separate from the water samples to avoid contamination), and frozen once back at the laboratory to await further analyses.

### *2.2.5 Laboratory analyses*

In the laboratory, the filtered water samples were analysed for inorganic nutrients (ammonia [$NH_4^+$], nitrite [$NO_2^-$], nitrate [$NO_3^-$], and phosphorus [$PO_4^{3-}$]), on a Lachat QuickChem 8000 Series FIA, using standard methods for seawater (Grasshoff et al., 2009). For chl *a* and phaeo analyses, the sediment was freeze dried, homogenised and measured before and after treatment with 0.1 N HCl, respectively, on a Turner 10-AU fluorometer, following the methods of Arar and

Collins (1997). The acidification step was used to allow for the extraction of phaeopigments. OM was determined through loss on ignition of a homogenised and dried (110°C for 24 h) subsample of sediment after combustion for 5.5 h at 550°C, as outlined in Dean (1974). GS samples were treated with 10% hydrogen peroxide to remove organic matter and calcareous material, and then analysed using a Malvern Mastersizer-S (300 FR lens, range 0.05 - 2000 µm). The macrofauna samples were stained with Rose Bengal (to aid in the sorting process) and identified to the lowest taxonomic level possible (usually species level).

### *2.2.6 Flux calculations and statistical analyses*

Flux calculations were made by subtracting the initial from the final concentrations and were standardised for incubation time, chamber water volume and the sediment surface area (Lohrer et al., 2010). In the light chambers, I measured NPP and net ammonium efflux (Net $NH_4^+$), while from the dark chamber fluxes I measured SOC, and gross ammonium efflux (Gross $NH_4^+$). GPP was calculated by subtracting the dark chamber DO flux from the light chamber DO flux and was standardised by chl *a* ($GPP_{chl\ a}$) to account for variations in MPB biomass and to provide a measure of photosynthetic efficiency. Concentrations of the other measured nutrients ($NO_2^-$, $NO_3^-$, $PO_4^{3-}$) were below or near the detection limits (< 0.001 µmol $L^{-1}$), and were therefore not considered for further analyses.

As a first step, one-way permutational analysis of variance (PERMANOVA) were carried out to ascertain if there were significant differences ($p$(perm) < 0.05) in the univariate response variables relating to the macrofaunal community (abundance and richness) and the sediment properties (i.e. % mud, GS, OM, chl *a*, phaeo), and multivariate measures of community (Bray-Curtis similarity) and sediment properties (Euclidean distance) between ambient and PC plots at d1 to determine if the mesh bags significantly altered any of the measured variables. The univariate data was untransformed while the macrofaunal and sediment property multivariate data was square root transformed and normalised, respectively.

Repeated measures PERMANOVA analyses were then carried out to test for significant differences between PC and UT plots through time on untransformed

univariate response variables relating to the macrofaunal community, sediment properties, and measures of ecosystem function (NPP, SOC, $GPP_{chl\ a}$, net $NH_4^+$, gross $NH_4^+$). A repeated measures PERMANOVA was also carried out separately on square root transformed multivariate macrofauna data (Bray-Curtis similarity), and normalised multivariate sediment and ecosystem function measures (both Euclidean distance). The experimental design comprised three factors; treatment (PC and UT) and time (d1 and d14), which were both treated as fixed factors, as well as plot (12 levels) which was treated as a random factor nested within treatment (Anderson et al., 2008). As both treatment and time only comprised two levels, pairwise tests were not needed when significant differences were detected. However, post-hoc pair-wise tests were carried out where the treatment x time interaction term was significant to ascertain the differences between treatments at each sampling date.

Non-metric multidimensional scaling analyses (nMDS) was performed on the transformed macrofauna community data to visualise the variation in community composition across different detrital addition treatments and through time (Anderson et al., 2008), and SIMPER analysis (Bray-Curtis similarity) was used to ascertain which species were predominantly contributing to significant differences between treatments, when they occurred. All the statistical analyses were carried out using the PRIMER 7 statistical software program with the PERMANOVA+ add on.

## 2.3 Results

### *2.3.1 Temporal variations in environmental conditions*

Mean light levels were approximately 40% lower on d1 compared to d14 (Table 2.1). Although temperature varied between the sampling dates (higher on d1 compared to d14), average temperatures on the two sampling dates were within 1°C of each other. Salinity was also slightly lower on d1 compared to d14 (Table 2.1).

**Table 2.1.** Ambient light, temperature and salinity at the sediment-water interface during benthic flux measurements on d1 and d14. Light and temperature measurements are presented as means (± SE; n = 6 loggers) recorded during the incubation period, while salinity was measured only once (at the start of each incubation period).

| **Time** | **d1** | **d14** |
|---|---|---|
| Light (Lux) | 18320 (1570) | 31439 (1284) |
| Temperature (°C) | 20.9 (0.06) | 20.4 (0.08) |
| Salinity | 21.5 | 27.6 |

### *2.3.2 Procedural effects vs Ambient conditions on d1*

There were some significant differences in sediment properties between ambient and PC sites (i.e. chl *a* and multivariate sediment properties) on the first sampling date, however there were no significant differences, and therefore no impact of the mesh bags, on the abundance of the two dominant macrofauna species, or overall species abundance or richness (Table 2.2). The mesh bags in the PC plots did, however, negatively impact on the overall macrofaunal community structure (multivariate measure) ($p$(perm) = 0.03) when compared to the ambient plots (Table 2.2). SIMPER analysis highlighted 5 species which significantly contributed to the differences between the communities in ambient and PC plots, with these species cumulatively contributing to 72% of the dissimilarity. These five species were; *Lasaea parengaensis*, *Aonides trifida*, Nereididae, *Scoloplos cylindifera* and *Prionospio aucklandica*. Only *Aonides trifida* however had significantly higher abundances ($p$(perm) = 0.02) in ambient plots compared to PC plots (Table 2.2).

GPP was also significantly different between the ambient and PC sites, with higher GPP recorded in PC compared to ambient plots (Table 2.2). Interestingly, when the GPP was corrected for chl *a* biomass ($GPP_{chl\ a}$), the ambient plots were shown to have significantly higher primary productivity compared to PC plots (Table 2.2). $NH_4^+$ fluxes in the dark chambers were also significantly higher in PC compared to the ambient plots (Table 2.2).

The significant differences recorded in the sediment properties, macrofaunal composition and the ecosystem function variables between the ambient and PC plots suggest that there was an effect of the mesh bags, and therefore, to account

for this effect, PC treatments were used as the 'control' treatments. PC plots were therefore compared with UT plots, to ascertain the impact of *Ulva* mats on benthic communities and their functions.

**Table 2.2.** Mean (± SE) sediment properties, macrofaunal community, and ecosystem function variables in ambient and PC plots on d1. Significant differences between treatments ($p$(perm) < 0.05) are indicated in bold (n = 12). SIMPER results show the % dissimilarity to standard deviation ratio (Diss/SD) and the contribution (%) of those species which collectively comprise 70% of the dissimilarity between treatments (average dissimilarity = 46.85%)

| Variable | Ambient | PC | $p$(perm) | Diss/SD | Cont. (%) |
|---|---|---|---|---|---|
| Median grain size (µm) | 182 (1) | 182 (2) | 0.84 | - | - |
| Mud content (%) | 1.8 (0.2) | 1.9 (0.2) | 0.57 | - | - |
| Organic content (%) | 1.69 (0.06) | 1.51 (0.06) | 0.06 | - | - |
| Chl *a* (µg $g^{-1}$ dw) | 4.6 (0.2) | 6.8 (0.4) | **0.0002** | - | - |
| Phaeophytin (µg $g^{-1}$ dw) | 3.0 (0.3) | 2.6 (0.4) | 0.70 | - | - |
| Multivariate sediment properties | - | - | **0.005** | - | - |
| Abundance (ind. $core^{-1}$) | 37.4 (3.2) | 31.5 (4.2) | 0.21 | - | - |
| Richness (sp. $core^{-1}$) | 9.1 (0.6) | 8.6 (0.7) | 0.53 | - | - |
| *A. stutchburyi* (ind. $core^{-1}$) | 1.3 (0.4) | 1.4 (0.3) | 0.54 | - | - |
| *M. liliana* (ind. $core^{-1}$) | 2.7 (0.3) | 2.5 (0.3) | 0.96 | - | - |
| Multivariate macrofauna community | - | - | **0.03** | - | - |
| *L. parengaensis* (ind. $core^{-1}$) | 8.3 (1.2) | 9.6 (2.2) | 0.59 | 1.26 | 21.01 |
| *A. trifida* (ind. $core^{-1}$) | 6.6 (1.6) | 1.7 (0.5) | **0.02** | 1.23 | 16.55 |
| Nereididae (ind. $core^{-1}$) | 7.3 (1.1) | 8.3 1.3) | 0.73 | 1.22 | 15.26 |
| *S. cylindifera* (ind. $core^{-1}$) | 4.5 (0.9) | 2.7 (0.7) | 0.37 | 1.09 | 11.75 |
| *P. aucklandica* (ind. $core^{-1}$) | 2.4 (0.7) | 1.0 (0.3) | 0.42 | 1.05 | 7.05 |
| NPP (µmol $O_2$ $m^{-2}h^{-1}$) | 1105.1 (227.5) | 1701.5 (228.1) | 0.08 | - | - |
| SOC (µmol $O_2$ $m^{-2}h^{-1}$) | -517.5 (25.4) | -571.7 (50.1) | 0.35 | - | - |
| GPP (µmol $O_2$ $m^{-2}h^{-1}$) | 1622.6 (211.1) | 2273.2 (240.3) | **0.05** | - | - |
| $GPP_{chl\ a}$ (µmol $O_2$ $m^{-2}h^{-1}$) | 353.1 (47.2) | 340.5 (36.2) | **0.04** | - | - |
| Gross $NH_4^+$ (µmol N $m^{-2}h^{-1}$) | 9.6 (3.7) | 10.9 (7.34) | **0.03** | - | - |
| Net $NH_4^+$ (µmol N $m^{-2}h^{-1}$) | -0.02 (4.7) | 1.77 (3.98) | 0.08 | - | - |

### *2.3.3 Effects of Ulva on sediment properties and macrofaunal community composition through time*

Sediment properties were variable, both as a function of treatment and time. Treatment effects were recorded for the median GS, which was significantly larger in UT compared to PC plots, while chl *a* was significantly higher in PC plots compared to UT plots (Tables 2.3 and 2.4). Interestingly, OM was

significantly higher in the PC compared to UT plots, but only on d1, as indicated by the significant interaction term (Tables 2.3 and 2.4). Treatment effects were also observed in the multivariate measure of sediment properties at both sample dates (Table 2.4).

**Table 2.3.** Mean (± SE) sediment properties and macrofaunal community variables as a function of time (d1 and d14), and treatment (procedural control [PC] and *Ulva* treatment [UT]).

| | **d1** | | **d14** | |
|---|---|---|---|---|
| **Variable** | **PC** | **UT** | **PC** | **UT** |
| Median grain size (µm) | 182 (2) | 185 (2) | 185 (2) | 194 (4) |
| Mud content (%) | 1.9 (0.2) | 1.4 (0.1) | 2.0 (0.2) | 1.9 (0.2) |
| Organic content (%) | 1.51 (0.06) | 1.29 (0.05) | 1.48 (0.09) | 1.71 (0.11) |
| Chl *a* (µg $g^{-1}$ dw) | 6.8 (0.4) | 4.7 (0.3) | 6.1 (0.4) | 3.9 (0.2) |
| Phaeopigment (µg $g^{-1}$ dw) | 2.6 (0.4) | 2.3 (0.2) | 2.6 (0.3) | 2.0 (0.2) |
| Abundance (ind. $core^{-1}$) | 31.5 (4.2) | 27.6 (2.9) | 35.5 (5.4) | 31.1 (4.8) |
| Richness (sp. $core^{-1}$) | 8.6 (0.7) | 7.8 (0.6) | 8.8 (0.8) | 8.5 (1.0) |

Temporal variation was observed in the univariate measures of median GS and % mud, which both increased through time (d1 < d14), while chl *a* decreased (d1 > d14) (Tables 2.3 and 2.4). Multivariate sediment properties varied significantly between d1 and d14, but only in the UT plots, as indicated by the significant interaction term (Table 2.4).

**Table 2.4.** Summary of repeated measures PERMANOVA results of univariate sediment properties and macrofaunal abundance and richness, as well as multivariate sediment and macrofaunal data, as a function of time (d1, d14), and treatment (PC, UT). Significant effects ($p$(perm) < 0.05) are indicated in bold, with pair-wise tests indicating where the significant differences occurred (n = 12).

| Variable | Source | df | MS | Pseudo-F | $p$(perm) | Pair-wise tests |
|---|---|---|---|---|---|---|
| Median GS | Time | 1 | 420.5 | 5.72 | **0.03** | d1 < d14 |
| (µm) | Treatment | 1 | 460.6 | 5.90 | **0.02** | PC < UT |
| | Time x Treatment | 1 | 98.0 | 1.33 | 0.26 | |
| | Plot (treatment) | 22 | 78.0 | 1.06 | 0.43 | |
| | Residual | 22 | 73.5 | | | |
| Mud content | Time | 1 | 1.2 | 4.78 | **0.04** | d1 < d14 |
| (%) | Treatment | 1 | 1.2 | 3.64 | 0.07 | |
| | Time x Treatment | 1 | 0.3 | 1.32 | 0.26 | |
| | Plot (treatment) | 22 | 0.3 | 1.32 | 0.27 | |
| | Residual | 22 | 0.3 | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Organic matter | Time | 1 | 0.4 | 5.99 | **0.02** | d1: PC > UT |
| (%) | Treatment | 1 | 0.0 | 0.00 | 0.95 | d14: PC = UT |
| | Time x Treatment | 1 | 0.6 | 8.27 | **0.009** | PC: d1 = d14 |
| | Plot (treatment) | 22 | 0.1 | 1.15 | 0.35 | UT: d1 < d14 |
| | Residual | 22 | 0.1 | | | |
| Chl *a* | Time | 1 | 6.9 | 4.95 | **0.04** | d1 > d14 |
| (µg $g^{-1}$ dw) | Treatment | 1 | 55.4 | 35.86 | **0.0001** | PC > UT |
| | Time x Treatment | 1 | 0.0 | 0.01 | 0.94 | |
| | Plot (treatment) | 22 | 1.5 | 1.12 | 0.40 | |
| | Residual | 22 | 1.4 | | | |
| Phaeophytin | Time | 1 | 0.4 | 0.37 | 0.58 | |
| (µg $g^{-1}$ dw) | Treatment | 1 | 2.3 | 1.72 | 0.21 | |
| | Time x Treatment | 1 | 0.3 | 0.27 | 0.63 | |
| | Plot (treatment) | 22 | 1.3 | 1.11 | 0.36 | |
| | Residual | 22 | 1.2 | | | |
| Sediment | Time | 1 | 15.7 | 4.13 | **0.003** | d1: PC ≠ UT |
| properties | Treatment | 1 | 31.0 | 7.11 | **0.0003** | d14: PC ≠ UT |
| (multivariate) | Time x Treatment | 1 | 8.6 | 2.28 | **0.05** | PC: d1 = d14 |
| | Plot (treatment) | 22 | 4.4 | 1.15 | 0.23 | UT: d1 ≠ d14 |
| | Residual | 22 | 3.8 | | | |
| Macrofauna | Time | 1 | 162.3 | 0.31 | 0.73 | |
| abundance | Treatment | 1 | 340.9 | 0.52 | 0.55 | |
| (ind. $core^{-1}$) | Time x Treatment | 1 | 163.6 | 0.31 | 0.72 | |
| | Plot (treatment) | 22 | 657.0 | 1.26 | 0.24 | |
| | Residual | 22 | 523.0 | | | |
| Macrofauna | Time | 1 | 42.1 | 0.21 | 0.72 | |
| richness | Treatment | 1 | 144.9 | 0.43 | 0.53 | |
| (sp. $core^{-1}$) | Time x Treatment | 1 | 16.6 | 0.08 | 0.88 | |
| | Plot (treatment) | 22 | 340.5 | 1.73 | 0.09 | |
| | Residual | 22 | 197.0 | | | |
| *Austrovenus* | Time | 1 | 2849.5 | 1.22 | 0.23 | |
| *stutchburyi* | Treatment | 1 | 2903.9 | 1.13 | 0.26 | |
| (ind. $core^{-1}$) | Time x Treatment | 1 | 3090.8 | 1.33 | 0.18 | |
| | Plot (treatment) | 22 | 2574.5 | 1.10 | 0.27 | |
| | Residual | 22 | 2331.8 | | | |
| *Macomona* | Time | 1 | 1328.4 | 1.17 | 0.32 | |
| *liliana* | Treatment | 1 | 1458.3 | 1.86 | 0.09 | |
| (ind. $core^{-1}$) | Time x Treatment | 1 | 1954.1 | 1.73 | 0.12 | |
| | Plot (treatment) | 22 | 784.4 | 0.69 | 0.97 | |
| | Residual | 22 | 1131.2 | | | |
| Macrofauna | Time | 1 | 1292.6 | 1.05 | 0.40 | |
| community | Treatment | 1 | 5078.8 | 3.57 | **0.0005** | PC ≠ UT |
| (multivariate) | Time x Treatment | 1 | 1711.4 | 1.39 | 0.20 | |
| | Plot (treatment) | 22 | 1421.3 | 1.16 | 0.16 | |
| | Residual | 22 | 1227.8 | | | |

Macrofaunal community data showed little treatment effects, while no temporal variation was recorded (Table 2.4; Figure 2.2A). The only significant treatment effect that was recorded was for the overall community composition (multivariate measure) (Table 2.4; Figure 2.2B).

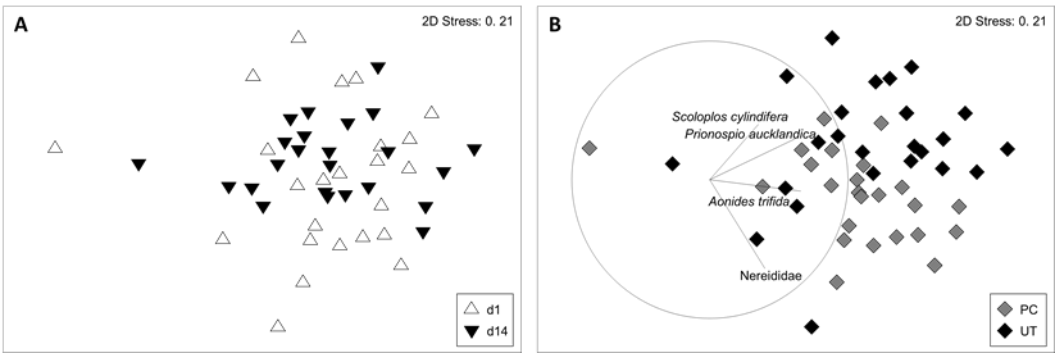


**Figure 2.2.** Non-metric MDS ordination plots of square-root transformed macrofaunal community data. Ordination plots (Bray-Curtis similarity) show community composition as a function of (A) time (d1 and d14), and (B) treatment (PC and UT). Where the community composition varied significantly ($p$(perm) < 0.05), the species that collectively contributed to 50% of the variation is indicated (Pearson's r < 0.5).

SIMPER analysis of the community data highlighted the same five macrofaunal species that were important contributors to ambient and PC dissimilarity (i.e. *Lasaea parengaensis*, *Aonides trifida*, Nereididae, *Scoloplos cylindifera* and *Prionospio aucklandica*) to be important contributors to the dissimilarity between PC and UT plots (Tables 2.2 and 2.5; Figure 2.2B), with significantly higher abundances of Nereididae in PC compared to UT plots (Table 2.5). *Lasaea parengaensis*, Nereididae and *Scoloplos cylindifera* collectively contributed to 48% of the dissimilarity between PC and UT plots.

**Table 2.5.** SIMPER analysis (Bray-Curtis similarity) showing the % dissimilarity to standard deviation ratio (Diss/SD) and the taxa which cumulatively contribute to 70% of the dissimilarity between PC and UT treatments, irrespective of sampling date (n = 24). The average dissimilarity between PC and UT treatments was 52.61%. Mean (± SE) abundances for each species is also shown, with significant differences between treatments ($p$(perm) < 0.05) indicated in bold.

| Species (ind. core$^{-1}$) | Mean abundance (ind. core $^{-1}$) (± SE) | | $p$(perm) | Diss/SD | Cont. (%) |
|---|---|---|---|---|---|
| | PC | UT | | | |
| *L. parengaensis* | 10.6 (1.3) | 7.2 (0.8) | 0.18 | 1.21 | 20.36 |
| Nereididae | 7.2 (1.0) | 2.7 (0.4) | **0.002** | 1.37 | 16.39 |
| *S. cylindifera* | 3.9 (0.7) | 4.6 (0.7) | 0.82 | 1.18 | 11.68 |
| *P. aucklandica* | 1.5 (0.4) | 3.8 (0.9) | 0.14 | 1.05 | 10.93 |
| *A. trifida* | 2.0 (0.6) | 3.7 (0.9) | 0.58 | 1.04 | 10.78 |

At the conclusion of the experiment, less than 5% of the originally added *Ulva* was recovered. After the removal of the bags containing the *Ulva*, the sediment surface in the PC and the UT did not show any visible differences.

### *2.3.4 Effect of Ulva on ecosystem function*

Ecosystem functions of benthic primary production and nutrient regeneration showed little significant variation between the PC and UT plots (Table 2.6; Figures 2.3A, B and C). At both sampling times, the PC plots were less productive compared to UT plots, when chl *a* was accounted for (i.e. $GPP_{chl\ a}$), however this result was only significant on d14 (Table 2.6; Figure 2.3B). This was the only significant treatment effect recorded for all the measures of ecosystem function.

Nutrient fluxes were variable, and although $NH_4^+$ fluxes were not significantly different between treatments (Table 2.6), some trends still emerged. In the light chambers, there was a positive efflux from the sediment into the water column in the PC plots, while the $NH_4^+$ flux was negative (influx into the sediment) in the UT plots at both sampling times (Figure 2.3C). In the dark chambers, the $NH_4^+$ flux was positive (efflux), regardless of treatment or sampling time (Figure 2.3C). A multivariate measure of ecosystem function indicated possible treatment effects ($p$(perm) = 0.1), however these results were not significant.

Temporally, significantly lower NPP and GPP were measured on d1 compared to d14 (Table 2.6; Figure 2.3A), which coincided with lower light levels (Table 2.1). Interestingly, the metabolic demand of the system (SOC), did not vary temporally (Table 2.6; Figure 2.3A). The high variability in the measured fluxes of $NH_4^+$ resulted in the absence of significant temporal variability (Table 2.5), although there was an overall higher efflux of $NH_4^+$ on d1 compared to d14 in dark chambers at both the PC and UT plots (Figure 2.3C).

**Table 2.6.** Summary of repeated measures PERMANOVA results of univariate and multivariate measures of ecosystem function, as a function of time (d1, d14), and treatment (PC, UT). Significant effects ($p$(perm) < 0.05) are indicated in bold, with pair-wise tests indicating where the significant differences occurred (n = 12).

| Variable | Source | df | MS | Pseudo-F | $p$(perm) | Pair-wise tests |
|---|---|---|---|---|---|---|
| NPP | Time | 1 | 5.3 E+07 | 164.00 | **0.0001** | d1 < d14 |
| (µmol $O_2$ $m^{-2}h^{-1}$) | Treatment | 1 | 5.4 E+05 | 0.55 | 0.46 | |
| | Time x Treatment | 1 | 2.9 E+05 | 0.91 | 0.35 | |
| | Plot (treatment) | 22 | 9.6 E+05 | 3.05 | **0.006** | |
| | Residual | 22 | 3.2 E+05 | | | |
| SOC | Time | 1 | 49129 | 2.36 | 0.14 | |
| (µmol $O_2$ $m^{-2}h^{-1}$) | Treatment | 1 | 36796 | 1.04 | 0.32 | |
| | Time x Treatment | 1 | 11166 | 0.54 | 0.47 | |
| | Plot (treatment) | 22 | 35234 | 1.69 | 0.11 | |
| | Residual | 22 | 20859 | | | |
| GPP | Time | 1 | 5.0 E+07 | 169.85 | **0.0001** | d1 < d14 |
| (µmol $O_2$ $m^{-2}h^{-1}$) | Treatment | 1 | 2.9 E+05 | 0.26 | 0.61 | |
| | Time x Treatment | 1 | 1.9 E+05 | 0.65 | 0.44 | |
| | Plot (treatment) | 22 | 1.1 E+06 | 3.90 | **0.001** | |
| | Residual | 22 | 2.9 E+05 | | | |
| $GPP_{chl\ a}$ | Time | 1 | 3.3 E+06 | 113.24 | **0.0001** | d1: PC = UT |
| (µmol $O_2$ $m^{-2}h^{-1}$) | Treatment | 1 | 5.8 E+05 | 9.85 | **0.006** | d14: PC < UT |
| | Time x Treatment | 1 | 2.0 E+05 | 6.89 | **0.01** | PC: d1 < d14 |
| | Plot (treatment) | 22 | 59358 | 2.07 | **0.05** | UT: d1 < d14 |
| | Residual | 22 | 28709 | | | |
| Gross $NH_4^+$ | Time | 1 | 594.6 | 1.44 | 0.24 | |
| (µmol N $m^{-2}h^{-1}$) | Treatment | 1 | 0.1 | 0.0002 | 0.99 | |
| | Time x Treatment | 1 | 217.9 | 0.53 | 0.48 | |
| | Plot (treatment) | 22 | 559.3 | 1.35 | 0.23 | |
| | Residual | 22 | 413.5 | | | |
| Net $NH_4^+$ | Time | 1 | 0.2 | 0.001 | 0.97 | |
| (µmol N $m^{-2}h^{-1}$) | Treatment | 1 | 603.8 | 2.39 | 0.13 | |
| | Time x Treatment | 1 | 23.9 | 0.12 | 0.73 | |
| | Plot (treatment) | 22 | 252.8 | 1.29 | 0.28 | |
| | Residual | 22 | 196.4 | | | |
| Ecosystem | Time | 1 | 58.7 | 20.26 | **0.0001** | d1 ≠ d14 |
| function | Treatment | 1 | 8.9 | 1.94 | 0.10 | |
| (multivariate) | Time x Treatment | 1 | 2.7 | 0.93 | 0.45 | |
| | Plot (treatment) | 22 | 4.6 | 1.58 | **0.02** | |
| | Residual | 22 | 2.9 | | | |

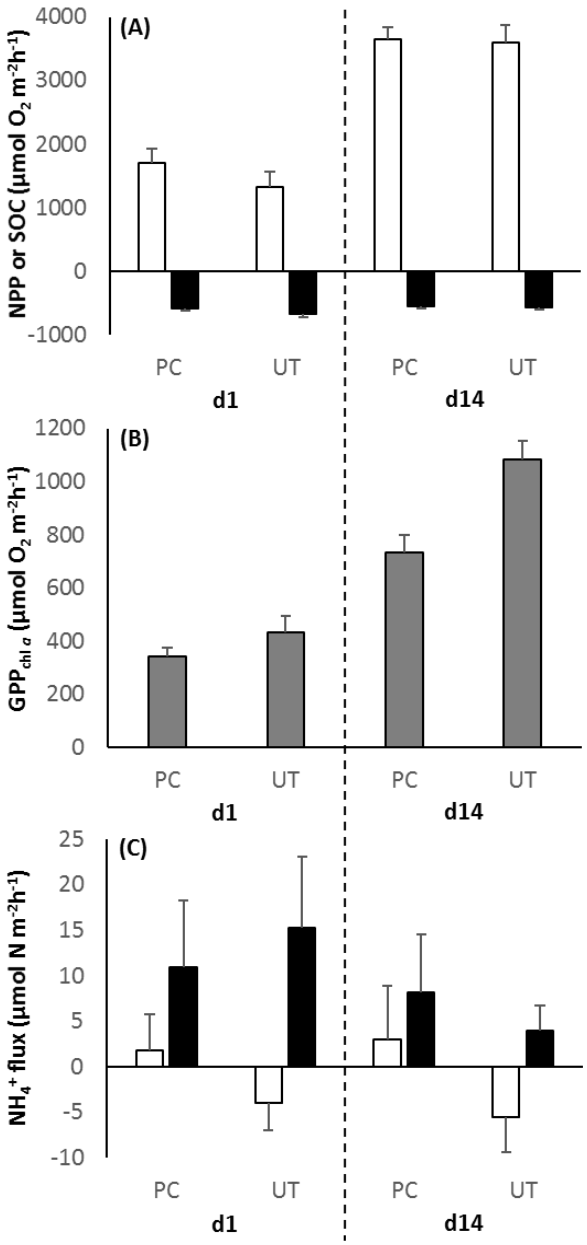


**Figure 2.3.** Solute fluxes (mean ± SE, n=12) as a function of treatment (PC and UT) and through time (d1, d14). (A) Net primary production (NPP from the light chambers, represented by the white bars) and sediment oxygen consumption (SOC from the dark chambers, represented by the black bars); (B) Gross primary production corrected for chl *a* biomass ($GPP_{chl\ a}$) and (C) $NH_4^+$ flux (where the white bars represent net $NH_4^+$ flux in the light chambers, and the black bars represent gross $NH_4^+$ flux measured in the dark chambers). Positive values indicate a flux from the sediment, whilst negative value indicate an uptake by the sediment.

## 2.4 Discussion

In this study, I examined the effects of macroalgal mats on the community composition, the ecosystem function and the sediment characteristics associated

with an intertidal macrofaunal community. I further examined the changes and recovery of the community and the ecosystem functions through time. Overall, impacts of *Ulva* were observed in the macrofaunal community and sediment characteristics, which related to subtle shifts in gross primary production, which served as a proxy for ecosystem function. As the system recovered over time, temporal variation was observed in most of the sediment properties, as well as in NPP and GPP.

Sediment properties were impacted by the *Ulva* treatments, with significant differences in the multivariate sediment characteristics. Most notably, MPB activity (measured as chl *a*) was significantly reduced under the *Ulva* treatment. This was most likely due to shading effects, which have been shown to reduce light conditions under algal mats (Sundbäck & McGlathery, 2005; Thrush et al., 2014). The reduction in available light in turn inhibit photosynthetic processes, hereby reducing MPB biomass (García-Robledo & Corzo, 2011). Although the composition of MPB was not differentiated in this study, other studies have reported shifts from a diatom dominated autotrophic to a cyanobacteria dominated heterotrophic MPB community under *Ulva* mats (García-Robledo et al., 2008; García-Robledo & Corzo, 2011). Reductions or shifts in MPB communities can have significant impacts on benthic communities, as they provide a significant food and energy source. The multivariate sediment characteristics further varied between the d1 and d14 post removal sampling dates in the UT plots, which was likely driven by the increase in OM from the d1 to the d14 sample date. It was expected that the OM loading would be the highest immediately following the removal of the mats, however, estuaries are dynamic and fast changing environments, and it is possible that the OM measured at d14 was unrelated to my experiment. Moreover, although significant, the differences in OM were relatively low (< 1%), suggesting either that a subtle difference in OM will have a strong impact on MPB, or, more likely, that MPB are driving the differences in sediment properties.

Species abundance and richness remained unaffected by the *Ulva* treatments, however there was a significant shift in community composition between the PC and UT plots. A shift in the abundance of key or dominant species has been

shown to be important drivers of metabolic processes such as respiration and $NH_4^+$ excretion (Banse, 1982; Norkko & Bonsdorf 1996b; Levin et al., 2001; Brown et al., 2004; Gammal et al., 2017). In this study the two size dominant bivalves (*A. stutchburyi and M. liliana*), which are known key contributors to ecosystem function in estuaries in New Zealand (Thrush et al., 2014), were not affected by the *Ulva* treatments. It is therefore not surprising that more dramatic changes in ecosystem functions of primary production, benthic metabolism and nutrient regeneration were not observed under the *Ulva* treatments, since notable changes in ecosystem functions are likely to be driven by *A. stutchburyi and M. liliana*. Although these key species were not impacted by the *Ulva* treatments, subtle shifts in other benthic macrofauna were observed.

The family of predator/scavengers Nereididae polychaetes was significantly impacted by the *Ulva* treatments, with lower abundances recorded in the UT compared to the PC plots. Nereididae are highly mobile, and have the ability to escape the *Ulva* mats by relocating to an area that was not affected. Studies from the Baltic Sea, have shown mobile macrofauna tend to move away when faced with macroalgal disturbances (Norkko & Bonsdorff, 1996c). Although the methods in this study could have been modified to limit macrofauna from freely moving away from the disturbance (e.g. cages), the aim of this research was to establish the impact of these mats, which often occur in patches that mimic the experimental treatments, on naturally occurring communities. Another species that was negatively impacted (although the result was not significant) was *Lasaea parengaensis*. *Lasaea parengaensis* is a shallow living (top 2cm) suspension feeder that has limited mobility, and likely lost its ability to effectively suspension feed under the mats. Conversely, macrofaunal species can also take advantage of the additional available organic matter (Thrush, 1986; Norkko & Bonsdorf, 1996b). These drifting mats can provide a relevant source of food, and mobile species can take advantage of the food source while avoiding any hypoxia that may ensue (Kautsky, 1995; Green & Fong, 2016). In this study, the abundances of the deposit feeders *Prionospio aucklandica*, *Scoloplos cylindifera* and *Aonides trifida* increased under the *Ulva* treatments, although these results were not significant. Mats have been shown to support higher abundances of surface deposit feeders (Green & Fong, 2016), especially under macroalgal densities of <

3 kg ww $m^{-2}$ (Hull, 1987). Surface deposit feeders in turn provide an important food source for predatory infauna, fish and shorebirds (MacKenzie, 2005; Powers et al., 2005), which highlights the bottom up effects macroalgal mats can have on food webs.

The changes in individual species abundance and community composition with the addition of *Ulva* largely did not translate into significant impacts on ecosystem functions, which suggests that the ecosystem continued to function normally under the mats. Ecosystem function variables were only significantly impacted temporally, although the temporal variation seen in primary productivity (measured in the light chambers) was likely driven by better light conditions at d14 compared to d1. Nutrient fluxes were highly variable, and although no significant temporal or treatment effects were measured, the trends observed suggest that nutrient regeneration was subtly impacted by the *Ulva* addition. The release of $NH_4^+$ was reduced in the light compared to the dark chambers, suggesting remineralisation and uptake of $NH_4^+$ by primary producers such as MPB, and highlighting the importance of nutrient regeneration during primary production (Blackburn, 1986). Furthermore, in the light chambers, the flux of $NH_4^+$ was negative at both sampling dates in the UT plots, whereas the PC plots showed a positive flux. These trends were reflected in $GPP_{chl\ a}$, which was higher in the UT compared to the PC plots at both sampling dates. Nitrogen is a limiting nutrient in marine environments (Howarth & Marino, 2006) and these results suggest that in the UT plots, where $GPP_{chl\ a}$ was higher, the demand for nitrogen is greater than the amount remineralised.

In previous studies, severe effects of macroalgal mats were observed after 16d of algal cover (e.g. Norkko & Bonsdorf, 1996b, c). It was therefore expected that the algal cover of 30d in my study would induce hypoxia and a notable shift in both macrofauna and ecosystem functions such as primary production, benthic metabolism and nutrient regeneration. The absence of a significant $NH_4^+$ efflux from the sediment, and other visual cues (blackened sediment) suggests that hypoxia was not achieved under the treatments (Childs et al., 2002), possibly due to the dynamic nature of the system and the constant flushing of the tides. Despite a reduction in the MPB biomass, there was no significant reduction in primary

production (NPP or GPP) under the *Ulva* treatments. To the contrary, GPP that was corrected for chl *a* biomass ($GPP_{chl\ a}$) was higher in UT compared to PC plots at d14. The higher productivity in treatment plots may be a function of the shift in the community composition that was observed between the UT and PC plots, or represent the availability of nutrients, as is shown by the influx of $NH_4^+$ into the sediment in the light chambers. Others have shown a negative relationship between $GPP_{chl\ a}$ and increased sediment mud content (Pratt et al., 2014), however in my study, the % mud content was unchanged between treatments. My results do however suggest that the mats have the ability to impact or change both the physical and biogeochemical environments to varying degrees.

Large blooms often occur during spring and summer, which coincides with the recruitment and settling periods of macrofauna, and can make community recovery more difficult (Norkko & Bonsdorf, 1996c). This study showed that while some macrofauna were negatively impacted by the *Ulva* treatment, the system as a whole continued to function normally as key species, which are most likely to significantly contribute to the overall ecosystem function, remained unaffected by the *Ulva* treatment. Patches of drifting mats are often found within Tauranga Harbour (Park, 2011). As a result, it is possible that this system has already lost the species which are sensitive to this type of disturbance, or that the species have become resilient to these types of disturbances, allowing for a more robust response against similar disturbances. This type of ecosystem resilience has been shown in previous studies (e.g. Kube & Powilleit, 1997; Rabalais et al., 2001). Although the plot sizes here were comparable to other studies (e.g. Everett, 1994; Green & Fong, 2016), the transport and movement of both sediment and macrofauna in dynamic systems like estuaries can be vast, and extend beyond the plot boundaries, which may have reduced treatment effects (e.g. Emerson & Grant, 1991; Lundquist et al., 2006; Sandwell et al., 2009). The nature and scale of the disturbance mimicked here was likely not severe enough given the dynamic nature of sandflats in New Zealand and the transport processes which dominate these environments.

## 2.5 Conclusions

This study examined the initial impact and subsequent recovery of a New Zealand soft sediment community following a macroalgal disturbance, and examined how these transient disturbances influence ecosystem functions such as primary production, metabolic respiration and nutrient regeneration. The *Ulva* mats, at the density I added, did not have significant impacts on the key macrofauna or the ecosystem functions I measured, however, a subtle shift in the benthic community was measured. The overall function of this particular ecosystem appeared to be robust and more difficult to shift. In the literature, a shift in community composition often assumes a shift in ecosystem function (Marinelli & Williams, 2003; Lohrer et al., 2004; Thrush et al., 2006; Sandwell et al., 2009; Jones et al., 2011), however, the results from this study propose that this is not always the case. Furthermore, temporal changes, largely governed by environmental conditions such as light availability, seems a more prominent driver in the differences observed in ecosystem function compared to the treatment effects of *Ulva* addition. My results suggest that, providing abundances of key species remain intact, the community composition can shift, and individual species can be lost, without a loss or shift in overall ecosystem function.

# 3.0 CHAPTER THREE: EFFECTS OF DETRITAL ENRICHMENT ON INTERTIDAL BENTHIC BIODIVERSITY AND ECOSYSTEM FUNCTION

## 3.1 Introduction

A common natural disturbance that affects the biodiversity of coastal benthic communities is drifting mats of macroalgae (e.g. Hull, 1987; Norkko & Bonsdorf, 1996b; Kelaher & Levinton, 2003; Olabarria et al., 2010). As macroalgae is carried by the tides and wind, it is deposited on the substrate in patches, or mats, which vary in density and biomass (Olabarria et al., 2010). When conditions for growth become less suitable, these macroalgal mats (wrack) will start to decompose and become detritus. During the decomposition phase, the decaying macroalgae may stimulate aerobic and anaerobic bacterial growth (Raffaelli et al., 1998), and through the release of nutrients, can enhance the growth of microalgal (microphytobenthos [MPB] and other primary producers) (Posey et al., 1999). Consequently, the increase of detritus, MPB and primary producers can be beneficial to grazers and deposit feeders, and increase the densities and biomasses of these trophic groups (Hull, 1987; Ford et al., 1999; Rossi & Underwood, 2002; Kelaher & Levinton, 2003). Conversely, high densities of decomposing detritus can cause anoxic conditions and sulphide production in the sediments (Nedergaard et al., 2002), which results in macrofaunal mortality and an overall decrease in biodiversity (Olabarria et al., 2010).

Changes in community composition and the loss of biodiversity can have significant impacts on ecosystem stability, which in turn, has social, environmental and economic consequences (Grime, 1997; McCann, 2000; Worm et al., 2006). Different species can contribute to similar ecosystem functions or have similar functional roles or traits, and can therefore compensate and perform the function of another species in that group should it be displaced as a result of disturbances. This is termed functional redundancy (Walker, 1992; Downing & Leibold, 2010). In estuarine-marine systems, however, a single species can often be the sole representative of a particular functional group. These species are considered 'key' contributors to ecosystem functions, and their demise can have catastrophic impacts on the health of the entire ecosystem (Bolam et al., 2002; Solan et al., 2004). As the impact on the macrofaunal biodiversity appears to be dependent on the amount of detritus that is being added to the sediment (Raffaelli, 2000; Bishop & Kelaher, 2007), it is important to understand the relationship between macrofauna and varying quantities of macroalgal detritus. Thus, I predict that the impact of the detritus on a system is largely dependent on the amount of wrack (biomass) that is added to the system.

A prominent macroalgal species that is found worldwide and that form excessive nuisance blooms is *Ulva* (Guidone & Thornber, 2013). The decomposition rate of *Ulva* has been reported to be weeks to months (e.g. Buchsbaum et al., 1991; Nedergaard et al., 2002; Rossi, 2007; Olabarria et al., 2007; Urban-Malinga et al., 2008) and therefore the impacts of the detritus on the macrofauna and the ecosystem services they provide are likely to vary with time. For example, it is possible that the initial breakdown of the macroalgae may induce hypoxia, which results in a shift in the macrofaunal community, as species are lost from the system. However, as the initial impacts subside, it is likely that some species may reclaim their position in the community. Despite the temporal changes that are likely to occur, most studies that have examined benthic community response to macroalgae detritus only focus on one or two fixed points in time (e.g. Rossi, 2006, 2007; Olabarria et al., 2010), whilst the ability to quantify temporal variation in the wider ecosystem function framework following macroalgal detrital additions has been completely overlooked. Gladstone-Gallagher et al. (2016) examined the temporal variation in macrofauna and ecosystem function

with the addition of three different types of detritus (mangrove, seagrass and kelp), however they did not investigate variations in the quantity (i.e. biomass) of detritus added to the system. In this study, I aim to quantify, for the first time, the density dependent effects of macroalgal detritus on benthic macrofauna and ecosystem function through time.

Benthic primary production, community respiration, and nutrient regeneration are considered good indicators of ecosystem functioning in soft-sediment systems (Lohrer et al., 2004, 2010; Hewitt et al., 2006; Thrush et al., 2006; Norling et al., 2007). Variations in these ecosystem functions are the result of multiple and complex interactions between bacteria, microphytes and macrofauna. Rates of benthic primary production and community respiration will ultimately specify whether a system is net autotrophic (oxygen producing) or heterotrophic (oxygen consuming) (Lohrer et al., 2010). Measuring the nutrient fluxes across the sediment-water interface provides an understanding of the bottom-up drivers that fuels primary production in the system (Lohrer et al., 2010). Ecosystem function is also an important indicator of the recovery of the ecosystem following changes to physical conditions, macrofaunal assemblages, or in this case, organic loading. Previous studies that have investigated the impact of organic loading on estuarine ecosystems have primarily examined the changes in macrofauna and sediment characteristics (e.g. Rossi, 2006; Olabarria et al., 2010; Taylor et al., 2010; Gladstone-Gallagher et al., 2014), but far fewer have linked shifts in the macrofaunal community to shifts in the overall functioning of the ecosystem (e.g. Karlson et al., 2010; Rossi et al., 2013; Gladstone-Gallagher et al., 2016). Functional redundancy is common within complex systems (Walker, 1992; Lawton, 1994), and it is therefore possible that a shift in macrofaunal communities will not always translate to a shift in ecosystem function.

In this study, I added three different quantities of *Ulva* detritus to the sediments of a sheltered intertidal sandflat and measured the impacts of these additions on the macrofaunal community and ecosystem function (i.e. primary production, metabolic respiration and nutrient regeneration) through time. I predicted that; (1) the macrofaunal community and ecosystem functions will vary between the different detrital treatments; and (2) that the impact of the different detrital loads

would be most obvious on the first sample date, with impacts becoming less obvious over time, as the system recovers.

## 3.2 Methods

### *3.2.1 Study site and experimental design*

The study was conducted on a sheltered mid-intertidal flat located at Tuapiro Point, in the northern part of Tauranga Harbour, on the east coast of New Zealand (S 37° 29.374, E 175°57.04, see Figure 1.2). The site is characterised by semi-diurnal tides, with an immersion period of approximately 8 h. A manipulative field experiment was carried out over an 8-week period between March - May 2012, which coincided with the end of the bloom season when *Ulva* biomass is on the decline (Park, 2011). The duration of the experiment was comparable to previous studies which indicated that the effects of macroalgal additions on macrofaunal assemblages are visible after 2 - 10 weeks (Thrush, 1986; Hull, 1987; Norkko & Bonsdorff, 1996b, c; Bolam et al., 2000; Rossi, 2006; Rossi et al., 2013), with peak decomposition occurring between 4 - 6 weeks (Buchsbaum et al., 1991; Nedergaard et al., 2002; Nielsen et al., 2004; Rossi, 2006; Olabarria et al., 2007).

*Ulva* was collected at low tide from the nearby Athenree intertidal area (S 37° 26.966, E 175° 58.152), dried at 60°C overnight, and made into detritus by shredding the dried *Ulva* sheets into pieces approximately 2 cm in diameter. The *Ulva* detritus was then placed back into the freezer until the start of the experiment. A 40 × 18 m area was established at the study site, and twenty-five 1 $m^2$ plots were randomly selected. Plots were marked with four tent pegs, which made them easy to relocate during subsequent sampling. Adjacent plots were at least 2 m apart. *Ulva* treatments comprised of detritus added to the sediment and hand churned into the top 2 cm. The detrital addition treatments each consisted of five randomly assigned replicates (n = 5) of; (1) low (60 g dw $m^{-2}$) (L); (2) medium (120 g dw $m^{-2}$) (M); and (3) high (240 g dw $m^{-2}$) (H) additions; (4) a procedural control (PC), where the sediment was churned but no *Ulva* was added;

and (5) ambient sediment, where the plots were left untouched (see Figure 3.1). The ambient plots were established to ensure that the process of mixing the detritus into the sediment did not create a noticeable disturbance which could impact on the results. The levels of addition were selected to correspond to naturally occurring *Ulva* biomass reported locally and internationally during mild to severe blooms (Busing, 1999; Kelaher & Levinton, 2003; Rossi, 2006, 2007; Olabarria et al., 2010; Park, 2011). Macrofaunal biomass at the sites was dominated by two bivalves; the suspension feeder *Austrovenus stutchburyi* and the deposit feeder *Macomona liliana*. For a species list of macrofauna found at this site see Appendix 1.

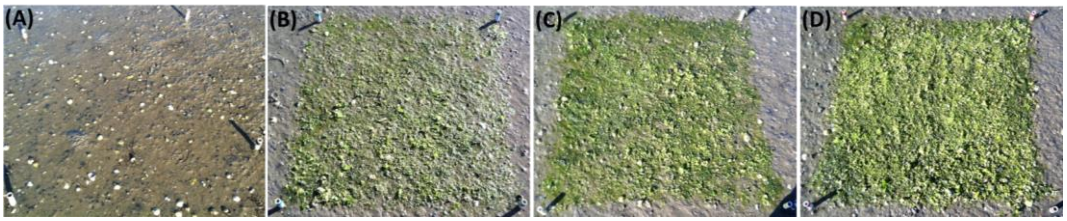


**Figure 3.1.** Experimental set up showing the (A) procedural control (PC), (B) low (60 g dw $m^{-2}$), (C) medium (120 g dw $m^{-2}$), and (D) high (240 g dw $m^{-2}$) (H) additions treatments prior to hand churning the sediment.

All plots were sampled 2, 4 and 8 weeks post *Ulva* addition, hereafter W2, W4, and W8 respectively, to ascertain temporal changes in macrofaunal biodiversity, ecosystem function and sediment properties. The sampling times were selected to be comparable with sampling periods used in previous studies examining the impacts of *Ulva* on macrofaunal assemblages (e.g. Kelaher & Levinton, 2003; Rossi et al., 2013), and to coincide with mid-day high tides in order to optimise the measures of ecosystem function. On each sample date, a different randomly selected quarter of the plot was sampled, and any sediment removed was replaced with clean azoic sand to prevent infilling from the surrounding sediment (e.g. Lohrer et al., 2010). The sampling positions were noted to prevent the re-sampling of those locations. HOBO data loggers were also deployed at the start of each sampling day at five randomly selected sites to collect ancillary data on bottom water temperature and light availability during the incubation period, as these factors can vary greatly and impact biological reactions and solute changes (Zlotnik & Dubinsky, 1989)

### *3.2.2 Field sampling*

On each of the three sampling dates, dissolved oxygen and nutrient fluxes were measured in two paired benthic chambers (one light and one dark) in each of the 25 experimental plots to quantify measures of primary production, benthic respiration and nutrient regeneration, according to the methods of Lohrer et al. (2010). One light and one dark chamber was placed in each plot on an incoming tide between 10:00 and 11:00 a.m., and once the water was deep enough to completely cover each chamber (approximately 20 cm). The chambers were pushed 2 cm into the sediment to ensure that the water within the chamber was contained. Each benthic chamber covered an area of 0.016 $m^2$, and trapped 0.85 L of seawater in the chamber above the sediment-water interface. The chambers had two ports, allowing water to re-enter the chamber (through the inlet port) as water was removed from the chamber via the sampling port.

Once the chambers were in place, they were left to incubate for approximately 4 to 5 h over a mid-day high tide. Water samples (60 ml) were collected at the beginning and the end of the incubation period by attaching a screw top syringe to the end of the sampling port, and drawing water into the syringe. Prior to the initial sample being collected, 20 ml water was drawn and discarded to flush any water that may have been contained in the tubing. I also assessed ambient water column oxygen and nutrient concentrations by placing a light and a dark bottle, each containing 1.5 L of ambient seawater, in the water column directly above the chamber at three locations in the experimental site. The light and dark bottles were sampled concurrently with the benthic chambers and used to correct the chamber solute fluxes for water column processes (usually < 5% of the measured sediment-water column fluxes).

Immediately following the collection of water samples, levels of dissolved oxygen (DO) were measured using a calibrated optical probe (RDO, In-Situ Incorporated, Fort Collins, Colorado, USA). Water samples were then filtered (Whatman GF/C grade filter, 1.2 µm pore size), kept on ice in darkness and subsequently frozen until inorganic nutrient analyses could be completed. Once the final water samples were collected, macrofauna were sampled from directly underneath the dark

chamber in each plot using a 13 cm diameter core to a depth of 10 cm. The sediment was sieved on a 500 µm mesh and the contents preserved in 70% isopropyl alcohol for subsequent macrofauna identification and analyses. Furthermore, three smaller cores (3 cm diameter) were taken adjacent to the chambers from each of the 25 treatment plots, to a depth of 2 cm using a 50 ml cut-off syringe, and amalgamated for analysis. The sediment samples were also kept in the dark and on ice (separate from the water samples to avoid contamination), and frozen once back at the laboratory to await further analyses. From these cores, chlorophyll *a* (chl *a*), phaeophytin (phaeo), organic matter (OM), median grain size (GS) and % mud was determined.

### *3.2.3 Laboratory analyses*

In the laboratory, the filtered water samples were analysed for inorganic nutrients (ammonium [$NH_4^+$], nitrite [$NO_2^-$)], nitrate [$NO_3^-$], and phosphorus [$PO_4^{3-}$]), on a Lachat QuickChem 8000 Series FIA, using standard methods for seawater (Grasshoff et al., 2009). For chl *a* and phaeo analyses, the sediment was freeze-dried, homogenised and measured spectrophotometrically before and after treatment with 0.1 N HCl on a Turner 10-AU fluorometer, following the methods of Arar and Collins (1997). The acidification step was used to allow for the extraction of phaeopigments. OM was determined through loss on ignition of a homogenised and dried (110°C for 24 h) subsample of sediment after combustion for 5.5 h at 550°C, as outlined in Dean (1974). GS samples were treated with 10% hydrogen peroxide to remove organic matter and calcareous material, and then analysed using a Malvern Mastersizer-S (300 FR lense, range 0.05 - 2000 µm). The macrofauna samples were stained with Rose Bengal (to aid in the sorting process) and identified to the lowest taxonomic level possible (usually species). Once the macrofauna were extracted from the samples, the remaining material was poured into a saturated sugar solution to separate the less dense detrital material from the heavier particles (shell hash and sediment). The floating matter was decanted into a separate container, and was subsequently dried (at 60°C) and weighed to determine the detritus (dry weight [dw]) remaining in each plot.

### *3.2.4 Flux calculations and statistical analyses*

Dissolved oxygen and nutrient fluxes were measured across the sediment-water interface to quantify measures of ecosystem functions. Fluxes were calculated by subtracting the initial from the final concentrations (after correcting for water column processes) and were standardised for incubation times, chamber water volume and the sediment surface area (Lohrer et al., 2010). In the light chambers, I measured net primary production (NPP) and net ammonium efflux (Net $NH_4^+$), while from the dark chamber fluxes I measured sediment oxygen consumption (SOC, which is indicative of benthic metabolism or respiration) and gross ammonium efflux (Gross $NH_4^+$). Gross primary production (GPP) was calculated by subtracting the SOC from the overall NPP. GPP was also standardised for chl *a* biomass ($GPP_{chl\ a}$) to account for variations in MPB biomass and therefore provide a measure of photosynthetic efficiency. Concentrations of the other measured nutrients (i.e. $NO_2^-$, $NO_3^-$, $PO_4^{3-}$) were below or near the detection limits (< 0.001 µmol $L^{-1}$), and were therefore not considered for further analyses.

Permutational analysis of variance (PERMANOVA) were carried out to ascertain whether there were any differences in the univariate response variables between the PC and ambient plots on the first sampling date. The data was left untransformed. The community structure and sediment properties associated with PC and ambient plots were further examined with a multivariate PERMANOVA using square root transformed macrofauna and normalised sediment data, based on a Bray-Curtis similarity matrices and Euclidean distance, respectively.

As a first step, repeated measures PERMANOVA was used to test for significant effects of detrital treatments through time on untransformed univariate response variables relating to macrofauna (i.e. abundance and richness), sediment properties (i.e. chl *a*, phaeo, OM, GS, % mud), and ecosystem function (i.e. NPP, SOC, GPP, $GPP_{chl\ a}$, Net $NH_4^+$ and Gross $NH_4^+$). A repeated measures PERMANOVA was also carried out on square root transformed multivariate macrofauna data (Bray-Curtis similarity) and normalised multivariate sediment and ecosystem function data (Euclidean distance). The experimental design comprised treatment (4 levels) and time (3 levels), which were both treated as

fixed factors, as well as plot (5 levels) which was a random factor nested within treatment. When significant differences were detected between treatments or through time, post-hoc pairwise tests were used to indicate these differences. Non-metric multidimensional scaling analyses (nMDS) was used on the transformed macrofauna community data to visualise the variation in community composition across different treatments and through time, and SIMPER analysis (Bray-Curtis similarity) was used to ascertain which species were predominantly contributing any differences. Furthermore, the functional roles of the 12 most abundant species were determined to aid in the interpretation of the community data (according to Greenfield et al., 2016). All the statistical analyses were carried out using the PRIMER 7 statistical software program with the PERMANOVA+ add on.

## 3.3 Results

### *3.3.1 Procedural effects vs ambient conditions at W2*

There were no significant differences in the univariate or multivariate response variables for the macrofauna community variables or sediment properties measured between the ambient and PC plots at W2 (all $p$(perm) $\geq$ 0.05) (Table 3.1). Data from the ambient plots were therefore excluded from further analyses.

**Table 3.1.** Mean (± SE) sediment properties and macrofaunal community data in ambient and procedural control (PC) plots at 2 weeks post addition (W2). Significant differences between sites ($p$(perm) < 0.05) are indicated in bold (n = 4).

| Variable | Ambient | PC | *p*(perm) |
|---|---|---|---|
| Median grain size (µm) | 189 (5) | 185 (2) | 0.43 |
| Mud content (%) | 3.1 (1.0) | 3.8 (0.5) | 0.54 |
| Organic content (%) | 1.89 (0.07) | 1.94 (0.11) | 0.65 |
| Chl *a* (µg $g^{-1}$ dw) | 16.6 (1.4) | 16.3 (1.7) | 0.91 |
| Phaeophytin (µg $g^{-1}$ dw) | 6.4 (0.8) | 5.3 (0.7) | 0.38 |
| Multivariate sediment characteristics | - | - | 0.29 |
| Abundance (ind. $core^{-1}$) | 148.8 (24.4) | 188.0 (5.4) | 0.15 |
| Richness (sp. $core^{-1}$) | 16.3 (2.1) | 14.3 (0.6) | 0.48 |
| *Austrovenus stutchburyi* (ind. $core^{-1}$) | 8.5 (2.5) | 12.0 (1.8) | 0.28 |
| *Macomona liliana* (ind. $core^{-1}$) | 8.5 (2.4) | 11.8 (2.3) | 0.34 |
| Multivariate community composition | - | - | 0.88 |

### *3.3.2 Sediment properties and environmental data*

The sediment properties from replicate plots were averaged and presented as a function of treatment and time (Table 3.2). The repeated measure PERMANOVA analyses indicated no significant differences in any of the univariate sediment properties between the treatments ($p$(perm) ≥ 0.78), however median grain size, mud content and phaeopigments varied significantly through time ($p$(perm) ≤ 0.001; Table 3.3).

**Table 3.2.** Mean (± SE) sediment property and macrofaunal community variables as a function of time (2, 4 and 8 weeks post addition), and treatment (PC = no addition, L = 60 g dw $m^{-2}$, M = 120 g dw $m^{-2}$, H = 240 g dw $m^{-2}$ *Ulva* addition).

| Week | Variable | PC | L | M | H |
|---|---|---|---|---|---|
| 2 | Median grain size (µm) | 185 (2) | 186(4) | 187 (6) | 186 (4) |
| | Mud content (%) | 3.8 (0.5) | 3.1 (0.5) | 3.3 (0.7) | 3.0 (0.3) |
| | Organic content (%) | 1.94 (0.11) | 1.91 (0.11) | 1.88 (0.14) | 1.82 (0.07) |
| | Chl *a* (µg $g^{-1}$ dw) | 16.3 (1.7) | 14.5 (0.5) | 15.9 (0.8) | 14.6 (1.5) |
| | Phaeopigment (µg $g^{-1}$ dw) | 5.3 (0.7) | 4.7 (0.1) | 4.8 (0.4) | 6.0 (1.1) |
| | Amount of detritus (g dw $core^{-1}$) | 1.41 (0.18) | 1.35(0.20) | 1.40 (0.15) | 1.34 (0.20) |
| | Macrofauna abundance (ind. $core^{-1}$) | 188.0 (5.4) | 161.0 (19.5) | 181.6 (13.7) | 172.8 (16.1) |
| | Macrofauna richness (sp. $core^{-1}$) | 14.3 (0.6) | 13.3 (0.9) | 13.4 (0.9) | 13.8 (0.7) |
| 4 | Median grain size (µm) | 191 (4) | 193 (6) | 194 (4) | 192 (3) |
| | Mud content (%) | 5.7 (0.7) | 5.9 (1.5) | 5.2 (0.9) | 5.5 (0.4) |
| | Organic content (%) | 1.84 (0.05) | 1.83 (0.07) | 1.88 (0.08) | 1.76 (0.08) |
| | Chl *a* (µg $g^{-1}$ dw) | 16.6 (1.3) | 16.2 (1.8) | 17.3 (1.3) | 15.2 (1.2) |
| | Phaeopigment (µg $g^{-1}$ dw) | 7.7 (0.6) | 8.6 (0.4) | 7.6 (0.7) | 7.3 (0.5) |
| | Amount of detritus (g dw $core^{-1}$) | 0.75 (0.16) | 1.18 (0.12) | 0.95 (0.20) | 0.86 (0.07) |
| | Macrofauna abundance (ind. $core^{-1}$) | 167.3 (17.2) | 154.0 (14.6) | 164.0 (9.2) | 135.4 (14.6) |
| | Macrofauna richness (sp. $core^{-1}$) | 18.3 (1.9) | 16.7 (0.7) | 14.8 (1.3) | 13.2 (1.4) |
| 8 | Median grain size (µm) | 191 (5) | 187 (6) | 187 (10) | 190 (4) |
| | Mud content (%) | 5.9 (1.0) | 7.1 (1.4) | 7.5 (2.0) | 5.7 (0.6) |
| | Organic content (%) | 2.05 (0.16) | 1.95 (0.24) | 1.76 (0.11) | 1.88 (0.07) |
| | Chl *a* (µg $g^{-1}$ dw) | 14.2 (2.4) | 14.3 (1.9) | 13.4 (1.5) | 15.6 (1.0) |
| | Phaeopigment (µg $g^{-1}$ dw) | 9.9 (2.2) | 12.4 (3.8) | 10.8 (1.5) | 9.0 (2.4) |
| | Amount of detritus (g dw $core^{-1}$) | 1.16 (0.19) | 1.49 (0.30) | 1.50 (0.45) | 1.30 (0.30) |
| | Macrofauna abundance (ind. $core^{-1}$) | 143.0 (14.5) | 175.7 (22.1) | 160.7 (19.1) | 170.0 (11.7) |
| | Macrofauna richness (sp. $core^{-1}$) | 14.8 (0.5) | 13.7 (0.7) | 15.0 (1.5) | 15.4 (1.7) |

Pair-wise tests showed a significant increase in the mud content and phaeopigments between 2 to 4 weeks post addition, and 2 to 8 weeks post addition. Median grain size significantly increased between 2 to 4 weeks post addition (Table 3.3). A multivariate analysis confirmed a significant difference in the overall sediment properties as a function of time, however there were again no treatment effects (Table 3.3). The amount of detritus recovered from the sediment did not vary between treatments, but did vary over time, with more detritus recovered across all the treatments at W2 and W8 compared to W4.

**Table 3.3.** Summary of repeated measures PERMANOVA results of univariate sediment properties and macrofaunal abundance and richness, as well as multivariate sediment and macrofaunal data, as a function of time (2, 4 and 8 weeks post addition), and treatment (PC = no addition, L = 60 g dw $m^{-2}$, M = 120 g dw $m^{-2}$, H = 240 g dw $m^{-2}$ *Ulva* addition). Significant effects ($p$(perm) < 0.05) are indicated in bold, with pair-wise tests indicating where the significant differences occurred (n = 5).

| Variable | Source | df | MS | Pseudo-F | *p*(perm) | Pair-wise tests |
|---|---|---|---|---|---|---|
| Median | Time x Treatment | 6 | 12.5 | 0.48 | 0.82 | |
| GS | Time | 2 | 201.8 | 7.73 | **0.001** | W2 < W4, W2 = W8, W4 = W8 |
| (µm) | Treatment | 3 | 4.5 | 0.02 | 0.99 | |
| | Plot (treatment) | 16 | 299.8 | 11.48 | **0.0001** | |
| | Residual | 32 | 26.1 | | | |
| Mud | Time x Treatment | 6 | 1.4 | 0.62 | 0.72 | |
| content | Time | 2 | 53.5 | 24.17 | **0.0001** | W2 < W4, W2 < W8, W4 = W8 |
| (%) | Treatment | 3 | 1.2 | 0.14 | 0.93 | |
| | Plot (treatment) | 16 | 8.9 | 4.00 | **0.001** | |
| | Residual | 32 | 2.2 | | | |
| Organic | Time x Treatment | 6 | 0.03 | 0.69 | 0.67 | |
| matter | Time | 2 | 0.04 | 1.00 | 0.39 | |
| (%) | Treatment | 3 | 0.1 | 0.39 | 0.78 | |
| | Plot (treatment) | 16 | 0.1 | 3.44 | **0.001** | |
| | Residual | 32 | 0.04 | | | |
| Chl *a* | Time x Treatment | 6 | 5.0 | 0.69 | 0.65 | |
| (µg $g^{-1}$ dw) | Time | 2 | 18.6 | 2.56 | 0.09 | |
| | Treatment | 3 | 1.6 | 0.09 | 0.97 | |
| | Plot (treatment) | 16 | 18.5 | 2.55 | **0.01** | |
| | Residual | 32 | 7.3 | | | |
| Phaeo- | Time x Treatment | 6 | 5.1 | 0.43 | 0.85 | |
| pigment | Time | 2 | 142.0 | 11.90 | **0.0002** | W2 < W4, W2 < W8, W4 = W8 |
| (µg $g^{-1}$ dw) | Treatment | 3 | 3.6 | 0.26 | 0.84 | |
| | Plot (treatment) | 16 | 13.6 | 1.15 | 0.35 | |
| | Residual | 32 | 11.9 | | | |
| Sediment | Time x Treatment | 6 | 1.6 | 0.59 | 0.92 | |
| properties | Time | 2 | 23.0 | 8.43 | **0.0001** | W2 ≠ W4, W2 ≠ W8, W4 ≠ W8 |
| (multivariate) | Treatment | 3 | 1.5 | 0.16 | 0.98 | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Plot (treatment) | 16 | 9.2 | 3.39 | **0.0001** | |
| | Residual | 32 | 2.7 | | | |
| Amount of | Time x Treatment | 6 | 0.1 | 0.39 | 0.88 | |
| Detritus | Time | 2 | 1.3 | 7.13 | **0.002** | W2 > W4, W2 = W8, W4 < W8 |
| (g dw core$^{-1}$) | Treatment | 3 | 0.2 | 0.38 | 0.78 | |
| | Plot (treatment) | 16 | 0.4 | 2.53 | 0.01 | |
| | Residual | 32 | 0.2 | | | |
| Macrofauna | Time x Treatment | 6 | 91.0 | 0.98 | 0.47 | |
| abundance | Time | 2 | 150.1 | 1.61 | 0.22 | |
| (ind. core$^{-1}$) | Treatment | 3 | 15.0 | 0.17 | 0.92 | |
| | Plot (treatment) | 16 | 82.3 | 0.88 | 0.59 | |
| | Residual | 32 | 93.4 | | | |
| Macrofauna | Time x Treatment | 6 | 84.6 | 1.04 | 0.42 | |
| richness | Time | 2 | 125.4 | 1.56 | 0.22 | |
| (sp. core$^{-1}$) | Treatment | 3 | 53.2 | 0.96 | 0.44 | |
| | Plot (treatment) | 16 | 52.3 | 0.64 | 0.81 | |
| | Residual | 32 | 80.1 | | | |
| Macrofauna | Time x Treatment | 6 | 306.6 | 1.21 | 0.19 | |
| community | Time | 2 | 659.2 | 2.72 | **0.007** | W2 ≠ W4, W2 ≠ W8, W4 ≠ W8 |
| (multivariate) | Treatment | 3 | 306.8 | 0.53 | 0.87 | |
| | Plot (treatment) | 16 | 702.1 | 2.81 | **0.0001** | |
| | Residual | 32 | 247.3 | | | |

Light levels and temperature also decreased steadily over time, while salinity was variable (Table 3.4).

**Table 3.4.** Ambient light, temperature and salinity at the sediment-water interface during benthic flux measurements. Light and temperature measurements are presented as means (± SE; n = 5 loggers) during the incubation period, while salinity was measured only once (at the start of each incubation period.

| Time | Light (Lux) | Temperature (°C) | Salinity |
|---|---|---|---|
| W2 | 46908 (712) | 21.2 (0.06) | 23.7 |
| W4 | 27600 (812) | 20.3 (0.03) | 21.5 |
| W8 | 17243 (468) | 16.9 (0.03) | 27.6 |

### *3.3.3 Macrofaunal community response*

Species abundance and richness did not vary significantly between treatments or through time ($p$(perm) ≥ 0.22; Table 3.3). Multivariate community composition, however, did vary significantly over the three sampling dates, although it did not vary significantly between treatments (Table 3.3; Figures 3.2A and B).

A total of 8163 individuals from 43 different species were collected from all the treatments across the three sampling dates. Twelve macrofaunal species from two groups (bivalves and polychaetes) comprised 95% of the abundance (Table 3.5). The numerically dominant macrofauna were the bivalves *Austrovenus stutchburyi*, *Macomona liliana*, *Lasaea* sp. and *Nucula hartvigiana*, and the polychaetes *Prionospio aucklandica*, *Aonides trifida*, *Heteromastus filiformis*, *Microspio maori*, *Paradoneis lyra*, *Scoloplos cylindrifer*, *Oligochaeta* and Nereididae.

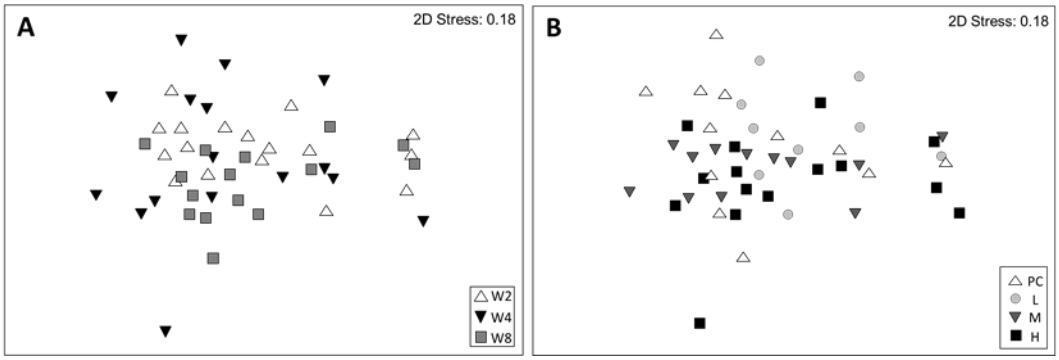


**Figure 3.2.** Non-metric MDS ordination plots of square-root transformed macrofaunal community data. Ordination plots (Bray-Curtis similarity) show community composition as a function of (A) time (2, 4 and 8 weeks post detrital addition), and (B) treatment (PC = no addition, L = 60 g dw $m^{-2}$, M = 120 g dw $m^{-2}$, H = 240 g dw $m^{-2}$ *Ulva* addition).

**Table 3.5.** Functional attributes and total abundance (# ind.) of the 12 most abundant macrofaunal species (which comprise 95% of the total abundance) based on adult body size (small < 5, medium 5 – 20 and large > 20 mm), motility within sediment (limited or freely), directional sediment particle movement (SPM) (SS = surface to surface; DD = depth to depth; SD = surface to depth; DS = depth to surface), and feeding mode (FM) (Sus = suspension feeder; Dep = deposit feeder; Pred = predator; Scav = scavenger).

| Species | Taxon | Size | Motility | SPM | FM | # ind. |
|---|---|---|---|---|---|---|
| *Austrovenus stutchburyi* | bivalve | large | free | SS/SD/DS | Sus | 434 |
| *Macomona liliana* | bivalve | large | limited | SD/DD | Dep | 473 |
| *Lasaea* sp. | bivalve | small | free | SS | Sus | 489 |
| *Nucula hartvigiana* | bivalve | small | free | SS | Dep | 90 |
| *Prionospio aucklandica* | polychaete | small | limited | SS/DD/SD/DS | Dep | 3466 |
| *Aonides trifida* | polychaete | small | limited | SS/DD/SD/DS | Dep | 824 |
| *Heteromastus filiformis* | polychaete | small | limited | DD | Dep | 466 |
| *Microspio maori* | polychaete | small | limited | SS/SD | Dep | 113 |
| *Paradoneis lyra* | polychaete | small | limited | SS/SD/DS | Dep | 76 |
| *Scoloplos cylindrifer* | polychaete | med | free | SS/DD/SD/DS | Dep | 248 |
| *Oligochaeta* | polychaete | small | limited | SS/DD/SD/DS | Pred/Scav | 126 |
| Nereididae (unspecified) | polychaete | med | free | SS/DD/SD/DS | Pred/Scav | 918 |

SIMPER analyses highlighted two polychaete species that were key contributors to dissimilarities between sampling dates; *Aonides trifida* and *Prionospio aucklandica*. These species collectively contributed to approximately 20% of the dissimilarity in the community composition between sampling dates (W2 and W4 = 22.1%; W2 and W8 = 23.3%; W4 and W8 = 19.0%), with the abundance for *Aonides trifida* decreasing by 50% from W2 to W4.

### *3.3.4 Ecosystem functions*

From the light and dark chambers, important ecosystem functions relating to primary production, community metabolism and nutrient regeneration and uptake were ascertained (Table 3.6). In the sunlit chambers, the net $NH_4^+$ flux varied significantly over time ($p$(perm) = 0.04), with more $NH_4^+$ released from the sediment at W2 and W4 compared to W8. At W8, there was an uptake of $NH_4^+$ in light chambers into the sediment at the PC, L and M treatments (Figure 3.3A). Gross (dark chamber) $NH_4^+$ flux also showed significant temporal variability ($p$(perm) = 0.0003), with pair-wise comparisons indicating that $NH_4^+$ flux from the sediments at W4 was greater compared to both other sampling dates (Table 3.6). The gross $NH_4^+$ flux from the sediment was also significantly higher at the W2 compared to W8 sample date. Furthermore, gross $NH_4^+$ flux showed significant treatment effects, with pair-wise test indicating significantly less gross $NH_4^+$ flux from the sediment in PC treatments compared to L density treatments (Table 3.6). Both net and gross $NH_4^+$ fluxes were, however, highly variable, as indicated by large standard errors associated with the means (Figure 3.3A). There were lower fluxes of $NH_4^+$ (in both the light and dark chambers) at W8 compared to either W2 or W4 (Figure 3.3A).

Although there were no significant differences in NPP, SOC, GPP or $GPP_{chl\ a}$ between any of the treatments ($p \geq 0.39$; Table 3.6; Figures 3.3B and C), some interesting trends still emerged. At both W2 and W8, NPP and GPP were lower in L compared to PC treatments and gradually increased as the amount of added detritus increased (Figures 3.3B and C). At W4, L treatments had the highest NPP and GPP (Figures 3.3B and C). All the treatments were net autotrophic (i.e. positive NPP from the sediment, indicating production > consumption).

**Table 3.6.** Summary of repeated measures PERMANOVA results of univariate and multivariate measures of ecosystem function, as a function of time (2, 4 and 8 weeks post addition), and treatment (PC = no addition, L = 60 g dw $m^{-2}$, M = 120 g dw $m^{-2}$, H = 240 g dw $m^{-2}$ *Ulva* addition). Significant effects ($p$(perm) < 0.05) are indicated in bold, with pair-wise tests indicating where the significant differences occurred (n = 5).

| Variable | Source | df | MS | Pseudo-F | $p$ (perm) | Post-hoc pair-wise tests |
|---|---|---|---|---|---|---|
| Net $NH_4^+$ | Time x Treatment | 6 | 3053.3 | 2.16 | 0.06 | |
| (µmol N $m^{-2}h^{-1}$) | Time | 2 | 4389.6 | 3.11 | **0.04** | W2 = W4, W2 > W8, |
| | Treatment | 3 | 589.1 | 0.34 | 0.83 | W4 > W8 |
| | Plot (treatment) | 16 | 1734.1 | 1.23 | 0.23 | |
| | Residual | 32 | 1410.9 | | | |
| Gross $NH_4^+$ | Time x Treatment | 6 | 726.4 | 1.62 | 0.13 | W2 < W4, W2 > W8, |
| (µmol N $m^{-2}h^{-1}$) | Time | 2 | 3009.4 | 6.73 | **0.0003** | W4 > W8 |
| | Treatment | 3 | 830.5 | 2.65 | **0.03** | PC < L, PC = M, PC = H, |
| | Plot (treatment) | 16 | 313.0 | 0.70 | 0.85 | L = M, L = H, M = H |
| | Residual | 32 | 447.2 | | | |
| NPP | Time x Treatment | 6 | 388740 | 0.46 | 0.84 | |
| (µmol $O_2$ $m^{-2}h^{-1}$) | Time | 2 | 563050 | 0.67 | 0.51 | |
| | Treatment | 3 | 667470 | 0.64 | 0.60 | |
| | Plot (treatment) | 16 | 1038700 | 1.23 | 0.31 | |
| | Residual | 32 | 844890 | | | |
| SOC | Time x Treatment | 6 | 270720 | 0.88 | 0.51 | |
| (µmol $O_2$ $m^{-2}h^{-1}$) | Time | 2 | 2371600 | 7.75 | **0.003** | W2 = W4, W2 > W8, |
| | Treatment | 3 | 43574 | 0.08 | 0.98 | W4 > W8 |
| | Plot (treatment) | 16 | 559750 | 1.83 | 0.07 | |
| | Residual | 32 | 306150 | | | |
| GPP | Time x Treatment | 6 | 974600 | 1.04 | 0.42 | |
| (µmol $O_2$ $m^{-2}h^{-1}$) | Time | 2 | 3383000 | 3.60 | **0.04** | W2 = W4, W2 = W8, |
| | Treatment | 3 | 454770 | 1.06 | 0.39 | W4 > W8 |
| | Plot (treatment) | 16 | 427640 | 0.46 | 0.95 | |
| | Residual | 32 | 939030 | | | |
| $GPP_{chl\ a}$ | Time x Treatment | 6 | 11150 | 1.41 | 0.25 | |
| (µmol $O_2$ $m^{-2}h^{-1}$) | Time | 2 | 3099 | 0.39 | 0.69 | |
| | Treatment | 3 | 2664 | 0.34 | 0.78 | |
| | Plot (treatment) | 16 | 7732 | 0.98 | 0.49 | |
| | Residual | 32 | 7893 | | | |
| Ecosystem | Time x Treatment | 6 | 5.6 | 1.29 | 0.18 | |
| function | Time | 2 | 14.7 | 3.35 | **0.002** | W2 = W4, W2 ≠ W8, |
| (multivariate) | Treatment | 3 | 3.1 | 0.61 | 0.84 | W4 ≠ W8 |
| | Plot (treatment) | 16 | 5.1 | 1.17 | 0.21 | |
| | Residual | 32 | 4.4 | | | |

There were, however, significant temporal differences in univariate measures of SOC and GPP (Table 3.6). Significantly higher SOC were recorded at both W2 and W4 compared to W8, whilst GPP was significantly higher at W4 compared to W8 (Table 3.6). A multivariate analysis of ecosystem function again showed significant temporal variation (more specifically between W2 and W8, and also W4 and W8), while no overall treatment effects were recorded (Table 3.6).

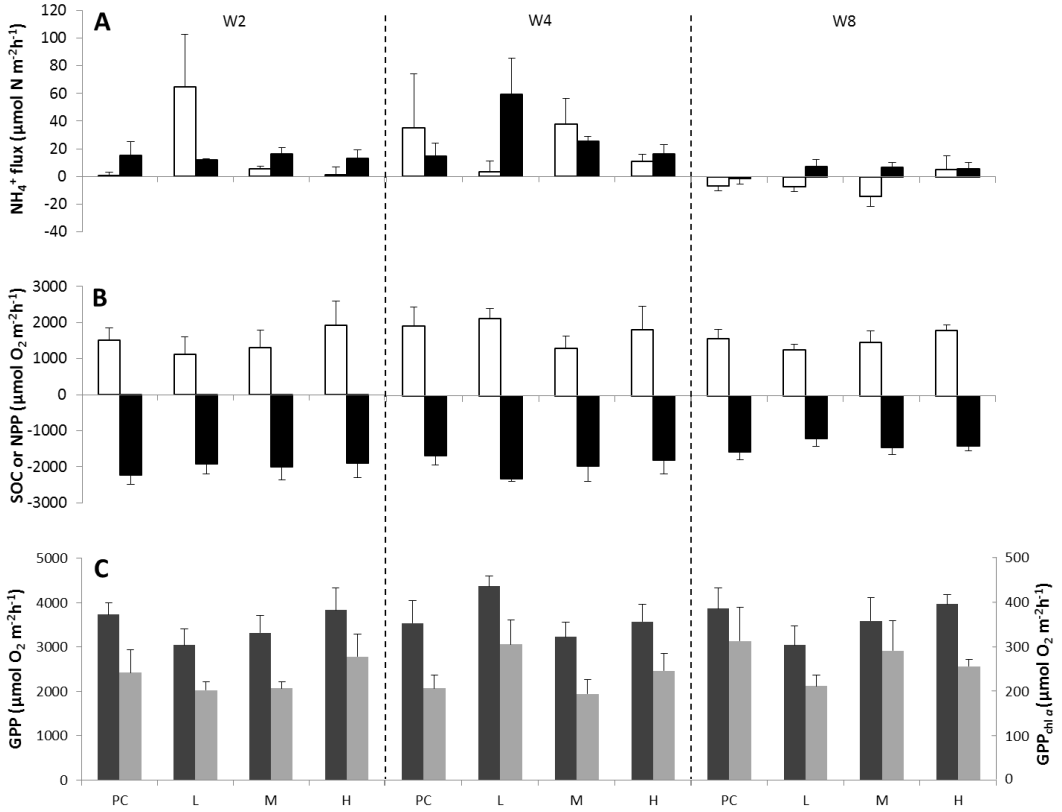


**Figure 3.3.** Solute fluxes (mean ± SE) as a function of treatment (PC = no addition, L = 60 g dw $m^{-2}$, M = 120 g dw $m^{-2}$, H = 240 g dw $m^{-2}$ *Ulva* addition) and through time (2, 4 and 8 weeks post addition). (A) $NH_4^+$ flux (white bars represent net $NH_4^+$ flux in the light chambers, and black bars represent gross $NH_4^+$ flux measured in the dark chambers), (B) net primary production (NPP, from the light chambers, represented by the white bars) and sediment oxygen consumption (SOC, from the dark chambers, represented by the black bars); and (C) gross primary production (GPP, light – dark chambers flux, represented by the dark grey bars), and GPP corrected for chl *a* biomass ($GPP_{chl\ a}$, represented by the light grey bars and on the secondary y-axis). (n = 5). Positive values indicate a flux from the sediment, whilst negative values indicate a flux into the sediment.

## 3.4 Discussion

This study investigated the impact of different amounts of macroalgal detritus on the biodiversity and ecosystem function of an estuarine community in Tauranga Harbour. More specifically, I examined the variations in the benthic community structure under different loads of *Ulva* detritus, and the associated changes in the ecosystem function (i.e. primary production, benthic respiration and nutrient regeneration). These responses were also measured through time. My results were not as predicted, with both macrofaunal community and measures of ecosystem functions showing no significant changes with the addition of detritus, however there were significant temporal effects. Multivariate macrofauna analyses showed significant differences between all three sample dates, while nutrient efflux from both the light and dark chambers, as well as SOC and GPP varied significantly between at least two of the sampling dates. The impacts of the added detritus were, however, not more obvious at W2 compared to W4 and W8, as predicted.

Sediment characteristics, including organic content and chl *a*, remained consistent through time and regardless of treatment, despite the input of large amounts of organic matter, especially in the high treatment plots. Inputs of organic matter often enhances the amount of nutrients available in a system, through nutrient leaching during the decaying process (Levinton et al., 1984; Rossi & Underwood 2002; Bishop & Kelaher 2007, 2013). Posey et al. (1999) found an increase in microalgae with increased nutrients. However, a significant difference in microalgae (measured as chl *a*) between treatments was not recorded, and gross $NH_4^+$ efflux was significantly higher in PC compared to L treatments, contrary to what was expected and reported in the literature. The different quantities of detritus that were added were comparable to other studies which did report elevated organic content in the sediment (Gladstone-Gallagher et al., 2016; Valença et al., 2016). However, our study site was primarily sand, with a low mud content, which suggests that much of the fine sediment and organic material at the surface are easily destabilised and eroded (Le Hir et al., 2008). As the *Ulva* was added to the sediment surface, it is realistic that the *Ulva* may also become resuspended along with the sediment and wash out of the treatment plots (Canal-Vergés et al., 2010). This wash out effect would also explain the non-significant

treatment effect on the amount of detritus that was recovered from the sediment at each sampling date.

In this study, the *Ulva* detritus was broken into small pieces (approx. 2 cm) to make it readily available to secondary consumers. While this prevented the onset of hypoxic conditions, it may have contributed to the easy resuspension and 'wash out' of the detritus. Similar studies have buried *Ulva* under the sediment surface as sheets (e.g. Rossi, 2006, 2007), however by adding the *Ulva* as detritus, I aimed to mimic the pathway by which it would enter the food web naturally, with minimal disturbance to the resident macrofaunal community. Another possible explanation for the lack of treatment effects measured in the sediment characteristics was that most of the added material may have already been processed when the first sampling took place. The half-life of *Ulva* is reported to be between 8 – 12 days (Buchsbaum et al., 1991; Nielsen et al., 2004), with the initial rapid leaching stage occurring in the first 4 days (Gladstone-Gallagher et al., 2016). It is therefore possible that the majority of the *Ulva* that was added had already been metabolised or recycled before the initial sampling date (2 weeks after the initial detrital addition). Franke et al. (2006) have shown that the processing of *Ulva* detritus in sandy sediments is rapid (< 12 d), whilst a study by Rusch et al. (2006) showed that processing of organic matter is rapid in sandy permeable sediment.

The macrofaunal community showed little variation across treatments, despite very obvious changes in macrofaunal communities reported in other studies that used similar quantities of detritus to my experimental treatments (e.g. Kelaher & Levinton, 2003; Olabarria et al., 2010). These results suggest that the relationship between detritus and macrofaunal communities is complex, and most likely a result of multiple factors, including the type of detritus and the specific macrofaunal community present. The macrofaunal community structure did vary significantly over time, and this variation was largely driven by changes in the abundances of two polychaete species; *P. aucklandica* and *A. trifida*. Both these species have limited motility (Greenfield et al., 2016) which may make them sensitive to changes in their environment, as they are less able to move when conditions become undesirable. The temporal variation observed was likely a

result of natural variations and the patchy nature of estuarine benthic communities, as these variations were consistent across the detrital addition and control plots (similar to Gladstone-Gallagher et al., 2016). The temporal variation may also have been due to a reduction in temperature and light conditions.

The feeding strategies of individual species have been suggested to play an important role in determining the source of organic matter (MPB vs *Ulva*) on which they will preferentially feed (Grall et al., 2006; Choy et al., 2008; Park et al., 2016). For example, deposit feeding gastropods and amphipods have been shown to have sequestered high concentrations of *Ulva*-derived carbon under bloom conditions, while a suspension feeding bivalve fed mainly on MPB, regardless of the presence or absence of *Ulva* (Park et al., 2016). Large deposit feeders were found to be the primary active consumers of *Ulva* detritus, but head-down deposit feeders and scavengers were also recognised as important in facilitating this uptake (see Chapter 5). Subtle shifts in diverse estuarine benthic communities can, however, be difficult to document over the small spatial and temporal scales examined in this study. Furthermore, many organisms are highly mobile and can easily move in and out of experimental plots over the time scales examined, and edge effects may have obscured treatment effects (Thrush et al., 2006; Sandwell et al., 2009). As a result, the recovery of macrofauna from disturbances of this scale can be rapid, and may have occurred within the first two weeks post-addition, and before our first sampling date (Thrush et al., 2006).

Ecosystem function variables again showed significant temporal variation, with little treatment effects. The $NH_4^+$ fluxes were highly variable and no clear pattern emerged as the quantity of added detritus increased. For example, the gross $NH_4^+$ efflux from the sediment was greater in all the treatments compared to the PC, but the only significant difference was between the PC and L addition treatments. This difference was likely driven by the elevated gross $NH_4^+$ efflux at W4. Temporally, at W2 and W4, the $NH_4^+$ efflux from the sediment was significantly greater in both the light and dark chambers compared to W8. Again, this is likely a result of the reduced light conditions and colder temperatures during the W8 sampling, as processes associated with nutrient regeneration are linked to these variables. NPP and $GPP_{chl\ a}$ remained consistent throughout the three sampling

times, but SOC was significantly lower at W8 compared to W2 and W4. Therefore, despite benthic metabolism and respiration decreasing over time, the primary production remained consistent. Interestingly, microalgal biomass, which directly impacts primary production, did not vary temporally or between treatments. The higher rate of primary production at W2 and W4 was therefore likely due to the increase in nutrient efflux and regeneration which was also recorded during those sampling times, while the decrease in metabolic processes could again be attributed to the reduction in temperature and light conditions. Although univariate ecosystem function variables were corrected for incubation time, temperature and light could not be accounted for in the calculations.

## 3.5 Conclusions

Detritus, at biomass levels which mimicked natural events, did not have significant impacts on macrofauna or the overall ecosystem function, yet some subtle shifts were observed. Estuarine sand flats are highly productive systems, and it is possible that the turnover of organic matter was rapid enough for the added detritus to be processed within the system before significant, obvious shifts occurred. By measuring community and ecosystem function parameters across three sampling dates I was able to gain a more accurate representation of how macrofaunal and ecosystem function variables are temporally changed or altered by detrital subsidies. It appears that the ecosystem was resilient to detrital inputs of this magnitude and scale, and hypoxia was not induced. Instead, rapid breakdown and assimilation of the organic matter occurred, which resulted in little impact on the macrofaunal community structure and ecosystem function. However, under greater stress (i.e. a higher intensity bloom), and over a larger spatial scale, more significant effects may be observed. My results highlight the importance of measuring temporal variability alongside treatment effects to gain a more in depth and holistic understanding of the impact of detrital subsidies on estuarine benthic communities and their associated ecosystem functions.

# 4.0 CHAPTER FOUR: THE DENSITY DEPENDENT EFFECTS OF TWO KEY BIVALVE SPECIES ON THE DISTRIBUTION AND PROCESSING OF MACROALGAL DETRITUS IN INTERTIDAL COMMUNITIES

## 4.1 Introduction

Bioturbation and sediment reworking by benthic communities is of global importance as it occurs in most oxic sediments and contributes greatly to ecosystem function (Lohrer et al., 2004; Maire et al., 2008; Braeckman et al., 2010). Bioturbation activities include the way organisms feed, burrow and move vertically and horizontally through the sediment, all of which can mix and oxidise the sediment (Kristensen & Blackburn, 1987; Sun et al., 1999; Ingalls et al., 2000). The bioturbation potential of a community (i.e. the specific ability of that community to move or displace sediment) is a function of the community composition and the specific traits of the species present, including body size, abundance, mobility and the way in which the species mix the sediment (Solan et al., 2004). By turning, mixing and displacing sediment, bioturbating activities impact key ecosystem services such as the rate of organic matter decomposition (Solan et al., 2004) and nutrient regeneration, which in turn sustains primary production (e.g. Mermillod-Blondin & Rosenberg, 2006; Needham et al., 2011).

Worldwide, increases in macroalgal blooms, due to increased nutrient loads, have resulted in large and sudden inputs of organic matter into coastal ecosystems (Bonsdorff, 1992; Fletcher, 1996; Norkko & Bonsdorff, 1996a, b, c; Raffaelli, 1999). There is therefore an urgent need to understand the breakdown pathways of this organic matter, and also any interactions which may facilitate this process (Maire et al., 2007). The bioturbation behaviour of a community will influence detrital processing rates by directly impacting on the mixing of the organic matter through the sediment, the oxygenation of the sediment (which in turn influences the rate organic matter degradation and nutrient regeneration) (Lohrer et al., 2004), and also shredding. Bioturbation behaviour can also contribute to the availability of organic matter to organisms living and feeding in the deeper layers of the sediment. For example, a community that contains many bioturbating individuals, may facilitate deeper mixing of organic matter into sediment, thereby making this organic matter less available to surface deposit feeders, but more accessible to sub-surface detritivores (Powell, 1979; Volkenborn et al., 2012). Ultimately, bioturbating behaviour will influence the aerobic and anaerobic mineralisation and the breakdown of any organic matter (Sun et al., 1999; Ingalls et al., 2000). The feeding modes of key macrofauna species become more relevant when animals are feeding directly on the detritus, and can either reduce the organic load (e.g. mobile grazers that feed and then move out of the system) or redistribute the organic matter (e.g. some deposit feeders will feed at the surface and defecate at depth, which provides another pathway for organic matter to mix into the sediment) (Wilcock et al., 1993; Thrush et al., 2006).

Studies examining sediment particle and organic matter mixing in sediments by macrofauna often focus on a single species (e.g. Duport et al., 2006; Braeckman et al., 2010), however, the effects of different intact communities are poorly understood, despite sediment mixing contributing significantly to our understanding of sediment biogeochemistry and ultimately ecosystem function (Boudreau, 1997 in Duport et al., 2006; Teal et al., 2010). Accurately tracing individual particles are an essential part in gaining an understanding of the ability of a particular community or individual species to move or distribute organic matter within the sediment. To date, many methods have been developed to assess the bioturbation potential of macrofauna, however no ‘standard’ method has been

achieved (Maire et al., 2008). Although many recent studies have used luminophores to trace sediment particle movement (e.g. Mahaut & Graf, 1987; Duport et al., 2007; Gilbert et al., 2007; Maire et al., 2007; Braeckman et al., 2010; Bernard et al., 2016), multiple other methods have also been successfully utilised. These methods have included glass beads (Levin et al., 1997), metal tracers (Wheatcroft et al., 1994), isotopic labelling (Blair et al., 1996; Josefson et al., 2002; Moodley et al., 2005; Rossi, 2007), and chlorophyll *a* (chl *a*) (Boon & Duineveld, 1998; Josefson et al., 2002). It has been suggested that multiple tracing techniques used simultaneously yield the most accurate quantification of macrofauna induced particle mixing (bioturbation potential) (Gérino et al., 1998). This experimental study aimed to trace the movement of organic matter within sediment and also into the food web (Chapter 5), therefore artificial tracers such as glass beads or luminophores could not be used. Instead, chl *a* and isotopic labelling of organic matter (in this case the macroalgae *Ulva* in detrital form) was used to quantify the movement of organic matter in the sediment associated with two functionally distinct soft sediment communities. Chl *a* is the most abundant photopigment in living microphytes and has proved to be a good tracer of fresh (high quality) organic matter (Gutiérrez et al., 2000; Ingalls et al., 2000), whilst isotope labelling of organic matter has been extensively used to trace organic matter in food webs.

The macrofaunal community composition on intertidal sandflats is often patchy and can be heavily impacted by adult macrofauna already present at a site. Key adult macrofauna can outcompete newly settling larvae and juveniles due to their size and higher feeding rates, and therefore the whole community can shift depending on the dominant adult macrofauna present (Thrush et al., 1992). In order to examine the impact of two key bivalve species and their associated communities on the processing of organic matter and the distribution of chl *a*, I used intact cores from two established communities (one dominated by the suspension feeding bivalve *Austrovenus stutchburyi* and the other by the deposit feeding bivalve *Macomona liliana*). Intact communities provide a more realistic approach and allow for an integration of complex interactions between naturally co-habitating organisms. *A. stutchburyi* is a shallow living bivalve (top 5 cm) that mixes the sediment through bioturbation as it moves (Powell, 1979), whilst *M.*

*liliana* is a deep living species (10 cm below the surface) that mixes sediment by feeding on organic matter and microphytobenthos (MPB) at the surface and defecating at depth (Powell, 1979; Volkenborn et al., 2012). These two species provided different pathways of sediment particle movement, have different associated communities (Thrush et al., 2006; Chapter 5), and thus are likely to vertically distribute organic matter differently in the sediment. Both species are ecologically important components of intertidal sand flats around New Zealand (Pridmore et al., 1990; Hickey et al., 1995). The density of key macrofaunal species have also been shown to have an impact on ecosystem functions such as nutrient cycling, MPB production and sediment reworking (Lohrer et al., 2004; Duport et al., 2006; Sandwell et al., 2009). As a result, my sampling purposely aimed to achieve a density gradient of the two key species, to examine if these impacts are also observed in terms of organic matter breakdown. A previous study has examined the functional importance of macrofaunal communities for sediment mixing and, using box cores containing intact subtidal communities, found higher mixing rates in a deposit feeding community compared to a suspension dominated community. They also recorded deeper mixing in the deposit feeding community (Josefson et al., 2002). To my knowledge, the bioturbation potential or the vertical mixing of sediment of two functionally varying intact intertidal estuarine communities under controlled laboratory conditions, have not been examined.

The aim of this experiment was to examine the similarities and differences in the vertical distribution of organic matter in the sediment occupied by the two distinct community types. More specifically, I wanted to quantify any differences in the processing rates of added organic matter and the sediment chl *a* profiles between the suspension and deposit feeder dominated communities. I predicted; (1) that less added organic matter (*Ulva* detritus) would be retained and recovered from the sediment of the deposit feeding dominated community as oppose to the suspension feeding dominated community as a result of active feeding, but the remaining organic matter (both chl *a* and *Ulva* detritus) would mix more evenly through the sediment dominated by the suspension feeder due to more active bioturbation; and (2) that there would be density dependent effects of the key species at each site (*A. stutchburyi* at site AS, and *M. liliana* at site ML) on the amount of organic matter retained in the sediments.

## 4.2 Methods

A full description of the *Ulva* labelling procedure and experimental setup can be found in Chapter 5, and only brief details are outlined here.

### *4.2.1 Macroalgae collection*

The macroalgae, *Ulva* sp., was collected from the Athenree intertidal area (S 37° 26.966, E 175° 58.152) in Tauranga Harbour, New Zealand, and subsequently labelled with stable carbon and nitrogen isotopes. For the preparation and labelling procedure, refer to Chapter 5.

### *4.2.2 Site description and collection of intact cores*

On 22 November 2011, at low tide, 76 macrofauna cores (12.5 cm diameter and 10 cm depth) were collected from Tuapiro Point (see Figure 1.2) and transported back to the laboratory intact. Of the 76 cores collected, 44 were from the *A. stutchburyi* site (AS; S 37° 29.390, E 175° 57.014), and 32 were from the *M. liliana* site (ML; S 37° 29.344, E 175° 57.094). Twelve of the 44 AS cores were collected from an area between the two sites (initially termed the mixed site), however after the similarities in the macrofaunal communities at the AS and the mixed site were established at the termination of the experiment, the decision was made to amalgamate these two sites. Both the AS and ML sites were located at the mid-intertidal region, and were approximately 50 m apart, with similar sediment properties. For a detailed description of the site and the core collection procedure, see Chapter 5.

### *4.2.3 Experimental setup*

Once back at the laboratory, the cores were randomly placed in fish bins (6 cores per bin) that were connected to a tidal system that created a 6 h emersion/immersion cycle, mimicking the inundation period observed at the collection site. For a complete outline of the experimental setup see Chapter 5.

Once the cores were acclimatized, 0.60 ± 0.01 g dw of the labelled, finely ground *Ulva* was added to each core by carefully spreading it onto the sediment surface using a Pasteur pipette (see Chapter 5). The amount of *Ulva* added was calculated based on reported natural quantities of *Ulva* observed in the field (Hull, 1987; Bolam et al., 2000; Kelaher & Levinton, 2003; Rossi, 2007). After 2 tidal cycles (24 h), 6 cores (3 from each site, hereafter referred to as reference cores [RC]) were sacrificed to determine the quantity of algae that was lost due to the simulated tidal inundation, assuming minimal faunal uptake (see results). The remaining 70 cores were inundated at 12 h periods for 10-d, after which the experiment was terminated. From each large core, one smaller sediment sample was taken using a cut off syringe core (2 cm diameter) to a depth of 10 cm, and immediately frozen in an upright position. Once the sediment samples were collected, the remaining sediment from the large core was sieved (500 µm mesh) for macrofauna. Sediment samples were later analysed for chl *a*, grain size, organic content and isotope analyses. Macrofauna were preserved in 70% ethanol for isotope analyses and identification (Chapter 5), and were identified to species level. Macrofauna that died during the 10-d period were noted and removed to avoid increased microbial activity due to decomposition processes. The dead macrofauna were not included in subsequent analyses.

### *4.2.4 Sediment sample preparation and analyses*

From each sediment core, we quantified mean grain size (µm), the percent mud (< 63 µm), organic content (%) and isotope composition ($\delta^{13}C$ and $\delta^{15}N$), and from a selected subset of samples we derived chl *a* concentration. Phaeophytin (phaeo) concentration was also determined alongside chl *a* to gain a measure of the degraded pigment biomass. Sediment samples were defrosted and sectioned into 1 cm intervals. Half of each section was used for grain size and organic content analyses whilst the other half of the sediment was freeze dried for isotope and pigment (chl *a* and phaeo) analyses, where applicable. To ascertain grain size and organic content, the vertically sectioned sediment was amalgamated and analysed using a Melvern mastersizer-S after digestion in 10% hydrogen peroxide (Singer et al., 1988) and through loss on ignition as outlined by Dean (1974), respectively. A subset of 35 samples (20 from AS and 15 from ML) was selected for chl *a* and

phaeo analyses, which were carried out on 0.5 cm increments for the top 2 cm and then 1 cm sections for the remaining 8 cm to produce profiles of pigment concentration. The 0.5 cm increments were subsequently averaged for presentation. These cores were specifically selected to represent a gradient in *A. stutchburyi* and *M. liliana* density. Isotope analyses were carried out on all of the cores. After outliers were removed (see statistical analysis section), 34 and 27 cores remained for statistical analyses from AS and ML, respectively, along with the three reference cores (RC) from each site.

To determine chl *a* and phaeo content, a known weight (approximately 0.1 g) of freeze-dried sediment was soaked in 90% acetone for 24 h before being centrifuged. Chl *a* and phaeo was then measured fluorometrically before and after acidification, respectively, on a Turner Designs 10-AU fluorometer (Arar & Collins, 1997). After subsamples were set aside for pigment analyses, the remaining freeze-dried sediment sections were amalgamated into two depths for isotope analyses; 0 - 1 and 1 – 5 cm. For each sample and depth, sediment was ground and a known weight (approximately 0.09 g) was placed inside a pre-weighed Ag-foil capsule. Inorganic carbon was removed using an HCl-fumigation method (Harris et al., 2001). Samples were then analysed at the Waikato Stable Isotope Unit in a Dumas elemental analyser (Europa Scientific ANCA-SL) interfaced to an isotope mass spectrometer (Europa Scientific 20-20 Stable Isotope Analyser). Samples were run in batches of 10 - 12 and bracketed by reference samples ($^{13}C$ is CSIRO sucrose with a $\delta^{13}C$ of -10.80‰ and for $\delta^{15}N$ is urea at -0.499‰) of known isotopic content, which have been calibrated with universal standards.

### *4.2.5 Isotope calculations*

The C and N isotope ratios are expressed in the ‰ notation, using the equation:

$$\delta R\ (‰) = ([R_{sample}/R_{standard}]^{-1}) \times 10^3$$

where R is the ratio between the heavy and light isotopes ($^{13}C$:$^{12}C$ or $^{15}N$:$^{14}N$). The stable isotope ratio, denoted by δ, is defined as the deviation in % from an

international reference standard of 0.0112372 for C (Vienna PeeDee Belemnite), and 0.0036765 for N (atmospheric nitrogen gas). Higher δ values indicate a higher proportion of the heavy isotope.

A linear two-source mixing model was used to quantify the *Ulva* C and N remaining in each core (Karlson et al., 2010) where:

$$f1 + f2 = 1$$

$$f1 = (\delta_{sample} - \delta_{source2})/(\delta_{source1} - \delta_{source2})$$

where f1 is the proportion of *Ulva* C or N in the sediment sample and f2 is the proportion of C or N derived from the initial sediment. The amount of *Ulva* was expressed as a percentage of the total added material that was recovered, and was calculated using the mixing model, assuming that 100% of the *Ulva* was recovered after 24 h (Chapter 5). The % $^{13}C$ and $^{15}N$ recovered were highly correlated ($R^2 = 0.93$, $p < 0.0001$), however the % $^{15}N$ that was recovered in all cores was consistently higher than the % $^{13}C$. This trend was similar to previous studies (e.g. Karlson et al., 2010, 2011). For this study only % $^{15}N$ data is presented in order to allow for comparisons to be made with other studies.

### *4.2.6 Statistical analyses*

Differences in community composition between sites were examined using non-metric multidimensional scaling (nMDS) using the Bray-Curtis similarity matrices (see Chapter 5). There was a clear distinction in the community composition between AS and ML sites and therefore for all subsequent analyses, cores were pooled based on their collection site (AS and ML).

The twenty most abundant mobile macrofaunal species (both limited or freely motile) were grouped into four separate functional groups based primarily on their mobility and also on their body size (Rodil et al., 2013; Harris et al., 2015; Table 4.1), as these two characteristics greatly contribute to their bioturbation potential. These twenty species accounted for 91.1% of the total abundance and 91.3% of the total richness from both sites. *M. liliana* and *A. stutchburyi* were treated as two

separate functional groups, as they comprised the highest biomass at both sites and were the key species of interest. The remaining 18 species, were grouped as either limited mobility/small bioturbators (LS), or freely mobile/large bioturbators (FL) (for a list of species within each functional group see Table 4.1). The LS group were mostly small individuals (< 5 mm, except for *Edwardsia* sp.), while the FL group comprised of medium and large bodied species (> 5 mm). *Nucula hartivigiana*, although freely mobile, were grouped in the LS group as these bivalves are very small in size. A complete analysis of the species community composition can be found in Chapter 5. Functional diversity between sites were examined using non-metric multidimensional scaling (nMDS) using the Bray-Curtis similarity matrices, and was fourth root transformed to standardise the data.

Chl *a* was examined as concentrations at specific depth intervals (averaged across cores from AS and ML, hereafter avg. chl *a*), and also as grouped averages over three sediment depths; 0 – 1 (upper), 1 – 5 (mid) and 5 – 10 cm (lower), in order to gain an understanding of the verticle distribution of the chl *a* in cores from the respective collection sites. $^{15}N$ data was expressed as the % of the total $^{15}N$ added that was recovered (hereafter % $^{15}N$), and was examined only at the upper and mid depth, as preliminary analyses showed very low recovery at the lower depth (i.e. < 5%). Although the chl *a* signature was a combination of MPB as well as the added labelled *Ulva*, comparing the chl *a* profile with the isotopic profile allowed for a differentiation between these two components and provided an insight into pre-experimental quantities of organic matter and sediment mixing in the cores. Data points for % $^{15}N$ that fell outside of 2 standard deviations of the mean were considered outliers and were subsequently excluded from all further analyses (AS n = 7; ML n = 2). The % $^{15}N$ that was recovered from the sediment and the macrofauna (Chapter 5) were combined to calculate a budget, and to estimate the amount of unaccounted added detritus.

Permutational univariate analysis of variance (PERMANOVA) (Anderson et al., 2008) was used to examine the inter-site differences in the community and functional group data (i.e. abundance and biomass of *A. stutchburyi* and *M. liliana*, the overall species abundance and richness, and the abundances of LS and FL bioturbators), pigment data (i.e. avg. chl *a* and phaeo at the upper, mid lower

sediment depth) and % $^{15}$N data (at the upper and mid depth). In each of these analyses, site and sediment depth remained fixed factors. PERMANOVA pair-wise test were performed to indicate where significant site and depth effects occurred.

The distance-based linear model (DistLM) function was used to conduct marginal tests to establish how much of the variation measured in the avg. chl *a* and % $^{15}$N at each depth could be explained by all the functional group data and the environmental factors measured at each site (Anderson et al., 2008). A Euclidean distance resemblance matrix based on 9999 permutations was computed independently for chl *a* and the % $^{15}$N at each depth, followed by 'marginal' tests (9999 permutations) which identified significant ($p < 0.05$) and marginally significant ($p < 0.1$) predictors of avg. chl *a* and % $^{15}$N. We used the corrected Akaike information selection criterion (AICc) which explained the greatest proportion of variability, while minimizing model complexity. All the statistical analyses were carried out using PRIMER v6 (with PERMANOVA+) software (Clarke & Gorley, 2006).

## 4.3 Results

### *4.3.1 Benthic species composition and sediment characteristics*

Species were grouped based on their functional traits (Table 4.1), and the communities at the two different sites showed significant differences in functional diversity (Figure 4.1).

Cores collected from AS sites had significantly higher numbers (PERMANOVA, $p$(perm) < 0.05) of *A. stutchburyi*, LS bioturbators, FL bioturbators, overall species abundance and richness compared with ML sites (Table 4.2; Figure 4.1). The species driving the significant differences between AS and ML cores in the LS bioturbator functional group were the polychaetes *H. filiformis* and *P. aucklandica*, while the differences between sites in the FL bioturbator group were driven by *Lysiannassidae* and *Naineris sp* (Table 4.1).

**Table 4.1.** Functional group classification of macrofaunal species based on motility within sediment (limited or freely) and adult body size (small < 5, medium 5–20 and large > 20 mm) (Rodil et al., 2013). The final two columns indicate the abundance of each species (and the range) at AS (n = 34) and ML (n = 27) sites. LS = limited mobility/small; FL = freely mobile/large.

| Functional Group | Species | Taxon | Size | Motility | Abundance (range) | |
|---|---|---|---|---|---|---|
| | | | | | AS | ML |
| ***A. stutchburyi*** | *Austrovenus stutchburyi* | bivalve | large | free | 6.9 (1 - 24) | 0.5 (0 - 2) |
| ***M. liliana*** | *Macomona liliana* | bivalve | large | limited | 3.5 (0 - 9) | 3.6 (0 - 7) |
| **LS bioturbators** | *Nucula hartvigiana* | bivalve | small | free | 1.1 (0 - 6) | 0.0 (0 - 0) |
| | *Heteromastus filiformis* | polychaete | small | limited | 10.2 (0 - 39) | 0.1 (0 - 1) |
| | *Prionospio aucklandica* | polychaete | small | limited | 21.7 (1 - 80) | 0.2 (0 - 2) |
| | *Magelona dakini* | polychaete | small | limited | 0.5 (0 - 6) | 0.0 (0 - 4) |
| | *Oligochaeta* | polychaete | small | limited | 0.2 (0 - 5) | 5.7 (0 - 18) |
| | *Aonides trifida* | polychaete | small | limited | 11.9 (0 - 52) | 0.0 (0 - 0) |
| | *Scolelepis* sp. | polychaete | small | limited | 4.7 (0 - 34) | 6.4 (0 - 14) |
| | *Edwardsia* sp. | Cnidaria | med | limited | 1.2 (0 - 7) | 4.0 (0 - 9) |
| **FL bioturbators** | Nemertea | polychaete | med | free | 2.7 (0 - 10) | 1.2 (0 - 4) |
| | Nereididae (unspecified) | polychaete | med | free | 7.2 (0 - 18) | 3.9 (0 - 14) |
| | *Naineris* sp. | polychaete | med | free | 0.8 (0 - 6) | 14.7 (1 - 33) |
| | *Scoloplos cylindrifer* | polychaete | med | free | 12.9 (1 - 30) | 5.3 (0 - 22) |
| | *Scolecolepides benhami* | polychaete | med | free | 0.7 (0 - 2) | 0.4 (0 - 2) |
| | *Orbinia papillosa* | polychaete | med | free | 1.5 (0 - 8) | 0.0 (0 - 1) |
| | *Lysianassidae* | amphipod | med | free | 10.6 (0 - 43) | 0.5 (0 - 2) |
| | *Phoxocephalidae* sp | amphipod | med | free | 2.2 (0 - 11) | 0.0 (0 - 0) |
| | *Zeacumantus lutulentus* | gastropod | large | free | 0.7 (0 - 6) | 0.2 (0 - 2) |
| | *Cominella glandiformis* | gastropod | large | free | 0.5 (0 - 3) | 0.3 (0 - 4) |

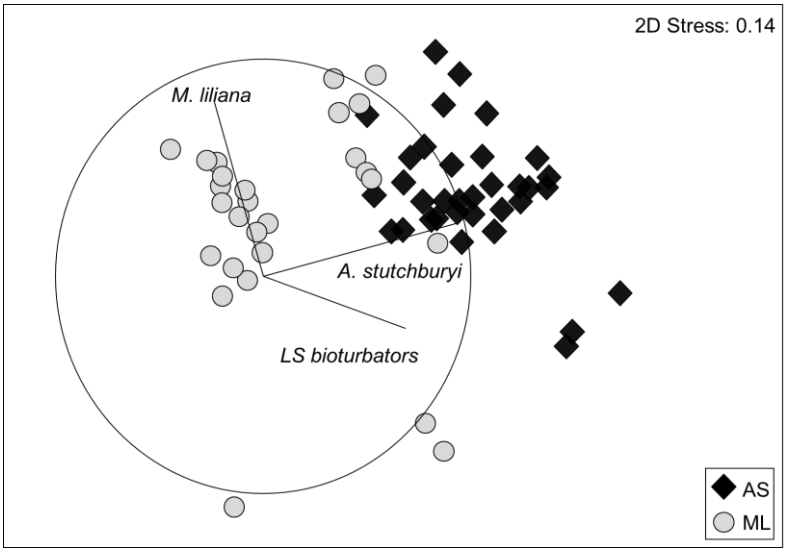


**Figure 4.1.** Non-metric multi-dimensional scaling (nMDS) analysis (Bray-Curtis similarity), showing differences in the functional characteristics of the community in each core, as a function of the site (AS and ML). The functional groups that collectively contributed to 50% of the variation is indicated (Pearson's $r \leq 0.5$).

The *M. liliana* abundance and the average combined biomass of *A. stutchburyi* and *M. liliana* was slightly higher in the cores collected from ML, but these differences were not significant between the two sites (Table 4.2). The non-significant result of the biomass in spite of a significantly higher *A. stutchburyi* abundance at AS was due to the *M. liliana* in AS cores being considerably smaller in size compared to those recovered from ML cores.

**Table 4.2.** Summary of the PERMANOVA results for differences between sites, as well as mean (± 1SE) community data (n = individual cores included in analyses). Significant effects (*p*(perm) < 0.05) are indicated in bold. LS bioturbators = limited mobility/small; FL = freely mobile/large. All units, unless otherwise specified, are ind. core$^{-1}$. The combined total biomass of *A. stutchburyi* and *M. liliana* is also given.

| | AS (n = 34) | ML (n = 27) | *df* | MS | *F* | *p*(perm) |
|---|---|---|---|---|---|---|
| *A. stutchburyi* abundance | 6.9 ± 1.1 | 0.5 ± 0.1 | 1 | 616.6 | 27.14 | **0.0001** |
| *M. liliana* abundance | 3.5 ± 0.4 | 3.6 ± 0.5 | 1 | 0.1 | 0.03 | 0.92 |
| LS bioturbators | 51.5 ± 4.6 | 16.3 ± 1.3 | 1 | 16333 | 34.93 | **0.0001** |
| FL bioturbators | 39.7 ± 2.3 | 26.7 ± 2.0 | 1 | 2535.6 | 17.01 | **0.0002** |
| Species abundance | 123.0 ± 8.0 | 47.2 ± 2.2 | 1 | 81521 | 62.05 | **0.0001** |
| Species richness | 15.6 ± 0.4 | 8.9 ± 0.3 | 1 | 669.5 | 169.50 | **0.0001** |
| Total biomass (mg) | 511.2 ± 41.6 | 523.0 ± 63.0 | 1 | 2089.9 | 0.03 | 0.87 |

The overall community and functional group composition also varied significantly at the two sites (see Chapter 5). Sediment properties were consistent at both sites, and comprised fine sand, with low organic and mud content (Table 4.3).

**Table 4.3.** Sediment property means (± 1SE) for cores collected from AS and ML. Median grain size, mud content and organic matter were calculated from AS (n = 34) and ML (n = 27), and averaged chl *a* and phaeo from AS (n = 20) and ML (n = 15).

| | AS | ML |
|---|---|---|
| Median grain size (µm) | 182 ± 1 | 192 ± 2 |
| Mud content (%) | 4.9 ± 0.4 | 3.3 ± 0.3 |
| Organic matter (%) | 2.08 ± 0.03 | 1.80 ± 0.03 |
| Avg. chl *a* (µg g$^{-1}$ dw) | 17.2 ± 2.1 | 6.0 ± 0.2 |
| Avg. phaeo (µg g$^{-1}$ dw) | 11.3 ± 1.0 | 4.4 ± 0.2 |

### *4.3.2 Pigments in the sediment*

Approximately three times higher avg. chl *a* and phaeo was measured in cores from AS compared to ML sites (Table 4.3), and this result was significant (Table

4.4). When chl *a* and phaeo was examined on a fine scale (i.e. every 1 cm), the sediment profiles from ML showed an exponential decrease with increasing sediment depth, however the cores collected from AS showed a more linear chl *a* profile (Figures 4.2A and B). There was a peak in phaeo biomass at the AS site between 8 and 9 cm (Figure 4.2B), however the results were highly variable between cores. The variation between replicate cores was significantly higher at AS compared to ML for both chl *a* (coefficient of variation = 0.56 and 0.34 respectively; Levene's test $p < 0.01$) and phaeo (coefficient of variation = 0.47 and 0.27, respectively; Levene's test $p < 0.01$). Analysis showed similar trends for the chl *a* and phaeo results and therefore only chl *a* data will be discussed.

**Table 4.4.** Summary of PERMANOVA and pairwise post-hoc results for two-way analyses of average chl *a* concentration in three depth intervals (upper, mid, lower) in cores from AS (n = 20) and ML (n = 15).

| Source | *df* | MS | Pseudo-F | *p*(perm) |
|---|---|---|---|---|
| Site | 1 | 2263.3 | 40.59 | **0.0001** |
| Depth | 2 | 1227.4 | 22.01 | **0.0001** |
| Site x Depth | 2 | 195.3 | 3.50 | **0.04** |
| Res | 99 | 55.8 | | |
| Post-hoc tests | Site AS | | Upper > Mid; Upper > Lower, Mid = Lower | |
| | Site ML | | Upper > Mid; Upper > Lower; Mid > Lower | |
| | All three depths | | AS > ML | |

The chl *a* data from each 1 cm section was pooled into three sediment depths; the upper (0 – 1 cm), mid (1 – 5 cm) and lower (5 – 10 cm). There was a significant difference in chl *a* with depth and these differences were variable between the two collection sites, as indicated by the significant interaction term (Table 4.4).

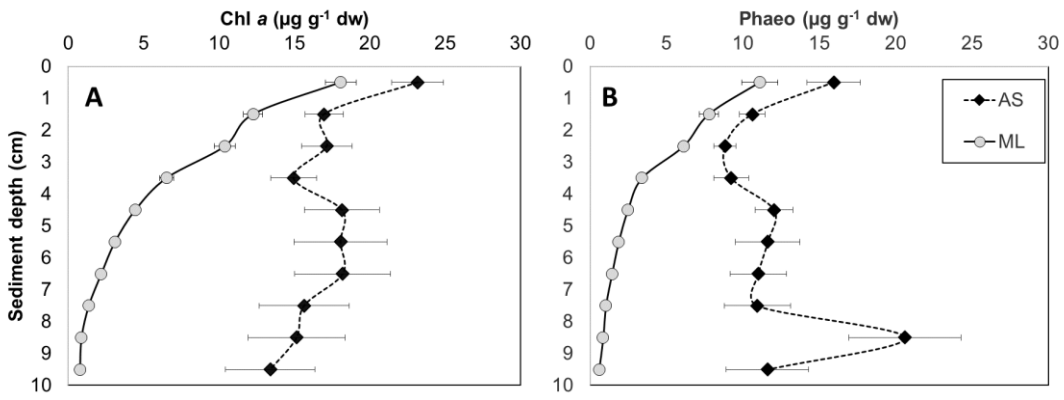


**Figure 4.2.** Sediment (A) chl *a* and (B) phaeo profiles of cores collected from AS (filled diamonds) and ML (shaded circles). AS: n = 20; ML: n = 15. Error bars represent 1 SE.

The chl *a* was more evenly distributed throughout the sediment in cores collected from AS, with approximately 40%, 30% and 30% of the chl *a* found in the upper, mid and lower depths, respectively (Figure. 4.3). Conversely, the chl *a* was concentrated in the upper 0-1 cm interval (60%) in cores collected from ML, with only 5% of the total chl *a* found at the lower depth (Figure 4.3).

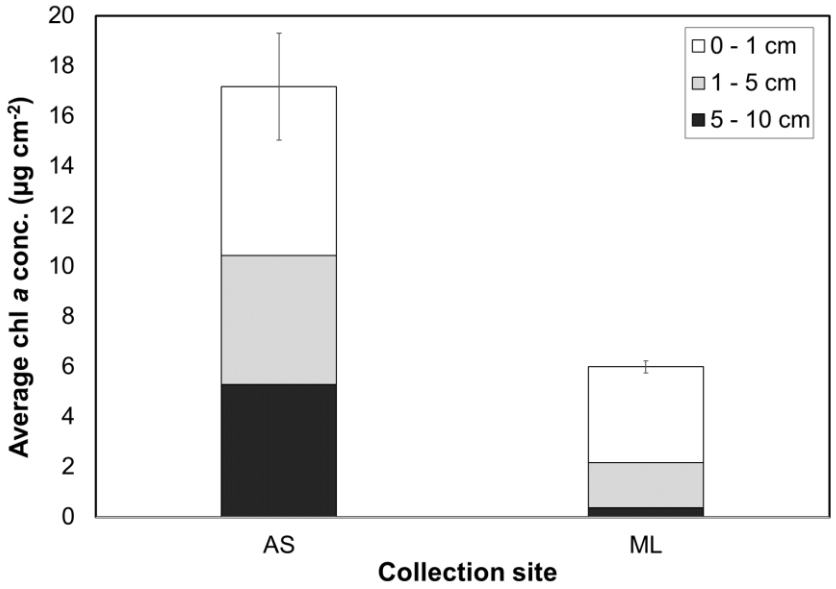


**Figure 4.3.** Bar graph showing the average chl *a* concentration in three depth intervals in cores from AS and ML, and the proportion of that average that was recovered from three specific sediment depths. AS: n = 20; ML: n = 15. Error bars represent ± 1 SE.

The distance based linear model function (DistLM) was used to conduct marginal test to explain the variation in the chl *a* distribution patterns using the functional groups and environmental variables as predictors in the model (Table 4.5). Marginal results showed that for cores collected from AS, FL bioturbators were significantly positively correlated with chl *a* concentrations in the upper 0 – 1 cm ($p < 0.05$), suggesting the facilitation of chl *a* production, and explained 23% of the total variation. *M. liliana* density was a significant predictor of chl *a* concentrations at the mid depth ($p < 0.05$) and marginally significant at the bottom depth ($p < 0.1$), explaining 22% and 20% of the variation, respectively (Table 4.5) and the correlation between chl *a* concentrations and *M. liliana* density was negative in both instances. For cores collected from ML, species richness was the only significant predictor of chl *a* concentrations, and was negatively correlated with chl *a* concentrations at the bottom depth, explaining 26% of the variation (Table 4.5).

**Table 4.5.** DistLM **'**marginal' test results for marginally significant ($p < 0.1$*) and significant ($p < 0.05$**) predictors of average chl *a* concentration in three depth intervals in cores from AS and ML. Prop. indicates the proportion of variation explained, with the direction of the correlation denoted in parentheses for significant variables.

| Site | Depth | Measure | Pseudo-F | Prop. |
|---|---|---|---|---|
| AS | Upper (0 – 1 cm) | *A. stutchburyi* | 0.07 | 0.004 |
| | | *M. liliana* | 1.16 | 0.06 |
| | | LS bioturbators | 0.09 | 0.01 |
| | | FL bioturbators | 5.26 | **0.23**(+)** |
| | | Abundance | 0.26 | 0.01 |
| | | Sp richness | 1.23 | 0.06 |
| | | Biomass | 0.16 | 0.01 |
| | | GS | 2.81 | 0.14 |
| | | % mud | 0.10 | 0.01 |
| | | Organics | 0.02 | 0.001 |
| | Mid (1 – 5 cm) | *A. stutchburyi* | 1.12 | 0.06 |
| | | *M. liliana* | 4.94 | **0.22**(-)** |
| | | LS bioturbators | 0.08 | 0.004 |
| | | FL bioturbators | 1.66 | 0.08 |
| | | Abundance | 0.55 | 0.03 |
| | | Sp richness | 0.31 | 0.02 |
| | | Biomass | 1.85 | 0.09 |
| | | GS | 1.40 | 0.07 |
| | | % mud | 0.35 | 0.02 |
| | | Organics | 1.61 | 0.08 |
| | Bottom (5 – 10 cm) | *A. stutchburyi* | 0.10 | 0.01 |
| | | *M. liliana* | 4.64 | **0.20*(-)** |
| | | LS bioturbators | 0.04 | 0.002 |
| | | FL bioturbators | 0.37 | 0.02 |
| | | Abundance | 0.02 | 0.001 |
| | | Sp richness | 0.00 | 0.0002 |
| | | Biomass | 2.09 | 0.10 |
| | | GS | 2.83 | 0.14 |
| | | % mud | 1.33 | 0.07 |
| | | Organics | 1.64 | 0.08 |
| ML | Upper (0 – 1 cm) | *A. stutchburyi* | 1.01 | 0.07 |
| | | *M. liliana* | 0.12 | 0.01 |
| | | LS bioturbators | 0.62 | 0.05 |
| | | FL bioturbators | 0.01 | 0.001 |
| | | Abundance | 0.10 | 0.01 |
| | | Sp richness | 1.25 | 0.09 |
| | | Biomass | 0.57 | 0.04 |
| | | GS | 3.08 | 0.19 |
| | | % mud | 0.12 | 0.01 |
| | | Organics | 0.42 | 0.03 |

| | | | |
|---|---|---|---|
| Mid (1 – 5 cm) | *A. stutchburyi* | 0.30 | 0.02 |
| | *M. liliana* | 0.04 | 0.003 |
| | LS bioturbators | 0.47 | 0.03 |
| | FL bioturbators | 1.55 | 0.11 |
| | Abundance | 0.48 | 0.04 |
| | Sp richness | 0.01 | 0.001 |
| | Biomass | 0.03 | 0.002 |
| | GS | 0.10 | 0.01 |
| | % mud | 0.15 | 0.01 |
| | Organics | 0.17 | 0.01 |
| Bottom (5 – 10 cm) | *A. stutchburyi* | 0.04 | 0.003 |
| | *M. liliana* | 0.66 | 0.05 |
| | LS bioturbators | 1.08 | 0.08 |
| | FL bioturbators | 0.41 | 0.03 |
| | Abundance | 0.06 | 0.005 |
| | Sp richness | 4.52 | **0.26**(-)** |
| | Biomass | 0.48 | 0.04 |
| | GS | 0.10 | 0.01 |
| | % mud | 1.66 | 0.11 |
| | Organics | 0.04 | 0.003 |

### *4.3.3 $^{15}N$ in the sediment*

The % $^{15}N$ results were examined as a total % recovery and were seperated into two sediment depths; upper (0 – 1 cm) and mid (1 – 5 cm) (Table 4.6; Figure 4.4). A recovery of 97% ± 3% was achieved in the reference cores, confirming minimal loss of *Ulva* through the tidal simulation process (Figure 4.4).

**Table 4.6.** Two-way PERMANOVA and pairwise post-hoc results for differences in the % of total $^{15}N$ recovered in two depth intervals (upper and mid) in cores from AS (n = 34) and ML (n = 27). Significant effects ($p$(perm) < 0.05) are indicated in bold.

| Source | *df* | MS | Pseudo-F | *p*(perm) |
|---|---|---|---|---|
| Site | 2 | 1182.2 | 6.21 | 0.12 |
| Depth | 1 | 53460 | 280.89 | **0.0001** |
| Site x Depth | 2 | 1132.8 | 5.95 | **0.01** |
| Res | 108 | 190.3 | | |
| Post-hoc tests | | | Site AS | Upper > Mid |
| | | | Site ML | Upper > Mid |
| | | | Upper depth | AS > ML |
| | | | Mid depth | AS = ML |

There was significantly less total % $^{15}N$ recovered from the cores collected from AS ($p$(perm) = 0.0002) and ML ($p$(perm) = 0.043) compared to the reference cores (Figure 4.4). Overall, a higher total % $^{15}N$ was recovered from the ML (78%) compared to the AS (67%) cores (Figure 4.4), however this result was not significant (Table 4.6). Furthermore, a significantly higher % $^{15}N$ was recovered from the upper compared to the mid depths in cores from both sites (Table 4.6).

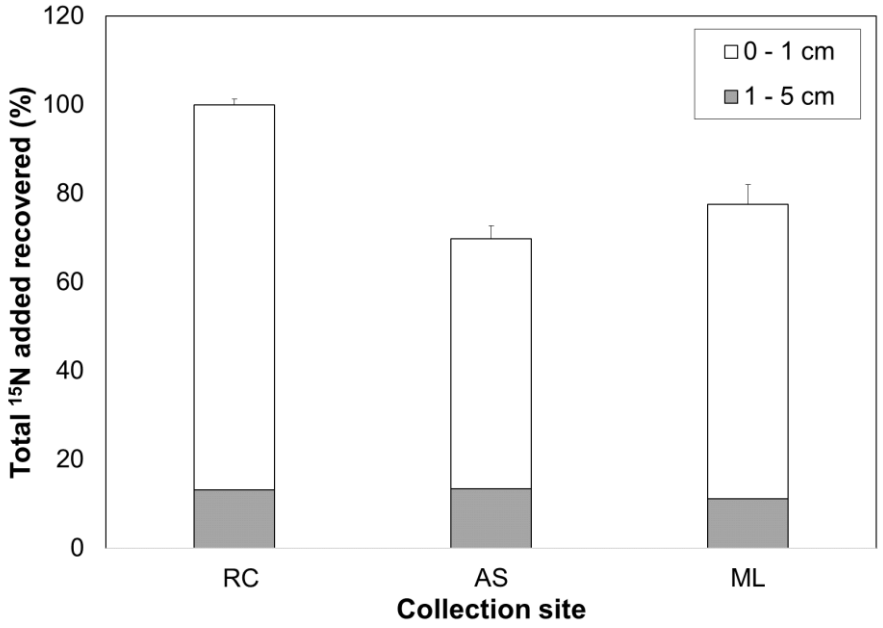


**Figure 4.4.** The % of the total $^{15}N$ that was added to each core that was recovered in two depth intervals in cores from RC, AS and ML. AS: n = 34; ML: n = 27; RC = 6.

The DistLM function was also used to quantify the variation in the $^{15}N$ distribution observed in Figure 4.4, using the same predictor variables as for chl *a*. Marginal test results showed that for cores collected from AS, *M. liliana* density was a marginally significant predictor ($p < 0.1$) of the % $^{15}N$ recovered at the mid depth, explaining 10% of the variation, while both LS bioturbators and biomass were significant predictors of $^{15}N$ recovered at this depth, each explaining 14% of the variation (Table 4.7). All these correlations were positive. No significant correlations were measured between any of the predictor variables and the % $^{15}N$ recovered in cores collected from ML, however organic content was marginally significant and explained 12% of the variation in the upper depth (Table 4.7).

**Table 4.7.** DistLM **'**marginal' test results for marginally significant ($p < 0.1$*) and significant ($p < 0.05$**) predictors of % total $^{15}N$ in two depth intervals in cores from AS and ML. Prop. indicates the proportion of variation explained, with the direction of the correlation denoted in parentheses for significant variables.

| Site | Depth | Measure | Pseudo-F | Prop. |
|---|---|---|---|---|
| AS | 0 – 1 cm | *A. stutchburyi* | 0.28 | 0.01 |
| | | *M. liliana* | 0.52 | 0.02 |
| | | LS bioturbators | 1.95 | 0.06 |
| | | FL bioturbators | 0.92 | 0.03 |
| | | Abundance | 0.85 | 0.03 |
| | | Sp richness | 2.34 | 0.07 |
| | | Biomass | 0.82 | 0.02 |
| | | GS | 0.64 | 0.02 |
| | | % mud | 0.41 | 0.01 |
| | | Organics | 0.01 | 0.0002 |
| | 1 – 5 cm | *A. stutchburyi* | 0.00 | 0.0001 |
| | | *M. liliana* | 3.68 | **0.10*(+)** |
| | | LS bioturbators | 5.15 | **0.14**(+)** |
| | | FL bioturbators | 0.78 | 0.02 |
| | | Abundance | 2.73 | 0.08 |
| | | Sp richness | 0.31 | 0.01 |
| | | Biomass | 5.29 | **0.14**(+)** |
| | | GS | 0.01 | 0.0005 |
| | | % mud | 0.88 | 0.03 |
| | | Organics | 0.11 | 0.004 |
| ML | 0 – 1 cm | *A. stutchburyi* | 0.28 | 0.01 |
| | | *M. liliana* | 0.12 | 0.005 |
| | | LS bioturbators | 0.46 | 0.02 |
| | | FL bioturbators | 0.45 | 0.02 |
| | | Abundance | 0.07 | 0.003 |
| | | Sp richness | 0.24 | 0.01 |
| | | Biomass | 0.03 | 0.001 |
| | | GS | 2.42 | 0.09 |
| | | % mud | 1.34 | 0.05 |
| | | Organics | 3.33 | **0.12*(+)** |
| | 1 – 5 cm | *A. stutchburyi* | 0.07 | 0.003 |
| | | *M. liliana* | 0.15 | 0.01 |
| | | LS bioturbators | 1.09 | 0.04 |
| | | FL bioturbators | 0.47 | 0.02 |
| | | Abundance | 0.01 | 0.0003 |
| | | Sp richness | 0.14 | 0.01 |
| | | Biomass | 0.16 | 0.01 |
| | | GS | 1.69 | 0.06 |
| | | % mud | 0.95 | 0.04 |
| | | Organics | 0.95 | 0.04 |

The total % $^{15}N$ that could be accounted for by macrofaunal uptake (from Chapter 5), and the total % $^{15}N$ found in the sediment, were combined and balanced against the total $^{15}N$ that was added at the start of the experiment in order to calculate the unaccounted for/respired total amount of $^{15}N$ at each site. Both sites had equal amounts of unaccounted $^{15}N$ (19.2% at AS and 19.5% at ML) (Figure 4.5), however significantly more $^{15}N$ were incorporated by macrofauna in the AS site compared to ML (Chapter 5).

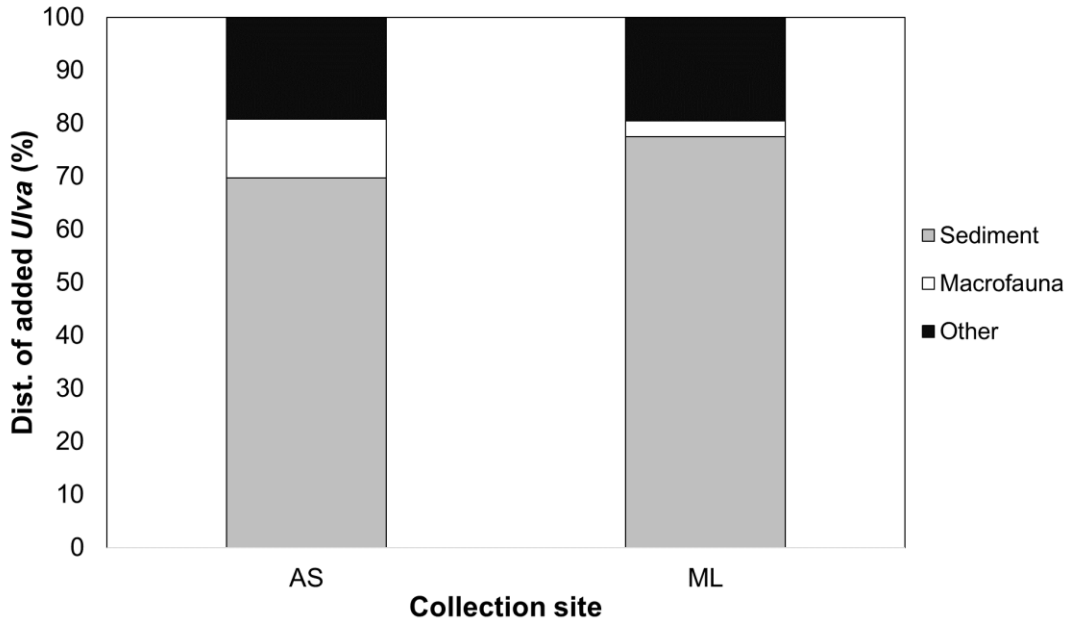


**Figure 4.5.** The % of the total added *Ulva* recovered (from the sediment and the macrofauna) and unrecovered (lost or metabolised, denoted as "other") at the two collection sites (AS and ML). AS: n = 34; ML: n = 27.

## 4.4 Discussion

Cores from intact benthic communities were used to determine the density dependent impacts of two functionally different macrofaunal species (the suspension feeder *A. stutchburyi* and deposit feeder *M. liliana*) and their naturally associated communities on the processing of organic matter and the vertical mixing of sediments. Naturally occurring MPB biomass (measured as chl *a*) and isotopically labelled organic matter (in this case *Ulva* detritus) was used to track the movement and distribution of surface organic matter in the sediment profile.

The first prediction, which suggested that less added organic matter (*Ulva* detritus) would be retained and recovered from the sediment dominated by the deposit feeder, was rejected. It is possible that *M. liliana* preferred feeding on MPB over *Ulva* detritus, which could explain why the first prediction was incorrect. In addition, significantly higher average chl *a* (MPB and *Ulva* detritus) was measured in cores collected from the *A. stutchburyi* dominated site (AS) compared to cores collected from the site dominated by the deposit feeder *M. liliana* (site ML). Lelieveld et al. (2004) found that deliberately excluding *M. liliana* from experimental plots doubled the chl *a* biomass, and attributed these trends in part to feeding on MPB by the *M. liliana*. In this experiment, *M. liliana* were not completely excluded from cores collected from AS in order to mimic naturally occurring communities, however the *M. liliana* in the cores from AS were considerably smaller than those found in cores collected from ML, and would therefore consume less MPB. Conversely, *A. stutchburyi* excretes $NH_4^+$, and this nutrient input has been shown to facilitate increased chl *a* production (Sandwell et al., 2009; Jones et al., 2011), which may also explain the increased chl *a* biomass in cores from the site where *A. stutchburyi* were prevalent. My findings were also in contrast to the findings of Josefson et al. (2002), which found overall higher chl *a* biomass associated with communities dominated by deposit feeders compared to suspension feeding dominated communities, however in their study the deposit feeding community was dominated largely by subsurface deposit feeders, which may not directly feed on surface chl *a*. Conversely, more added *Ulva* (higher % $^{15}N$) was measured in cores collected from ML compared to cores collected from AS, although these differences were not significant. It has been shown, however, that community interactions may be more important for MPB biomass and distribution than direct feeding effects (Pratt et al., 2014).

The depth distribution profiles of chl *a* were markedly different in cores collected from the two sites, and supported the prediction that organic matter would mix deeper into sediments dominated by *A. stutchburyi*. Cores from ML showed an exponential decrease in chl *a* biomass with depth, a trend which is consistent with other studies (Boon & Duineveld, 1998; Ingalls et al., 2000), while chl *a* was almost evenly distributed throughout the 10 cm in cores collected from AS sites. *A. stutchburyi* is a shallow living, efficient bioturbator, that turns and mixes the

sediment as it moves (Morton & Miller, 1973; Sandwell et al., 2009), which supports the well mixed sediment chl *a* patterns observed in my study. *M. liliana*, however, is a deep living species that mainly mix the top few mm of sediment through its feeding behaviour (Lelieveld et al., 2004), which explains the exponential decrease in chl *a* biomass with depth. Furthermore, the bioturbating activities of *A. stutchburyi* increases the permeability of the sediment which may drive chl *a* further down in the sediment (Lohrer et al., 2004; Pratt et al., 2014). *A. stutchburyi* were also found in higher densities per core compared to *M. liliana* at the AS site (approximately 7 and 3, respectively) and therefore occupied a higher biovolume, which has been suggested as an important predictor of sediment reworking (Gilbert et al., 2007). The chl *a* biomass from cores collected from AS were much more variable compared to cores collected from ML. This is likely due to the patchy distribution of the macrofauna (Boon & Duineveld, 1998) as well as the bioturbating activity exhibited by *A. stutchburyi*.

Significantly less *Ulva* was recovered in the top 0 – 1 cm at the end of the 10-day isotope experiment compared to the reference cores which were sampled 24 h post addition. Interestingly, the labelled *Ulva* was not distributed throughout the core at the end of the experiment, with less than 15% of the total added recovered at depth (1 – 5 cm) for cores from AS, ML and RC, and this result was not significant between sites. The non-significant result between the RC and the cores from AS and ML, which were sampled at the end of the experiment, suggests that any of the labelled *Ulva* recovered at this depth after 10-d was not due to sediment reworking by the communities present, but most likely a smearing effect of the core collection procedure. As most of the labelled *Ulva* was clearly still concentrated in the surface sediment, the depth distribution pattern of chl *a* below a 1 cm depth can be attributed to MPB biomass, and does not include the labelled material. It is likely that the duration of the study (10-d) was too short to document these trends in the added isotopically labelled *Ulva*, but was evident in the already present MPB community, which constitutes a large proportion of the chl *a* signature. It is also possible that the macrofauna were less active or modified their behaviour under the laboratory conditions. Approximately the same amount of the isotopically labelled *Ulva* was unaccounted for at the termination of the experiment at AS and ML and the differences in macrofaunal uptake (see Chapter

5) were balanced by what was found in the sediment. These findings indicate that organic matter and detrital processing was slower at ML, and suggest that the unaccounted amount was likely not metabolised, but simply lost from the system.

Community composition has also been shown to be an important factor in the fate of phytodetritus in a system (Josefson et al., 2002), which further explains the differences in the chl *a* profiles between the two sites, which showed significantly different species richness (higher in AS cores). FL bioturbators, LS bioturbators and *A. stutchburyi* were all significantly more abundant in cores collected from AS compared to ML. However, our results showed that there was no density dependent effect of *A. stutchburyi* or LS bioturbators on the distribution of organic matter (chl *a* or *Ulva*) in the sediment. Chl *a* was negatively correlated with *M. liliana* density, however, this negative correlation was only observed in cores collected from AS sites, but no correlation was found between *M. liliana* density and chl *a* biomass in cores collected from ML.

Studies have shown higher ingestion rates in deposit feeders when more food is available (Taghon & Jumars, 1984; Boon & Duineveld, 1998), which could explain why *M. liliana* density was only relevant in cores collected from AS, which had four times more chl *a* compared to cores from ML, and why the correlation was negative (i.e. active feeding). Unlike the negative correlations observed between chl *a* biomass and *M. liliana* densities, the % $^{15}$N labelled *Ulva* that was recovered at the 1 – 5 cm depth was marginally positively correlated with *M. liliana* density at the AS site. There was also a positive correlation between the *Ulva* recovered and LS bioturbators at this site. Similar findings have been reported by Josefson et al. (2002), where the vertical distribution of $^{14}$C labelled phytodetritus was positively correlated with subsurface deposit feeders. *M. liliana* feeds directly on the sediment surface and defecates at depth, thus potentially increasing the concentration of organic matter at depth (Wilcock et al., 1993; Thrush et al., 2006; Volkenborn et al., 2012). *A. stutchburyi* has been shown to act as a facilitator for detrital uptake in benthic communities. For example, uptake of *Ulva* by the community was three times greater in cores collected from the AS compared to the ML site (see Chapter 5). This may explain why *M. liliana*

densities were only relevant in the cores from AS, as these cores also contained relatively high densities of *A. stutchburyi*.

The conflicting trends between the chl *a* and *Ulva* recovered suggests that the fate of organic matter in sediments with intact communities are complex and cannot simply be explained by active feeding or bioturbating behaviour. Other studies have reported both positive and negative relationships between the mixing of chl *a* and artificial tracers in sediments with increased abundances of large deposit feeding bivalves (Ingalls et al., 2000; Duport et al., 2006). Unlike the chl *a* biomass, there were no negative correlations between the amount of labelled *Ulva* that was recovered from the sediment and *M. liliana* densities, suggesting that *M. liliana* may preferentially feed on MPB and not the labelled *Ulva*. This is further supported by low recorded $^{15}N$ uptake in *M. liliana* (Chapter 5).

## 4.5 Conclusions

This study examined the density dependent effects of two key bivalve species on the distribution and processing of macroalgal detritus in intertidal communities. Less of the added material was not recovered from the ML compared to the AS site as predicted, however, the chl *a* was more evenly mixed through the sediment profile in AS cores as predicted. My prediction that the amount of material recovered would be dependent on the density of the most dominant macrofauna (in biomass) at each site (*A. stutchburyi* at AS and *M. liliana* at ML) was not supported by the results, however community composition as a whole were important. In conclusion, the results show that the presence of key species and community composition are important factors in sediment mixing and the processing of organic matter, but that this relationship is complex.

# 5.0 DENSITY OF KEY-SPECIES DETERMINES EFFICIENCY OF MACROALGAE DETRITUS UPTAKE BY INTERTIDAL BENTHIC COMMUNITIES

## 5.1 Introduction

Considering the major community changes, including species losses, documented worldwide in recent years there is an urgent need to gain a mechanistic understanding of the relationship between biodiversity and ecosystem functioning which ultimately affects the ecological services provided to humanity (Cardinale et al., 2006, 2012). Studies show that increased biodiversity has a positive effect on ecosystem functions, such as primary production, decomposition of organic matter and nutrient regeneration, but the pattern of response varies depending on the ecosystem and species investigated (Balvanera et al., 2006; Cardinale et al., 2006, 2012; Hiddink et al., 2009). Much of what we know about the role of biodiversity in mediating ecosystem functioning stems from manipulative laboratory experiments. Although they have helped articulating hypotheses and provided mechanistic explanations for observed patterns they do not incorporate habitat complexity or allow long-term community dynamics and feedback processes to develop (Thrush & Lohrer, 2012; Snelgrove et al., 2014; Thrush et al., 2014). A key challenge in the field of biodiversity-ecosystem function research is to demonstrate whether the observed importance of biodiversity in controlled experimental assemblages also persists in natural systems (Larsen et al., 2005; Hiddink et al., 2009; Lohrer et al., 2010; Naeem et al., 2012).

Biodiversity explains variation from the level of genes to ecosystems, with species richness (number of species) being the most commonly used measure in studies examining biodiversity and ecosystem function relationships. Species richness is representative of an environment as it is determined by prevailing biotic and abiotic conditions, as well as being a logistically achievable measure, and is therefore appropriate for such studies. Three main hypotheses have been proposed that relate the responses of ecosystem functioning to species richness. First, the linear or "rivet" hypothesis suggests that all species contribute critically and approximately equally to ecosystem function (Lawton, 1994). Second, the "redundancy" hypothesis suggests that ecosystems can lose many species with no consequences for ecosystem performance, as long as the major functional groups are still present, i.e. it is not the number of species per se which is important but the functional traits of the species (Walker, 1992; Lawton, 1994). Redundant species are considered necessary only to ensure ecosystem resilience to perturbation (Walker, 1992). Third, the "idiosyncratic" hypothesis states that species diversity affects ecosystem functioning, but not in a predictable direction, because the roles of individual species are complex and context-dependent (Lawton, 1994; Naeem et al., 1995). Biodiversity-ecosystem function relationships within any system may be determined by any combination of these three hypotheses. However, there are further important components of biodiversity that may affect these relationships, including the density of a species (Chapin et al., 2000; Dangles & Malmqvist, 2004). In many cases it has been shown that certain key-species, rather than species richness, can have a disproportionate effect on ecosystem functioning such as nutrient cycling and productivity (Widdicombe & Austen, 1998; Lohrer et al., 2004; Thrush et al., 2006; Rossi et al., 2013). Loss of a key-species would result in a rapid decline in ecosystem functioning ([Naeem et al., 2002] c.f. rivet hypothesis) as this species is unique and cannot be replaced by another species with similar functional traits (c.f. redundancy hypothesis). Changes in species abundance patterns may have important consequences for ecosystems long before a species is threatened by extinction (Chapin et al., 2000). At local scales, variations in the absolute density and relative abundance of species can modify biodiversity-ecosystem functioning relationships. For example, the per capita performance of individual species may increase as their density declines, reflecting reduced intraspecific competition

(Karlson et al., 2010). Also, decreased relative abundance of one species may likely alter complementary resource use or facilitation (McKie et al., 2008). The hypotheses listed above would have difficulty in accounting for these common shifts in biodiversity.

Marine soft sediments cover more than 70% of Earth's surface and play a critical role in the global storage and cycling of nutrients and energy (Snelgrove et al., 1997; Covich et al., 2004; Larsen et al., 2005). Benthic invertebrate species often contribute idiosyncratically to ecosystem functioning with their impact strongly dependent on species identity and their functional role (Emmerson & Raffaelli, 2000; Emmerson et al., 2001; Bolam et al., 2002). There is also some support for the redundancy hypothesis. Raffaelli et al. (2003) grouped species by functional group according to their mode of bioturbation and found that increased species richness of benthic macrofauna belonging to different functional groups had a significant effect on nutrient fluxes from sediments, while increased species richness within the same functional group had no effect. Complete extinctions of regional species pools are however comparatively rare in the marine benthos whereas compositional changes and reductions in abundance and biomass are common (Carlton, 1993; Elahi et al., 2015). These changes in benthic abundance and biomass can be important drivers of ecosystem functioning as they direct species dominance patterns and functioning (e.g. infaunal community structure and diversity (Widdicombe & Austen, 1999), bioturbation potential, and degradation patterns (Lohrer et al., 2004; Sandwell et al., 2009; Norkko et al., 2013).

New Zealand sandflats provide an ideal system to investigate the contribution of species composition and abundance to ecosystem functioning because the macrofaunal community is species rich and has diverse functional groups. Using small-scale patchiness (0.01 $m^2$) in the density of key-species, we compared the uptake of macroalgal detritus by the benthic infaunal community. This process is a fundamental ecosystem function where benthic infauna converts dead organic material to secondary production, which is available for higher trophic levels such as fish. The isotope tracing technique enables quantifiable measurement of detrital uptake by all species in the community; resolving trophic relationships and the

outcomes of species interactions which amount to uptake at the community level (Karlson et al., 2010). This approach also enables the detection of subtle diversity effects, which could be masked from key-species effects when studying cumulative processes only (e.g. nutrient fluxes or bioturbation depth [Raffaelli et al., 2003]). The use of intact cores with natural infaunal communities under controlled laboratory conditions and the ability to relate macroalgal uptake to the behaviour of individual species and their distribution in the sediment gives greater insight into the mechanisms underlying the relationships between biodiversity and ecosystem functioning than is typical of studies where the contribution of individual species to community interactions cannot be disentangled.

The two dominant bivalves on New Zealand intertidal sandflats, the large, mainly surface deposit-feeding deep-burrowing tenellid *Macomona liliana* and the large suspension-feeding endemic venerid *Austrovenus stutchburyi*, influence sediment characteristics and community composition, which affects ecosystem functions such as nutrient fluxes, metabolism and primary production (Thrush et al., 2006; Sandwell et al., 2009; Jones et al., 2011). We predict that densities of these large key-species will drive the patterns of uptake of algal detritus by macrofauna (c.f. the key-species hypothesis, a variant of the rivet hypothesis [Naeem et al., 2002]) but that higher functional group diversity as measured by diversity indices will also contribute in explaining uptake (redundancy hypothesis). Our knowledge of the natural history of key-species allows us to hypothesise that (i) higher densities of *M. liliana* will facilitate uptake of macroalgae detritus by sub-surface deposit feeders, since they draw organic material from the sediment surface with their inhalant siphon and defecate at depth, enhancing the concentration of organic matter at 5–10 cm below the sediment surface (Volkenborn et al., 2012). In contrast, (ii) high densities of *M. liliana* should decrease uptake by small surface-feeding infauna due to exploitative and interference competition in the surface layer (as found for macrofauna-meiofauna interactions [Nascimento et al., 2011]). Furthermore, we hypothesise that (iii) high densities of the clam *A. stutchburyi* will facilitate macroalgal uptake by surface-feeding infauna, since clams, if feeding on resuspended macroalgal detritus, would produce organic-rich deposits in the surface sediment thereby facilitating uptake by other infauna (Norkko et al., 2001). However, in laboratory conditions where resuspended material will settle

again, their bioturbation and mixing in the upper centimetres of sediment (Whitlatch et al., 1997; Sandwell et al., 2009) may rework algal detritus into the sediment and eventually increase food access also for sub-surface feeders. To summarize, in this study we investigate whether relationships between species diversity, functional group diversity and densities of key-species and ecosystem functioning (detritus uptake) occur in natural communities. We test this using a multiple regression approach; uptake of algal detritus at both an individual level (per capita uptake by each species) and at the community level (total uptake by the whole community) is evaluated in relation to these measures of community structure.

## 5.2 Methods

### *5.2.1 Macroalgal labelling*

The macroalgal species *Ulva* sp. which blooms in estuaries (Teichberg et al., 2010) and later decomposes in soft sediments was collected on 10$^{th}$ Oct, 2011, from northern Tauranga Harbour, New Zealand, at low tide. Healthy looking thalli were rinsed in GFC filtered seawater and distributed among aquaria comprising an *Ulva* to seawater ratio of 10 g ww $L^{-1}$. Two days later, we labelled *Ulva* with stable carbon and nitrogen isotopes by adding 5% $Na^{15}NO_3$, 10% $(^{15}NH_4)_2SO_4$ and 99% $NaH^{13}CO_3$ to the seawater in quantities similar to Rossi (2006). We also added $KH_2PO_4$ according to the Redfield ratio to improve growth condition and hence ensure that assimilation of isotopes would result in sufficient isotope enrichment. *Ulva* was placed in a constant temperature room set at 18$^0$C under on a 12 h light:dark cycle for 6-d. The thalli were then carefully and repeatedly rinsed in MilliQ water, quickly dried using paper towels, freeze-dried and ground to a fine homogenised powder using a ball mill. The labelled macroalgae was sampled for stable isotope analyses (see below) and stored frozen until the start of the experiment. Isotope analyses confirmed a strong labelling of the *Ulva* material; $\delta^{15}N$ = 9597 ± 95‰, $\delta^{13}C$ = 1745 ± 11‰ compared to unlabelled *Ulva*; $\delta^{15}N$ = 8 and $\delta^{13}C$ = -12.

### *5.2.2 Collection of intact cores*

On 22nd Nov, 2011, we collected intact sediment cores from Tuapiro Point, Tauranga Harbour (see Figure 1.2). Animal ethics approval/permits were not sought as benthic invertebrate fauna used in this study are exempt from the Animal Welfare Act 1999. The collection of benthic fauna was undertaken with a Ministry of Primary Industries Special Permit (560) Client Number 8770024. At low tide, 78 cores (12.5 cm in diameter, 20 cm deep) were selectively taken from a known *Austrovenus stutchburyi* bed (S 37° 29.390, E 175°57.014) and *Macomona liliana* bed (S 37° 29.344, E 175°57.094) located approx. 50 m apart. Sediment properties were similar at both sites; the median grain size was 183 and 192 µm, mud content (< 63 µm) 5.2 and 3.3% and organic matter content 2.1 and 1.8% at the *Austrovenus* and *Macomona* sites, respectively.

Salinity and temperature was 29.3 and 16.7°C on the outgoing tide and 25.9 and 20.1°C on the incoming tide on the day of sampling. The distinct feeding tracks of *M. liliana* and the holes created by anemones (*Anthopleura aureoradiata)* attached to *A. stutchburyi* enabled estimates of their respective abundances so that cores ranging from low to high bivalve density could be collected and to avoid destructive sampling of individuals close to core edges. Preliminary sampling indicated higher species richness at the *Austrovenus* than the *Macomona* site so the former site was sampled more intensively. After sacrificing some cores for initial analyses (see below) there were 41 and 29 experimental cores for the *Austrovenus* and *Macomona* site respectively to which labelled *Ulva* were added.

Back at the laboratory, cores from the two sites were randomly allocated to 12 tanks that were connected to a flow-through seawater system that generated a 12 h tidal cycle with a 6 h immersion/emersion period. The cores were fitted with an 800 µm mesh net around the circumference of the core that was extended above the simulated "high tide" mark to prevent amphipods escaping. An 800 µm mesh net also covered the base of each core so water could drain through the sediment with the rise and fall of the "tide". The thermo-constant laboratory had windows, which allowed natural light to reach the cores (PAR 4.3 ± 2 µE, 15 cm above the

sediment surface). The light:dark cycle was 12:12 h (8 am: 8 pm). Artificial saltwater was used in the experiment (salinity 29.4) and temperature set at 19°C.

### *5.2.3 Start of experiment*

The cores were left to acclimatize for two tidal cycles. At low tide the next day (23 Nov), 0.60 ± 0.01 g dw of the labelled, finely ground *Ulva* was mixed with 20 ml seawater and added to each core by carefully spreading it evenly on the sediment surface using a Pasteur pipette. Recovery of added *Ulva* from sub-sampling sediment in six cores containing few (< 2) *M. liliana* and *A. stutchburyi* after 24 h was 97 ± 3%, supporting visual observations and verifying that detrital recovery at the end of the experiment could be attributed to faunal activity rather than resuspension and loss due to the simulated tidal cycles.

### *5.2.4 Experimental procedures and termination of experiment*

The experiment was checked twice a day and occasionally dead *A. stutchburyi* were carefully removed from the surface sediment. After 10-d each core was sieved on a 500 µm mesh and fauna preserved in 70% ethanol until sorted to species level under a stereomicroscope. All specimens were counted and biomass measured (after drying at 60°C) or estimated. For larger polychaetes which were often incomplete, a width-biomass relationship ($r^2$ = 0.84-0.92) was established from intact individuals of each species (Paavo et al., 2008). Bivalves were weighed without shells since we were interested in macroalgal uptake in organic material.

The eleven most common macrofaunal species were selected for isotope analyses. Within species, similar sized individuals were selected to minimize biomass/growth dependent enrichment (Wolf et al., 2009). Only adult individuals were used for isotope analyses with the exception of *Naineris* sp. which were present as juveniles only. For abundant species with a small biomass (*Aonides trifada, Prionospio aucklandica, Naineris* sp.), the first 10-20 individuals encountered (to obtain enough biomass for analyses) from each core were

collected and transferred to a pre-weighed tin capsule. For amphipods (*Parawaldeckia* sp.), about 6 individuals core$^{-1}$ were used. Larger species (*Scoloplos cylindrifer, Orbinia papillosa, Nereis* sp., *Heteromastus filiformis, Nucula* sp. *M. liliana* and *A. stutchburyi*) were weighed or measured individually, pooled then homogenised to get a representative sample for isotope analyses from each core. Other species either had too small biomass for isotope analyses or did not occur in enough cores to allow statistical analysis, however a few of these additional species were screened for enrichment to improve community uptake estimates.

### *5.2.5 Isotope analyses and calculations*

Aliquots (about 2 mg dw) of samples for isotope analyses of carbon and nitrogen were packed in tin capsules and analysed at the Chemistry Department, University of Otago, in a Carlo Erba NA1500 elemental analyser coupled to a Europa 20/20 mass spectrometer. Internal standards, which were calibrated against international standards, were run in each batch of samples. The average standard deviation for all runs was ± 0.2 for $\delta^{15}N$ and ± 0.1 for $\delta^{13}C$. The C and N isotope ratios are expressed in the ‰ notation, using the equation:

$$\delta R\ (‰) = ([R_{sample}/R_{standard}]^{-1}) \times 10^3 \quad (1)$$

where R is the ratio between the heavy and light isotopes ($^{13}C$:$^{12}C$ or $^{15}N$:$^{14}N$). The stable isotope ratio, denoted by δ, is defined as the deviation in ‰ from an international reference standard (Vienna PeeDee Belemnite for C, and atmospheric nitrogen gas for N). Higher δ values indicate a higher proportion of the heavy isotope.

To quantify the macroalgal (*Ulva* sp.) nitrogen (N) taken up in faunal tissue, a linear two-source mixing model was used (Karlson et al., 2010):

$$f1 + f2 = 1;\ f1 = (\delta_{sample} - \delta_{source2})/(\delta_{source1} - \delta_{source2}) \quad (2)$$

where f1 is the proportion of *Ulva* N in the animal sample and f2 is the proportion of N derived from the initial sediment. The amount (mg) of *Ulva*-N taken up in each animal was calculated from the mixing model (proportion N from *Ulva*) and the total N content (mg) in the animal. This amount was extrapolated to the number of individuals of this species found in this core. To obtain community uptake of *Ulva*-N the species-specific total uptake values (based on core-specific density) were summed. Uncorrected δ values were used in the mixing model, since species-specific differences in fractionation (Goedkoop et al., 2006) and fat content (Post et al., 2007) were negligible compared to the strong labelling. C uptake is not shown since $\delta^{13}C$ and $\delta^{15}N$ enrichment were highly correlated for all species, Pearson product moment correlation $r > 0.95$.

### *5.2.6 Functional group categorisation and selection of species fro statistical analyses of uptake*

All species were included in a biological traits matrix containing 32 traits based on an organism's living position, sediment topographic features created, the direction of sediment particle movement, the degree of motility, feeding behaviour, body size, shape and hardness (Hewitt et al., 2008). Based on these traits, species were assigned to functional groups (Greenfield, 2014), see Table 5.1. The large key-species (*M. liliana* and *A. stutchburyi*) were separated into single species functional groups (deposit-feeding bivalves and suspension-feeding bivalves). The overall prediction was that densities of these two functional groups (species) as well as the functional group and species diversity of the community would determine per capita uptake by infauna, and hence total community uptake (summed uptake by all community members). Species diversity and functional group diversity were calculated for each core using Shannon's H' which accounts for both abundance and evenness of the species (or functional groups) present and is therefore a density-independent measure of community composition.

**Table 5.1.** Species and functional group assignment. Each species was assigned to a functional group (FG) based on Greenfield et al. (2014). Densities of FGs in bold (1-6) were included as explanatory variables in statistical analyses. Only adult specimens of *S. cylindrifer*, *M. liliana* and *A. stutchburyi* were included in the FG, as juveniles were expected to confound possible density effects. For *Naineris* sp. only juveniles were found. Species in bold were selected for isotope analyses and used as response variables in separate statistical tests. Underlined species were screened for isotope enrichment (per capita uptake) but not included in statistical analyses because of insufficient enrichment (e.g. *A. stutchburyi*) or low abundance.

| FG | Body | Feeding | Position | Movement | Species |
|---|---|---|---|---|---|
| **1** | **calcified** | **suspension** | **top 2 cm** | **freely mobile** | *Austrovenus stutchburyi* |
| **2** | **calcified** | **deposit** | **deep** | **limited mobility** | ***Macomona liliana*** |
| **3** | **soft** | **deposit** | **below surface** | **freely mobile** | ***Orbinia papillosa*** |
| | | | | | ***Scoloplos cylindrifer*** |
| | | | | | *Scolecolepides benhami* |
| **4** | **soft** | **deposit** | **below surface** | **limited mobility** | *Paradoneis lyra* |
| | | | | | *Magelona dakini* |
| | | | | | ***Aonides trifida*** |
| | | | | | ***Prionospio aucklandica*** |
| | | | | | *Scolelepis* sp. |
| | | | | | ***Naineris*** sp. |
| **5** | **soft** | **deposit, head-down** | **below surface** | **limited mobility** | ***Heteromastus filiformis*** |
| | | | | | *Heteromastus sp.* |
| **6** | **soft** | **predator/ scavenger** | **below surface/deep** | **freely mobile** | ***Nereididae*** (unspecified) |
| | | | | | *Phyllodocidae* |
| | | | | | *Nemertean* |
| 7 | rigid | deposit/ predator/ scavenger/ grazer | top 2 cm | freely mobile | ***Parawaldeckia spp*** |
| | | | | | *Phoxocephalidae spp* |
| 8 | calcified | deposit | top 2 cm | limited mobility | ***Nucula hartvigiana*** |
| 9 | calcified | suspension | attached | not mobile | *Barnacle* (unspecified) |
| | | | | | *Limpet* (unspecified) |
| 10 | calcified | deposit/ predator/ scavenger/ grazer | above surface | freely mobile | *Cominella glandiformis* |
| | | | | | *Diloma subrostrata* |
| | | | | | *Zeacumantus lutulentus* |
| 11 | soft | suspension | attached | not mobile | *Anthopleura aureoradiata* |
| 12 | soft | suspension | attached, tube | not mobile | *Boccardia syrtis* |
| 13 | soft | deposit | deep | freely mobile | *Capitella sp.* |
| 14 | soft | predator/ scavenger | top 2 cm | limited mobility | *Edwardsia sp.* |
| | | | | | *Oligochaeta* |
| 15 | rigid | predator/ scavenger | above surface | freely mobile | *Halicarcinus* (unspecified, juvenile) |
| 16 | rigid | predator/ scavenger | below surface, burrow | freely mobile | *Heterosquilla* |

In addition to key species density and diversity indices we also included the densities of another four functional groups as explanatory variables in statistical analyses since their biomass and/or abundance dominated community structure (i.e. they constituted 73 ± 13% and > 95% of total abundance and biomass, respectively). The additional four functional groups selected were: "Head-down deposit feeders" (also represented by only one species, the capitellid *Heteromastus filiformis*), "Large, mobile deposit-feeding polychaetes" (mainly Orbiniids, dominated by *S. cylindrifer*), "Large, mobile predators/scavengers" (mainly Nereids and Nemertines) and "Small, surface-deposit-feeding polychaetes" (mainly spionids, highly abundant). See Table 5.1 for details on the classification of all functional groups and Table 5.2 for site macrofauna metadata.

As response variables, in the statistical approach taken (described under Data analyses and statistics), we used $\delta^{15}N$ enrichment of the ten most abundant species (Table 5.1, one test for each species), and total uptake of *Ulva*-derived nitrogen by the macrofaunal community. Only three of the selected species were abundant at both sites (see results) and so statistical analyses were restricted to within-site comparisons with the exception of community uptake. Differences in isotope enrichment among species depends partly on differences in feeding mode (Karlson et al., 2010, 2011) and partly on differences in growth rate and metabolic turnover, resulting in differences in time to reach isotopic equilibrium with the diet (Wolf et al., 2009). For this reason, we avoided statistical comparisons in $\delta^{15}N$ enrichment among species (for simplicity, referred to as per capita uptake throughout the paper). Since *A. stutchburyi* did not show any per capita uptake it was excluded as a response variable (but still included as a predictor variable, see above).

### *5.2.7 Data alalyses and statistics*

#### *5.2.7.1 Differences in macrofaunal community composition, biomass and total macroalgal N uptake between sites*

Multidimensional scaling using principal coordinate analysis (PCO) and permutational ANOVA (Permanova) as implemented in PERMANOVA+ of

PRIMER v6 (Anderson et al., 2008) were used to assess inter-site differences in community biomass and species and functional group composition. Analyses were based on the Bray-Curtis Similarity Index and fourth-root transformed abundance data (Anderson et al., 2008). PCO analyses considered species/functional groups with a Spearman correlation $\rho > 0.6$ with any of the first two ordination axes as significantly contributing to the difference between sites. Community biomass and community uptake of *Ulva*-derived N calculated for each core (see methods) was tested for differences between sites using Permanova. Biomass normalised N uptake (core-specific total uptake divided by total biomass of the core using only those species contributing to uptake (i.e. excluding *A. stutchburyi* biomass)) was tested in the same way to account for inter-site differences in biomass.

#### *5.2.7.2 Predictors of community macroalgal N uptake*

To test the overall prediction that community macroalgal N uptake was determined by density of functional groups and the functional diversity of the community, the relationship between community macroalgal N uptake and selected explanatory variables (that included total community biomass (all species) and those listed in Table 5.2) was assessed for each site separately, using distance-based linear models (DistLM) in PERMANOVA+ of PRIMER v6 (Anderson et al., 2008). DistLM is a multiple regression routine where a resemblance matrix (in this case based on Bray-Curtis distance of community macroalgal N uptake values using cores as samples) is regressed against a set of explanatory variables. Prior to analyses both response data and explanatory variables were square-root transformed to conform to normality. Skewness of the explanatory variables was inspected using pair-wise Draftsman plots of all variable combinations. The explanatory variables were generally not strongly correlated to each other (Pearson's $r <$ the critical 0.95 according to [Anderson et al., 2008]) and distributions were not strongly skewed. See Table 5.3 for relationships between the explanatory variables at each site and for sites combined. Marginal DistLM was first used to determine which variables accounted for a significant proportion of N uptake when considered alone in the model, ignoring all other variables. The variables included in the final DistLM-models for each species and site were selected using the 'best' selection procedure, which utilizes all possible combinations of explanatory variables to

determine which combination accounts for the greatest proportion of uptake explained in the models $R^2$ based on the corrected Akaike information criterion (AICc). To remove the effect of differential biomass between sites, biomass-normalized community uptake from both sites was tested in a DistLM with the addition of a categorical factor, site (*Macomona* or *Austrovenus*).

#### *5.2.7.3 Predictors of per capita uptake (individual $\delta^{15}N$ enrichment)*

The association between $\delta^{15}N$ isotope enrichment (per capita uptake) and the selected explanatory variables was assessed for each species and site separately using DistLM, as described above. This resulted in 12 species-specific models; nine for the *Austrovenus* site and three for the *Macomona* site. Since the main purpose of these individual uptake models was to generalize among responses and predictors we present only significant marginal results and the variables included in the 'best' model based on AICc. For those models where AICc values were within 2 units, the model with highest explanatory power was chosen rather than the most parsimonious model, since the purpose was to find the combinations of species that would best explain enrichment patterns. The specific hypotheses related to the effects of key species feeding mode on macroalgal N uptake by surface- and subsurface feeding infauna were determined by comparing whether *A. stutchburyi* (FG1) or M. *liliana* (FG2) were included in the best model for a species with those particular feeding modes (Table 5.6).

## 5.3 Results

### *5.3.1 Community composition and sediment characteristics at the Macomona and Austrovenus sites*

The *Austrovenus* site contained 30 macrofaunal species and 13 functional groups, while at the *Macomona* site only had 22 species and 9 functional groups (Table 5.2). There was a significant difference in macrofaunal community composition (based on species abundance) between the *Macomona* and *Austrovenus* sites (Pseudo-$F_{1,67} = 63.28$, $p = 0.0001$, Figure 5.1A).

**Table 5.2.** Macrofaunal metadata. Differences between the *Austrovenus* and *Macomona* sites in terms of infaunal species richness, functional group richness (FG), Shannon diversity index for species (H'SP) and functional groups (H'FG), total density of individuals and the density of the key FG, (see Table 5.1 for explanations to abbreviations). Values are mean ± 1 SD. Headings in bold are predictors for statistical analyses.

| | ***Austrovenus* site** | ***Macomona* site** |
|---|---|---|
| Species richness (# core-1) | 15.9 ± 2.4 | 8.9 ± 1.5 |
| FG richness (# core-1) | 9.9 ± 1.3 | 5.9 ± 1.2 |
| **H' SP** (core$^{-1}$) | 2.2 ± 0.2 | 1.8 ± 0.2 |
| **H' FG** (core$^{-1}$) | 1.7 ± 0.2 | 1.2 ± 0.2 |
| Density (ind. core$^{-1}$) | 125 ± 46 | 49 ± 16 |
| **FG1** (ind. core$^{-1}$) | 7.0 ± 6.1 | 0.5 ± 0.7 |
| **FG2** (ind. core$^{-1}$) | 3.6 ± 2.2 | 3.6 ± 2.3 |
| **FG3** (ind. core$^{-1}$) | 14.6 ± 7.3 | 6.3 ± 5.9 |
| **FG4** (ind. core$^{-1}$) | 40.5 ± 24.8 | 20.7 ± 9.3 |
| **FG5** (ind. core$^{-1}$) | 11.0 ± 8.6 | 0 |
| **FG6** (ind. core$^{-1}$) | 10.9 ± 6.3 | 5.0 ± 2.6 |

The same clear separation between sites was obtained when functional group composition was used (Permanova Pseudo-$F_{1,67}$ = 64.54, $p$ = 0.0001, Table 5.2; Figure 5.1B). However, the species which dominated the biomass (*A. stutchburyi* and *M. liliana* and the orbiniid *Scoloplos cylindrifer*) were present at both sites. Other common infaunal species and taxa commonly occurring at both sites were the polychaetes *Nereis* sp., *Prionospio aucklandica*, *Scolecolepides benhami*, *Scolelepis* sp., *Naineris* sp., the amphipod *Parawaldeckia* sp., the anemone *Edwardsia* sp. and Nemertines and Oligochaetes.

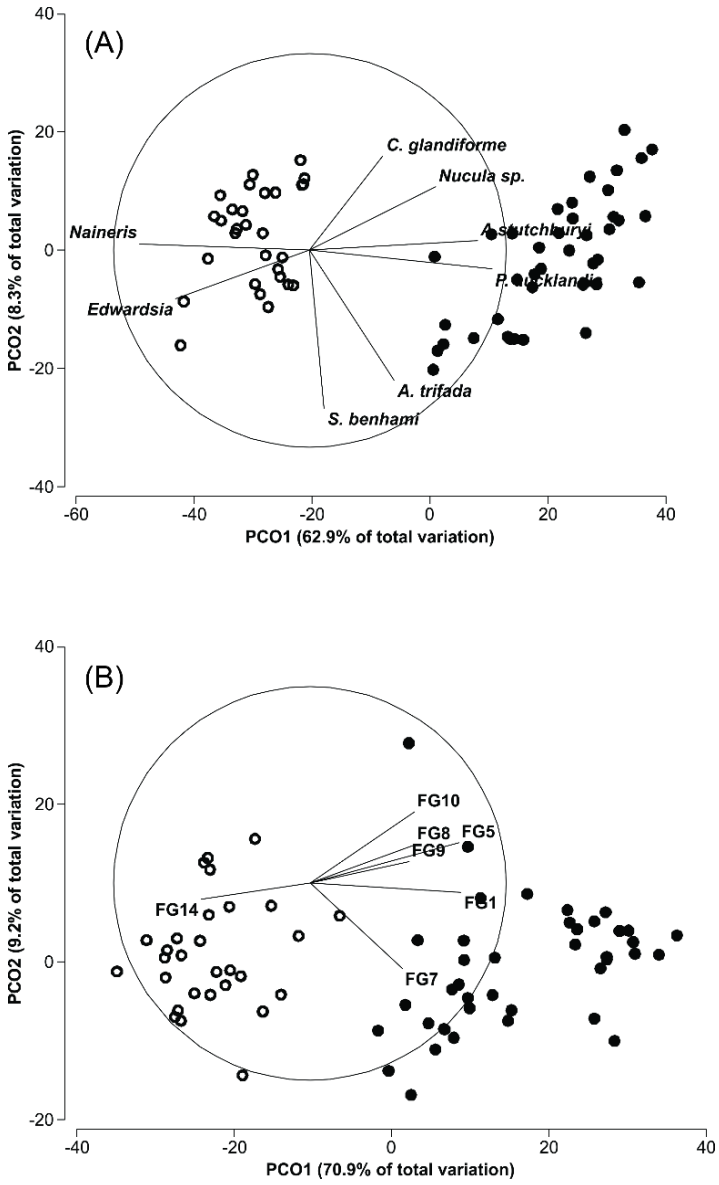


**Figure 5.1.** Results of a PCO analysis of (A) species composition and (B) functional group composition. Empty symbols represent the *Macomona* site and filled symbols the *Austrovenus* site. Only species or functional groups with a Spearman correlation $\rho > 0.6$ are shown. To improve clarity, *A. aureoradiata* and *Phoxocephalidae* were removed from (A) since they are highly correlated and nearly identical to the distribution of *A. stutchburyi*. Similarly, the distribution of *O. papillosa* was identical to *P. aucklandica* and Oligochaetes were identical to *Naineris* sp. See text for Permanova results and Table 5.1 for species and functional group explanations.

### *5.3.2 Isotope enrichment of Ulva and infauna*

The *Ulva* was highly enriched ($\delta^{15}N$ = 9597 ± 95‰, $\delta^{13}C$ = 1745 ± 11‰, mean ± SD, n = 3) relative to the sediment (c. $\delta^{15}N$ = 6‰, $\delta^{13}C$ = -15‰) and initial values for fauna (Figure 5.2A), enabling quantification of macroalgal uptake by benthic infauna (section below). Initial isotope values differed among species (Figure

5.2A). The isotope enrichment of the species at the end of the experiment (per capita uptake) varied both among and within species (Figure 5.2B). All species selected for statistical analyses were highly enriched compared to initial values, although in a few individuals (< 5%) of *M. liliana, O. papillosa* and *H. filiformis* minimal enrichment occurred. Anemones (*Anthopleura aureoradiata*) attached to *A. stutchburyi* were not expected to feed on *Ulva* detritus however the samples analysed for screening purposes revealed substantial enrichment ($\delta^{15}N = 56 \pm 30$, $\delta^{13}C = -5 \pm 2$, mean ± 1 SD, n = 6).

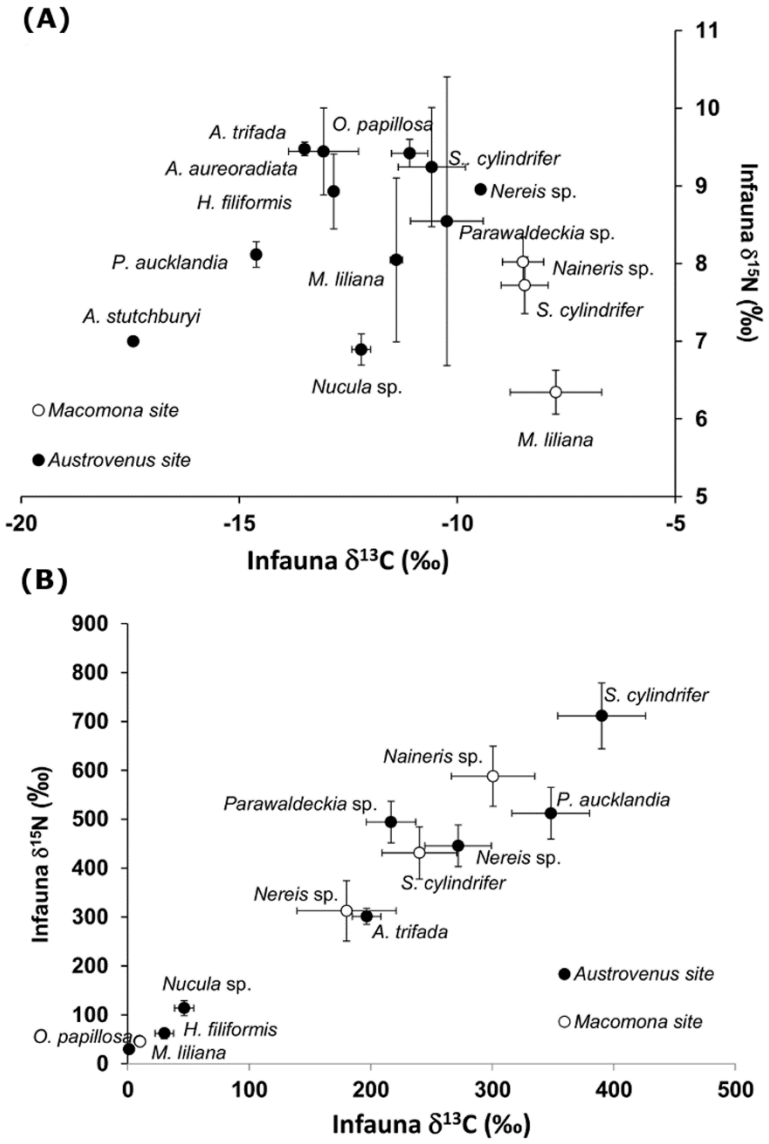


**Figure 5.2.** Initial natural abundance isotope values (A) and final enriched isotope values for infauna after the addition of isotope enriched *Ulva* detritus (B). The initial isotope values (mean ± 1 SD, n = 2-6) are shown for common species at both sites. The final values include only those species selected for statistical analysis (see methods) and the data represent the mean ± 1 SE (n = 23-35 except for *A. trifada* where n = 11 and *Nucula* sp. where n = 18).

### *5.3.3 Community uptake of macroalgal N in relation to predictors*

Community infaunal biomass was similar between sites, 0.56 ± 0.25 (*Austrovenus* site) and 0.53 ± 0.32 mg core$^{-1}$ (*Macomona* site) (Figure 5.3A), however the macrofaunal community at the *Austrovenus* sites had taken up approximately three times more *Ulva*-N than at the *Macomona* site (0.83 ± 0.86 vs 0.25 ± 0.13 mg; Permanova Pseudo-F = 16.779, $p$ = 0.0001). This difference was even more pronounced (5 times) after normalizing uptake by the enriched biomass since *A. stutchburyi* did not contribute to uptake (Pseudo-F = 21.877, $p$ = 0.0001). Two species, *M. liliana* and *S. cylindrifer*, were mainly responsible for the amount of *Ulva*-derived N taken up in faunal biomass during the experiment (Figure 5.3B). *S. cylindrifer* took up on average 89% of this nitrogen at the *Austrovenus* site and 33% at the *Macomona* site, whereas *M. liliana* took up 6% and 55% at the respective sites. This can be compared with the average contribution to community biomass by the same species; at the *Austrovenus* site, *A. stutchburyi*, *M. liliana*, and *S. cylindrifer* constituted 39%, 33% and 17% respectively; and at the *Macomona* site, 7%, 90% and 1%, respectively (Figure 5.3A).

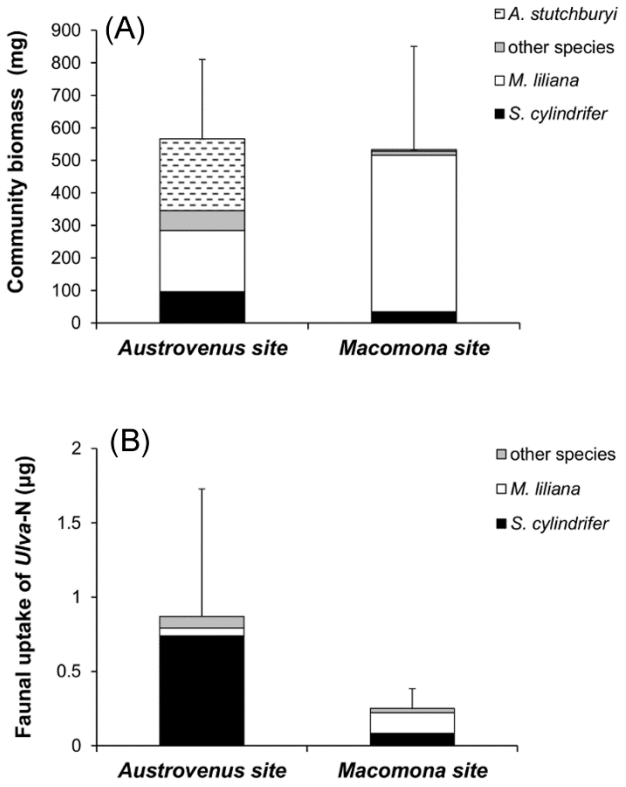


**Figure 5.3.** Infaunal community biomass and uptake of *Ulva*-derived nitrogen. Community biomass (shell-free dry weight) (A) and community uptake of macroalgal nitrogen (B) with the species contributing most at each site shown (mean ± 1 SD.)

Of the other species, only *Naineris* sp. (*Macomona* site, 7%) and *Nereis* sp. (*Austrovenus* site, 3%) contributed more than 1% to total community uptake. Accordingly, marginal tests showed that FG3, (i.e. *S. cylindrifer*) explained most of the variance in total community uptake at the *Austrovenus* site whereas *M. liliana* explained most of the variance at the *Macomona* site (Tables 5.3 and 5.4; Figure 5.4).

**Table 5.3.** Correlations between the predictors used in statistical analyses. Spearman rank correlations (ρ) between explanatory variables (densities of functional groups and diversity indices) used in DistLM analyses (Tables 5.4, 5.5 and 5.6). Values in bold are significant at $p < 0.05$. (A) *Austrovenus* site, (B) *Macomona* site and (C) both sites pooled. Abbreviations are defined in Tables 5.1 and 5.2. na = non-applicable predictor (this FG was missing for this site).

| **(A)** | **H'SP** | **H'FG** | **FG1** | **FG2** | **FG3** | **FG4** | **FG5** |
|---|---|---|---|---|---|---|---|
| **H'FG** | **0.63** | | | | | | |
| **FG1** | **0.32** | **0.61** | | | | | |
| **FG2** | 0.16 | -0.06 | -0.09 | | | | |
| **FG3** | -0.05 | 0.29 | 0.07 | -0.17 | | | |
| **FG4** | -0.3 | **-0.59** | -0.09 | -0.08 | -0.14 | | |
| **FG5** | 0.24 | **0.49** | **0.56** | -0.27 | 0 | 0.07 | |
| **FG6** | **0.32** | **0.34** | 0.21 | **-0.39** | 0.1 | -0.12 | **0.43** |
| **(B)** | | | | | | | |
| **H'FG** | **0.88** | | | | | | |
| **FG1** | 0.19 | 0.34 | | | | | |
| **FG2** | 0.33 | **0.44** | 0.01 | | | | |
| **FG3** | 0.25 | 0 | -0.26 | 0.31 | | | |
| **FG4** | **-0.57** | **-0.64** | -0.19 | -0.09 | 0.03 | | |
| **FG5** | na | na | na | na | na | na | |
| **FG6** | -0.07 | -0.11 | -0.07 | -0.23 | -0.04 | 0.19 | na |
| **(C)** | | | | | | | |
| **H'FG** | **0.84** | | | | | | |
| **FG1** | **0.68** | **0.79** | | | | | |
| **FG2** | 0.16 | 0.08 | -0.04 | | | | |
| **FG3** | **0.4** | **0.48** | **0.4** | 0 | | | |
| **FG4** | 0.12 | 0 | **0.32** | -0.11 | 0.21 | | |
| **FG5** | 0.24 | **0.49** | **0.56** | -0.27 | 0 | 0.07 | |
| **FG6** | **0.49** | **0.53** | **0.46** | **-0.25** | **0.3** | **0.25** | **0.43** |

**Table 5.4.** Predictors of total community uptake of Ulva-derived nitrogen. DistLM marginal test results reporting the proportion of total community N uptake at the *Austrovenus* (n = 41) and *Macomona* (n = 29) sites and both sites combined (biomass normalized) explained by diversity indices and FG densities). Marginal tests results describe how much variation each variable explains when considered alone, ignoring other variables. The (+) or (-) sign denote direction of the relationship, na = non-applicable predictor. Significant relationships are shown in Figure 5.4.

| Explanatory variable | *Austrovenus* site | *Macomona* site | Both sites |
|---|---|---|---|
| **Total biomass** | | na[1] | na[2] |
| **H'SP** | 0.08 (-)* | 0.10 (+)* | |
| **H'FG** | | 0.13 (+)** | |
| **FG1** | | | 0.20 (+)*** |
| **FG2** | 0.17 (-)*** | 0.58 (+)*** | 0.43 (-)*** |
| **FG3** | 0.44 (+)*** | 0.17 (+)** | 0.22 (+)*** |
| **FG4** | | | 0.04* |
| **FG5** | | na[3] | 0.30 (+)*** |
| **FG6** | | | |
| **Site** | na | na | 0.24*** |

*$p < 0.1$; **$p < 0.05$; ***$p < 0.01$

na[1] total biomass and *M. liliana* density were highly correlated ($p > 0.95$), thus, total biomass was not included in the analyses.

na[2] uptake was normalized for biomass when combining both data from both sites.

na[3] FG3 was absent from this site.

**Table 5.5.** 'Best' model of community uptake of Ulva-derived nitrogen for different numbers of predictor variables at the *Austrovenus* site, *Macomona* site and both sites pooled (biomass normalized). AICc denote corrected Akaike information criterion and $R^2$ is the total cumulative variance explained by the model.

| Number of variables | AICc | $R^2$ | Predictor variables |
|---|---|---|---|
| ***Austrovenus* site** | | | |
| 1 | 227.86 | 0.44 | FG3 |
| 2 | 224.18 | 0.52 | FG3, FG2 |
| 3 | 219.73 | 0.59 | FG3, FG2, H'SP |
| 4 | 220.60 | 0.61 | FG3, FG2, H'SP, FG1 |
| 5 | 221.22 | 0.63 | FG3, FG2 H'SP, FG1, FG6 |
| ***Macomona* site** | | | |
| 1 | 155.17 | 0.58 | FG2 |
| 2 | 151.35 | 0.66 | FG2, FG3 |
| 3 | 152.09 | 0.69 | FG2, FG3, FG1 |
| 4 | 153.11 | 0.71 | FG2, FG3, FG1, FG4 |
| 5 | 155.06 | 0.72 | FG2, FG3, FG1, FG4, H'FG |
| **Both sites** | | | |
| 1 | 417.68 | 0.43 | FG2 |
| 2 | 397.16 | 0.55 | FG2, site |
| 3 | 395.18 | 0.61 | FG2, site, FG3 |
| 4 | 393.65 | 0.63 | FG2, FG5, H'SP, site |
| 5 | 392.28 | 0.65 | FG2, FG3, FG5, H'SP, site |

Using biomass-normalized data across sites, the same two species as well as head-down feeders (i.e. *H. filiformis*), and site explained most of the variance. When the biomass effect of *M. liliana* is removed, the combined sites analysis shows that it has a negative effect on community uptake (in agreement with per capita uptake which is biomass independent). Even though not ranked as the most important predictors, it is worth noting that both functional group diversity (as predicted) and species diversity were significant in marginal tests or included in the 'best' model (Tables 5.4 and 5.5; Figure 5.4).

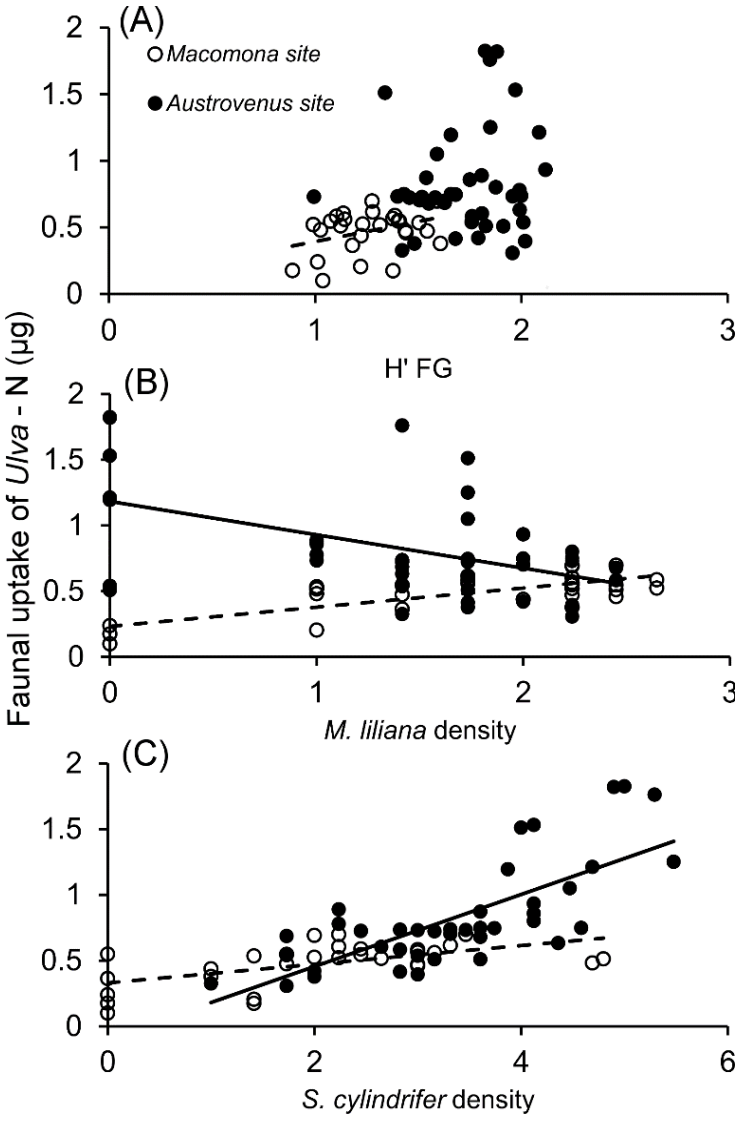


**Figure 5.4.** Total community uptake of *Ulva*-derived nitrogen in relation to (A) Shannon's H'FG; (B) *M. liliana* density and (C) *S. cylindrifer* density. Empty symbols and dotted lines represent the *Macomona* site and filled symbols and solid lines the *Austrovenus* site. Both uptake and densities are square-root transformed. Only these relationships were significant ($p < 0.05$) according to marginal tests in DistLM. See Table 5.4 for details on the statistical models.

### *5.3.4 Per capita uptake in relation to predictors*

The results from site and species specific DistLM analyses of per capita uptake ($\delta^{15}N$ enrichment on species level) are summarised in Table 5.6 (both marginal tests and 'best' models) and significant relationships are shown in Figure 5.5.

**Table 5.6.** Predictors of per capita uptake ($\delta^{15}N$ enrichment). A summary of marginal test and 'best' model results for (A) *Austrovenus* site and (B) *Macomona* site for the per capita uptake by each species (rows) as explained by species and functional group (FG) density and diversity indices (see Tables 5.1 and 5.2 for definitions of abbreviations). Numbers are the proportion of variance explained by single predictors (marginal tests) and the (+) or (-) denote direction of significant relationships. Values in bold denote parameters selected by $AIC_C$ to be included in the 'best model' and the $R^2$ is the total cumulative variance explained by the 'best model'. Non-significant variables or variables not included in the 'best model' are not shown. na = non-applicable predictor. Significant relationships are shown in Figure 5.5.

| Variable | H' SP | H' FG | FG1 | FG2 | FG3 | FG4 | FG5 | FG6 | $R^2$ |
|---|---|---|---|---|---|---|---|---|---|
| **A) *Austrovenus* site** | | | | | | | | | |
| *P. aucklandica* n=26 | | 0.32 (+)** | 0.12 (+)* | **0.22 (-) **** | 0.10 * | **0.31 (-) ***** | 0.20 (+)** | 0.21 (+)** | 0.51 |
| *A. trifada* n=11 | | **> 0.01** | | | | **0.18** | | | 0.39 |
| *Parawaldeckia* sp. n=24 | | | | **0.32 (-)***** | **0.13 *** | | 0.14 (+)* | | 0.35 |
| *Nucula* sp. n=18 | **0.48 (+)***** | 0.31 (+)*** | | **0.02** | | | **0.26 (+)***** | | 0.68 |
| *M. liliana* n=31 | | | **0.07** | | | | **0.06** | **0.04** | 0.17 |
| *S. cylindrifer* n=26 | | | | | | | **0.05** | **0.03** | 0.12 |
| *O. papillosa* n=23 | **0.10 (+)** | **0.01** | **0.04** | | | | | | 0.31 |
| *Nereis* sp. n=23 | | | | | | | **0.13 (+)*** | **0.22 (+)**** | 0.29 |
| *H. filiformis* n=35 | 0.09 * | **0.29 (+)***** | 0.17 (+)** | **0.19 (+)***** | | | **0.35 (+)***** | **0.23 (+)***** | 0.54 |
| **B) *Macomona* site** | | | | | | | | | |
| *Naineris* sp. n=25 | **0.02** | **0.03** | | | | | na | | 0.14 |
| *M. liliana* n=26 | | | **0.15 (+)**** | **0.01** | | | na | | 0.17 |
| *S. cylindrifer* n=21 | **0.01** | | | | | **0.22 (+)**** | na | **0.04** | 0.38 |

*$p < 0.1$; **$p < 0.05$; ***$p < 0.01$

At the *Austrovenus* site, *M. liliana* had, as hypothesised, a negative effect on the per capita uptake of the two surface feeders (*P. aucklandica* and *Parawaldeckia*

sp.) and a positive effect on *H. filiformis* (Table 5.6). *H. filiformis* in turn, was positively associated with per capita uptake in species representing different feeding modes (Figure 5.5). As predicted, this was the case also for species and functional group diversity, which were positively correlated with per capita uptake in one and three species, respectively. Per capita uptake of larger species (*S. cylindrifer, M. liliana*) had a lower proportion of variance explained than smaller species (this was true for both sites). *Naineris* sp. (abundant only at the *Macomona* site) had also no clear relationship to any of the explanatory variables included in the analyses.

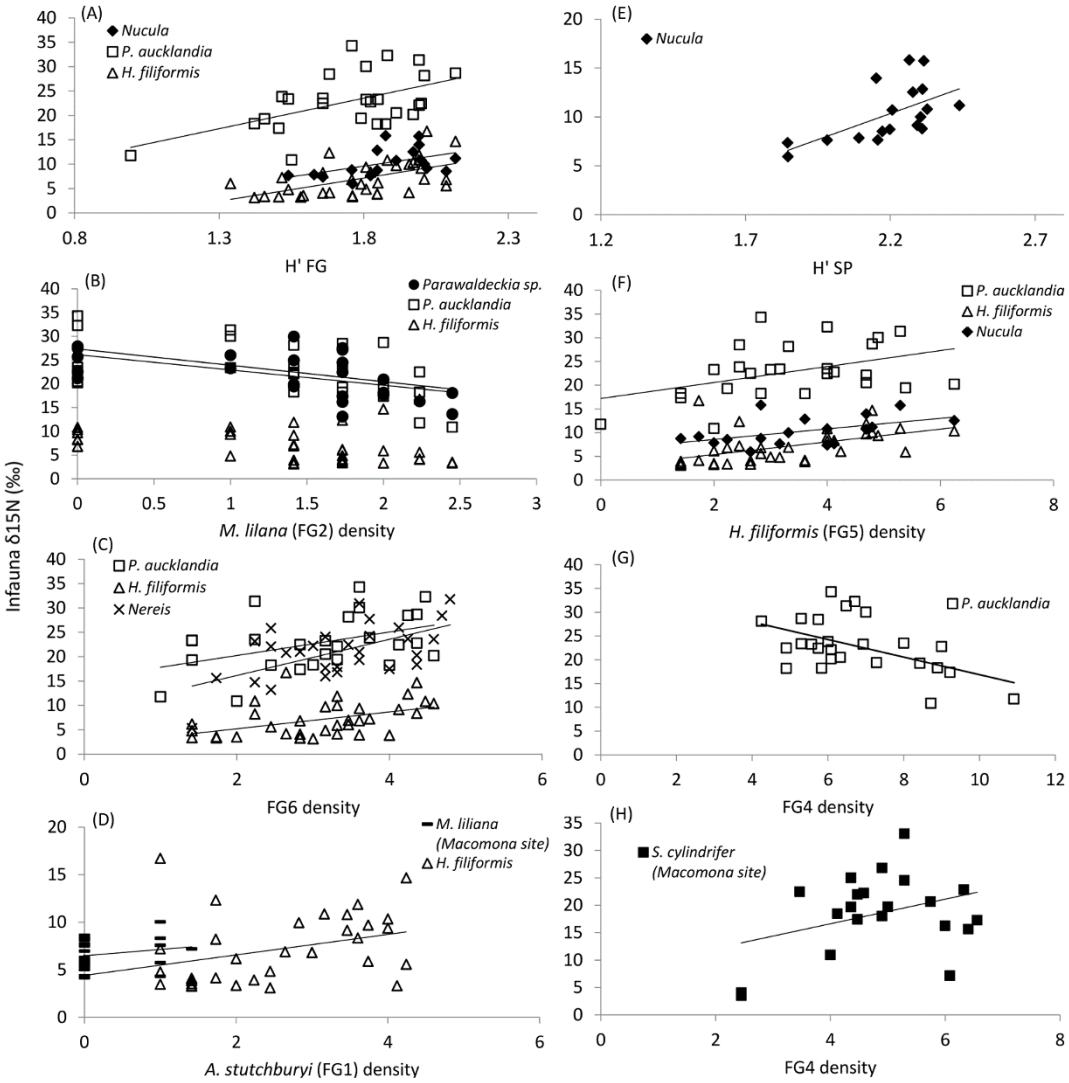


**Figure 5.5.** Per capita uptake ($\delta^{15}N$ enrichment in individual species) in relation to species and functional group (FG) density and diversity indices. Species are represented by different symbols. All species are from the *Austrovenus* site except for *M. liliana* (in D) and *S. cylindrifer* (H) in the bottom panels. Both uptake and densities are square-root transformed. Only significant relationships ($p < 0.05$) according to marginal tests in DistLM are shown. See Table 5.6 for details on the statistical models and Tables 5.1 and 5.2 for definitions of abbreviations.

## 5.4 Discussion

This study shows that densities of only a few species in natural communities strongly influence the community uptake of macroalgal detritus. Using isotopically labelled macroalgae, we were able to relate the macroalgae detrital uptake to the ecological role of individual species and demonstrate the importance of densities of key-species for influencing ecosystem functioning. Using natural communities restricts us to a correlative statistical approach which cannot be confused with the species substitution approach commonly used in traditional biodiversity-ecosystem functioning studies. Still, the use of an isotope tracer provides greater insight into the mechanisms underlying the relationships between biodiversity and ecosystem functioning than is typical of studies where cumulative processes such as nutrient fluxes are the only endpoints measured (e.g. Raffaelli et al., 2003). Further, by using intact benthic communities with known functional traits, complex direct and indirect interactions among naturally co-occurring species could be discerned.

Although the core constrains mobility of the species, those selected a priori as key-species are sedentary and likely to be less affected. Similarly, most of the species analysed for isotope enrichment are small in body size and the cores could be thought of as mesocosms rather than microcosms. The only exceptions are the few large and mobile polychaete species encountered and the uptake for these species accordingly had poorer statistical models in terms of proportion variance explained. It is however possible that their uptake rates are more influenced by environmental conditions rather than community structure in the field due to their mobility. By sampling gradients in density of a priori selected key-species and measuring detrital uptake (the first step in benthic secondary production), our study bridges a gap between controlled experiments with selected species combinations and field data, where environmental conditions are difficult to control.

Uptake of macroalgal (*Ulva*) nitrogen by the whole community was three-fold (or five-fold when normalized for biomass) greater in the *Austrovenus* dominated site compared to the *Macomona* site. Previous studies have documented the

importance of *Austrovenus stutchburyi* for ecosystem functioning due to physical properties of the bivalve bed and biological activities such as elevating sediment organic content through bio-deposition (Thrush et al., 2006; Sandwell et al., 2009). Our study demonstrates that *A. stutchburyi* also indirectly facilitates detrital uptake and food web efficiency in benthic infaunal communities, since its density was positively associated with higher functional diversity, species diversity and higher densities of the head-down feeder *Heteromastus filiformis* (FG5, Table 5.3) which, in turn, were all positively correlated to higher isotope enrichment on the individual level (hereafter referred to as per capita uptake of the *Ulva* nitrogen) for several species (Table 5.6). Further, *A. stutchburyi* density was positively related to per capita uptake in four species and it was the variable contributing most in explaining per capita uptake by *M. liliana* (at both sites). Although *A. stutchburyi* is a suspension-feeder, we expected some of the detritus, which was added as a fine powder to the sediment surface, to be resuspended from bioturbation activities and thereafter consumed and assimilated by the clam (Leduc et al., 2006). This was, however, not the case during the experimental period, perhaps due to their slow growth and metabolic turnover of bivalve foot muscle tissue (up to 1 year to reach isotopic equilibrium, [Raikow & Hamilton, 2001]) but perhaps also due to the sheltered hydrodynamic condition in the experimental set-up minimizing resuspension processes, meaning that our results potentially underestimate its direct contribution to community uptake. Below we discuss mechanistic reasons for higher community uptake in the *Austrovenus* site compared to the *Macomona* site by examining the species level data.

Higher densities of the head-down feeder *H. filiformis*, which was absent from the *Macomona* site, was positively related to per capita uptake in three of the surface-dwelling deposit-feeders (the spionid *Prionospio aucklandica*, the amphipod *Parawaldeckia* sp. and the small bivalve *Nucula* sp.) as well as in the omnivorous *Nereis* sp. and *H. filiformis* itself at the *Austrovenus* site. It was also included as a predictor of macroalgal uptake in the best model for five species (Table 5.6). Possibly, buried *Ulva* detritus was brought to surface layers again through the feeding mode of this species. Similar positive interactions between head-down feeders and other species performance have been found, e.g. Weinberg and Whitlatch (1983) reported increased growth of small suspension-feeding bivalves

when kept in close proximity to a polychaete with this feeding-mode. The other small polychaete *Aonides trifada* was only abundant when *H. filiformis* densities were low so this particular relationship could not be properly tested here. It is however possible that *A. trifada* also feeds deeper in the sediment and should not be categorised as a surface dwelling deposit-feeder since these two species had very similar initial isotope signatures (Figure 5.2B); relatively depleted $\delta^{13}C$ while enriched $\delta^{15}N$ values, indicating feeding primarily on aged organic matter in the sediment (Goedkoop et al., 2006; Karlson et al., 2015). In support of this, per capita uptake by *A. trifada* was not influenced negatively by *M. liliana* which feeds mainly in the surface sediment. In other systems, deposit-feeders separate resources by depth in sediment and/or by feeding on different fractions of the organic matter e.g. fresh and aged (Rudnick, 1989; Byrén et al., 2006; Nascimento et al., 2011). Such niche differentiation increases resource utilization and thus promotes a positive biodiversity–ecosystem functioning relationship, as suggested by Karlson et al. (2011). The initial isotope values of fauna suggest that there is a broader range of primary producers supporting the food web at the more species rich *Austrovenus* site compared to the *Macomona* site. Although the aim of this study was not to disentangle the importance of different primary producers to the diet of macrofauna, the more depleted $\delta^{13}C$ of *A. stutchburyi* indicates phytoplankton and macroalgae are the primary food sources whereas the enriched $\delta^{13}C$ of *M. liliana* (at both sites) suggests feeding on microphytobenthos and seagrass detritus (Leduc et al., 2006). The generally more enriched $\delta^{15}N$ values at the *Austrovenus* site compared to the *Macomona* site could indicate larger microbial conditioning of detritus that enrich nitrogen isotope values (Goedkoop et al., 2006), perhaps also an effect of the higher density of individuals and higher species richness at this site. Interpretation of these differences, however, requires caution since the fauna were preserved in ethanol prior to analyses which may enrich $\delta^{13}C$ values by a few ‰ (Kaehler & Pakhomov, 2001) although other studies have found negligible effects from ethanol preservation on $\delta^{13}C$ or $\delta^{15}N$ (e.g. Lau et al., 2012).

In contrast to the positive effect of the head-down feeding *H. filiformis*, as hypothesised, higher densities of *M. liliana* were negatively associated with per capita uptake in two surface-feeding species; *P. aucklandica* and the amphipod

*Parawaldeckia* sp. (both in marginal tests and in the best model results). This is likely due to the removal of added detritus from the surface sediment to deeper layers by the bivalve, and partly through consumption and defecation (as evident from the enriched isotope signal in *M. liliana* tissues demonstrating uptake of *Ulva*-derived nitrogen). There is a similar situation in the species-poor Baltic Sea, where the functionally and morphologically similar deposit-feeding bivalve *Macoma balthica* reduces access to food for other surface-feeding species, including amphipods (Òlafsson et al., 2005; Karlson et al., 2011) and through interference competition lowers uptake rates of phytodetritus by meiofauna (Nascimento et al., 2011). An alternative explanation is that increased oxygenation from the feeding mode of bivalves results in rapid mineralization of the organic matter by the bacterial community (Karlson et al., 2010). *M. liliana* generates pore-water pressure gradients during their feeding and burrowing behaviour that may stimulate bacterial activity through alteration of sediment oxygen dynamics (Volkenborn et al., 2012). The hypothesised increase in macroalgal uptake by sub-surface feeders, i.e. *H. filiformis*, along with higher densities of *M. liliana* (defecating at depth) was partly supported by our data (Table 5.6). Even more important in predicting *H. filiformis* per capita uptake was, however, higher functional group diversity (as Shannon H'FG), suggesting that more of the added material reached deeper in the sediment when more bioturbation modes are present. In a modelling study, Solan et al. (2004) found that loss of species richness leads to a decline in bioturbation depth.

Larger species, e.g. *M. liliana, S. cylindrifer* and *Nereis* sp. had a generally lower proportion of their respective per capita uptake explained by densities of other species/functional groups. For polychaetes, this is most likely due to their mobility, which enables them to feed in the whole sediment column. Interestingly, the functional group of large scavengers were selected in the best model for these species as well as for *H. filiformis*. We speculate that pre-conditioning of the refractory macroalgal food source resulting from feeding activities by e.g. *Nereis* sp., which is an opportunistic omnivore (the first species to show high uptake of isotopically labelled *Ulva* in the field after only 1 d of incubation), will facilitate uptake for the other species. This pre-conditioning is not likely to influence the isotope signal of the *Ulva* food source, since isotope fractionation effects are

negligible compared to the strong enrichment from the labelled macroalgae, especially in a 10-d experiment. Bioturbation activities by *Nereis* sp. result in spatially redistributed food sources, improving its availability to bacteria and hence promoting stable co-existence through such scale-based partitioning of resources (van Nugteren et al., 2009).

Species diversity (as Shannon H'SP) was positively associated with isotope enrichment in only one species, *Nucula* sp. (both in marginal tests and selected in the best model) while functional group diversity was significant for three species (*Nucula* sp., *P. aucklandica, H. filiformis*), although only selected in the best model for *H. filiformis*. Interestingly, not only per capita uptake but also density itself of *H. filiformis* was significantly positively correlated to functional group diversity (Table 5.3). The negative (*M. liliana*) and positive (*H. filiformis*) effects of key-species density on per capita uptake in smaller surface-feeders was also mirrored when their density was considered as a response variable. For example, *P. aucklandica* density was also negatively correlated with *M. liliana* density (Spearman $\rho = -0.34$, $p < 0.05$) whereas *Nucula* sp. and *P. aucklandica* densities were positively correlated to *H. filiformis* density ($\rho = 0.50\text{-}0.75$, $p < 0.05$). On a larger scale, these similarities between uptake and abundance could help explain why few spionids were found at the *M. liliana* dominated site. Thrush et al. (2006) in a field experiment removed large *M. liliana* which resulted in increases in the density of *P. aucklandica* and *A. trifada*. In agreement with these findings Baltic Sea clam and amphipod abundances are negatively correlated in the field, and so are their uptake rates in laboratory experiments (Karlson et al., 2011). Moreover, the negative relationship between meiofauna uptake rates and macrofaunal species diversity due to interference competition found in experimental work agree well with field data on meiofaunal abundance and biomass; both decreasing with higher macrofaunal diversity (Nascimento et al., 2011).

Although the spionid *P. aucklandica*, the amphipod *Parawaldeckia* sp. and the orbiniid *Naineris* sp. all had high per capita uptake and high densities, their small body mass (and hence low body nitrogen content), still down-weigh the importance of these species to total community uptake of macroalgal nitrogen. In contrast, *M. liliana*, which had a low per capita uptake during the experiment,

most likely due to its slower growth and turnover relative to polychaetes and amphipods, nevertheless was the top or second most important species for total macroalgal nitrogen uptake in the community, when taking its large body size into account. The orbiniid *S. cylindrifer* had both highest per capita uptake and a large body size meaning that a large amount of *Ulva*-derived nitrogen was taken up in its tissues (Figures 5.2 and 5.3), suggesting it is a key-species for the conversion of detritus to secondary production in this ecosystem. Due to competition, or other factors, the other orbiniid species had either too small body mass (*Naineris* sp.) or too low abundance and low per capita uptake (*O. papillosa*) to replace the function of *S. cylindrifer*. The fact that *S. cylindrifer* dominated macrofaunal community uptake suggests little redundancy for this particular ecosystem function during the initial rapid breakdown of macroalgal detritus. *M. liliana* and *H. filiformis* on the other hand, were the only representatives of their respective functional groups, meaning that species and functional identity cannot be differentiated, hence it is impossible to distinguish between the redundancy and rivet hypotheses. Indirectly, however, our results lend some support for the redundancy hypothesis, since functional group diversity (Shannon's H'FG) contributed significantly in explaining both per capita uptake and total community uptake. As expected from the redundancy hypothesis, functional group diversity correlated positively with community uptake only in the *Macomona* site which had low numbers of species and functional groups (Figure 5.4A). This observation that an ecosystem process rate saturates at a rather low number of species has been shown from experimental work with synthetic assemblages representing e.g. soil communities, but is rarely shown in natural assemblages (Chapin et al., 2000; Nielsen et al., 2011; Cardinale et al., 2012). However, the relatively short duration of the experiment limits uptake of the labelled nitrogen by slow growing or predatory (or omnivorous) animals. It is likely that the importance of species richness for detrital uptake increase over larger spatial and temporal scales, as has been shown for ecosystem processes (e.g. biomass production and cover) in both terrestrial and aquatic systems (Tilman et al., 2001; Stachowicz et al., 2008; Cardinale et al., 2012).

*S. cylindrifer* had no effect on per capita uptake in other species (with the possible exception of *Parawaldeckia* sp.). *H. filiformis,* on the other hand, did not have a

large uptake itself but instead facilitated uptake for surface-feeders through its unique bioturbation mode or by pre-conditioning the detritus into finer particles or more palatable material. The effect of *M. liliana*, also the only representative of its functional group (deposit-feeding large bivalves) was more ambivalent, since it negatively influenced per capita uptake of other, smaller surface-feeders through either exploitative and/or interference competition. However, as hypothesised it had a positive effect on *H. filiformis*, which in turn was positively associated to uptake rates of other community members. Finally, the large size of *M. liliana* resulted in this species dominating community uptake of macroalgal nitrogen at the *Macomona* site, supporting the importance of large body size for ecosystem functioning (Nascimento et al., 2011; Norkko et al., 2013; Nordström et al., 2015).

## 5.5 Conclusion

In conclusion, our results demonstrate the importance of species identity, body size and density for ecosystem functioning, showing that large key-species determine uptake of algal detritus by macrofauna. These findings highlight the complex interactions underlying loss of ecological services and underscore the importance of understanding compositional and density changes of key-species with declining biodiversity.

# 6.0 CHAPTER FIVE: SUMMARY, RECOMMENDATIONS AND CONCLUSIONS

## 6.1 Summary

The overall aim of my thesis was to examine the effects of macroalgae, in particular *Ulva*, on estuarine communities and the ecosystem functions they provide, and how these effects may change through time. Each research chapter encompassed *Ulva* in a different form, from when it first accumulates in estuaries as large sheets (Chapter 2), through the decomposition phase as it becomes detritus (Chapter 3), and finally where it is incorporated and moved into the sediment (Chapter 4), and into the food web (Chapter 5). The location of the field experiments (Chapters 2 and 3), as well as the mesocosm collection area for Chapters 4 and 5, was from the same estuary in Tauranga Harbour, to allow for cross-study comparisons.

Although the impacts of macroalgae (such as *Ulva*) on intertidal communities have been well documented (e.g. Everett, 1994; Norkko & Bonsdorff, 1996a, b, c; Cardoso et al., 2004), the flow on effects on wider ecosystem functions are poorly understood. In Chapter 2, I examined the effects of intact macroalgal mats on the sediment characteristic, macrofaunal community composition, and ecosystem functions (i.e. primary production, benthic respiration and nutrient regeneration) in an intertidal estuary in Tauranga Harbour, and how these effects changed through time. Subtle changes were observed in the macrofaunal community and sediment characteristics under the *Ulva* treatments, which in turn resulted in subtle shifts in gross primary production. Furthermore, temporal variation in macrofaunal community, sediment properties and ecosystem function were also measured.

As large sheets of *Ulva* begin to break down and decompose, it becomes detritus, and although some studies have examined the impact of detritus on macrofaunal communities, few have examined how these impacts vary over time (e.g. Rossi, 2006, 2007; Olabarria et al., 2010), and no previous research has incorporated temporal variation as well as different amounts of macroalgal detritus in a wider ecosystem function framework. In Chapter 3, I aimed to bridge this knowledge gap by examining changes in the sediment characteristics, community structure, and the ecosystem functions (as in Chapter 2) in an intertidal benthic community that was subject to different loads of *Ulva* detritus, and again document these changes through time. I found no significant differences in macrofaunal community composition or measures of ecosystem function with the different detrital addition treatments, however interesting temporal variations emerged. The macrofaunal communities as a whole differed between all three sampling dates, whilst ecosystem functions varied significantly between at least two of the sampling dates (i.e. nutrient efflux from the light and dark chambers, SOC and GPP). The impact of the detritus was, however, not more obvious at the first sampling date (i.e. W2) compared to later sampling periods (i.e. W4 and W8), as predicted.

As the detritus settles on the sediment surface, bioturbation and physical sediment mixing will start to incorporate the detritus into the sediment profile, where it is processed and reworked. In Chapter 4, I examined the density dependent effects of two key bivalve species (*A. stutchburyi* and *M. liliana*) on the distribution and processing of macroalgal detritus in intertidal communities by isotopically labelling and tracking *Ulva* detritus in a mesocosm experiment. Results showed that less labelled *Ulva* was retained in the sediment of the site dominated by *A. stutchburyi*, but that these sediments also had higher overall chl *a* biomass and chl *a* was distributed evenly throughout the sediment profile (10 cm core). These results highlighted the complex relationships between the specific community present and the way in which the *Ulva* is reworked and processed, as well as the importance of considering whole communities, and not just key species, when trying to understand sediment mixing and organic matter processing.

In Chapter 5, the natural, small-scale patchiness in the density of the suspension-feeding *A. stutchburyi* and deposit-feeding *M. liliana* was used to examine the density dependent effect of these bivalves on the community uptake of nitrogen from macroalgae detritus (i.e. measure of ecosystem function and food web efficiency). This was done in the same 10-d laboratory isotope-tracer experiment as Chapter 4. Results showed that *M. liliana* and *S. cylindrifer* dominated macroalgal nitrogen uptake in the community due to their large biomass. However, their densities were mostly not important or negatively influenced the macroalgal uptake by other species. Instead, the density of a head-down deposit-feeder (*H. filiformis*), scavengers (mainly Nemertines and Nereids) and species and functional group diversity best explained overall community uptake rates. These results demonstrated the importance of individual species, density and large body size for ecosystem functioning and highlighted the complex interactions underlying loss of ecological functions with declining biodiversity and compositional changes.

## 6.2 Macroalgae, benthic communities and ecosystem function

Although macroalgal blooms are a common occurrence worldwide, and the impacts of these natural disturbances on benthic communities are well documented (e.g. Everett, 1994; Norkko & Bonsdorff, 1996a, b, c; Cardoso et al., 2004; Valença et al., 2016), my research found only subtle shifts in the benthic communities under simulated *Ulva* bloom conditions, and I did not observe significant changes in key species within these communities. Furthermore, these shifts generally did not lead to big shifts in ecosystem function. Key species are often large contributors to the overall ecosystem function, and it is therefore not surprising that ecosystem functions like primary production, benthic metabolism and nutrient regeneration remained largely unchanged by the addition of *Ulva*, both in large sheets (Chapter 2) and as detritus (Chapter 3).

I expected noticeable impacts, especially with the addition of large mats of *Ulva*, which has been shown to smother the benthic community and lead to anoxic sediments (Perkins & Abbott, 1972; Norkko & Bonsdorff, 1996b; Reise, 2012).

Such changes would have clear impacts on the ability of the ecosystem to function, and would significantly alter benthic primary production, benthic respiration and nutrient regeneration. Although no major impacts on benthic ecosystem function was observed, subtle changes were recorded as a result of both *Ulva* mats (Chapter 2) and *Ulva* detritus (Chapter 3). These results suggest that the relationship between benthic estuarine communities, measured ecosystem functions and *Ulva* mats are complex and unpredictable. Factors that may have contributed to the unpredictability of the results across the studies were identified and have been discussed in individual chapters (e.g. the methods used to retain the *Ulva* in the experimental plots, the spatial scales of the experimental plots and the environmental conditions at each of the two sites). A holistic review of the results from the three experimental chapters allows for general conclusions to be drawn on the importance of *Ulva*, during different stages of the decomposition cycle, in shaping the macrofaunal community and the associated ecosystem functions.

As conditions for growth deteriorates, large *Ulva* sheets will settle on the sediment surface and quickly start to break down and become detritus. The transition of *Ulva* from sheets to detritus, allows for easier incorporation into the sediments, and eventually into the food web (Chapter 4). Under larger sheets or mats, hypoxia may occur, however this is less likely to happen once the *Ulva* becomes detritus. As a result, it was predicted that the macrofaunal community would respond differently depending on the physical form of the *Ulva* (i.e. sheets versus detritus). The studies conducted here found that the abundance and species richness of the macrofaunal community at this site, were not significantly impacted by either in-tact sheets of *Ulva* or *Ulva* detritus, as shown by the results from Chapters 2 and 3, respectively. However, the overall community structure was impacted by the large sheets of *Ulva*, while the detrital additions did not have a significant impact on overall community structure. The reworking of *Ulva* into sediments, that was examined in Chapters 4 and 5, highlighted the importance of community composition in the processing of *Ulva* detritus. The community composition in Chapter 3 more closely resembled the AS site of Chapter 4, where bioturbation and sediment reworking were rapid. Chapter 4 ran for 10-d, and over this time scale much of the originally added *Ulva* was either reworked into the sediment or incorporated into the food web (Chapters 4 and 5, respectively). It is

therefore perhaps not surprising that more significant shifts in ecosystem function was not measured, when Chapters 2 and 3 covered even longer time scales. Future research should consider the rapid turnover of organic matter in dynamic system such as intertidal sandflats.

## 6.3 Recommendations and concluding remarks

*Ulva* that was still visible in the experimental treatments by the time the first samples were collected were minimal in both Chapters 2 and 3. The detrital addition experiment (Chapter 3) was carried out one year prior to the experiment where whole *Ulva* mats were added (Chapter 2). The results from Chapter 3 suggested that perhaps two weeks was too long to wait before the first sampling, and that the dynamic nature of the system, and the small spatial scales of the experimental treatments, could have resulted in the recovery of affected macrofauna or ecosystem functions before the first samples were taken. The first sampling for the experiment in Chapter 2 was therefore carried out 1 d after the mats were removed, to ensure that initial impacts on the biodiversity and ecosystem functions were captured. In this experiment, however, much of the *Ulva* appeared to have been washed out of the mesh bags as the *Ulva* began to break down into smaller pieces. Although this accurately depicts the natural breakdown of *Ulva* in estuarine systems, it eased the disturbance pressure intended in the experiment, which probably impacted on the overall results of the study. Although logistics are often challenging in the natural environment, the results from both studies still suggest that the *Ulva* had some impacts on benthic communities and the functions they provide, although future studies should build on the methods used here to contain the *Ulva* more effectively within the experimental plots.

Another avenue that could be explored in future research is the spatial scale of the macroalgal disturbance. Studies have shown that macrofaunal recovery after a disturbance will largely depend on the spatial extent of the event (e.g. Thrush et al., 1996; Whitlatch et al., 1998; Norkko et al., 2006), however the impact of spatial scale manipulations in the larger ecosystem function framework has not

yet been examined. It will therefore be interesting to examine the impacts of varying spatial scales of macroalgal disturbances on macrofaunal biodiversity and ecosystem functions.

Another potential avenue for research is to compare the impact of macroalgae on communities and ecosystem functions associated with different habitat types, e.g. sand flats *vs* mud flats *vs* seagrass beds. Chapter 4 highlighted the impact differences in community composition can have on the processing and reworking of *Ulva*, and this could be explored further. In estuarine systems, macrofaunal assemblages, richness and abundance vary greatly depending on habitat type and local environmental conditions (Whitlatch, 1977; Thrush et al., 2003, 2008; van Houte-Howes et al., 2004; Alfaro, 2006; Hewitt et al., 2008). Some habitats, e.g. mudflats, are naturally more anoxic compared to sandflat and contain more opportunistic species or species that are adapted and resilient to hypoxic conditions, and so may have a better recovery rate following a disturbance which induces hypoxia (Dauer, 1984; Norkko & Bonsdorff, 1996b; Whomersley et al., 2010). It is therefore expected that the recovery of biodiversity and ecosystem functioning following a disturbance event will vary as a function of habitat (Cardoso et al., 2004; Hewitt et al., 2008; Lohrer et al., 2010), although this has not previously been explored. Cross habitat comparisons are therefore needed to accurately predict the impact of disturbances on estuarine communities (Giller et al., 2004; Hewitt et al., 2008). Estuarine intertidal habitats are ideal systems for examining the diversity-functioning relationship across different habitats, as a single intertidal area often comprise a variety of habitats (sand, mud, seagrass, mangroves), have extremely high levels of biodiversity (encompassing numerous functional groups), are relatively easy to manipulate, and periodically experience sharp reductions in biodiversity following natural disturbance events.

Overall, the results from the three studies highlighted the important and complex interactions between biotic and abiotic components in estuaries. The functions of this particular ecosystem appeared to be robust and difficult to shift. In the literature, a shift in community composition often assumes a shift in ecosystem function (Marinelli & Williams, 2003; Lohrer et al., 2004; Thrush et al., 2006; Sandwell et al., 2009; Jones et al., 2011), however, the results from these studies

propose that this is not always the case. Furthermore, temporal changes, largely driven by environmental conditions such as light availability and temperature, are more prominent drivers in the differences observed in ecosystem function compared to the treatment effects of *Ulva* addition. My results also highlighted the impacts of community composition on the processing and reworking of macroalgal detritus, which further underlines the complexity of estuarine systems. Ultimately, the results from all three studies suggest that in this particular system, providing abundances of key species remain intact, the community composition can shift and species can be lost without a loss or shift in overall ecosystem function.

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# APPENDIX 1: BENTHIC MACROFAUNAL SPECIES LIST AT TUAPIRO POINT

**Table A1.1. Benthic macrofaunal species list at Tuapiro point.**

| Species | Ind. core$^{-1}$ | SE |
|---|---|---|
| *Macomona liliana* | 2.70 | 0.24 |
| *Austrovenus stutchburyi* | 1.70 | 0.28 |
| *Lasaea parengaensis* | 9.68 | 0.94 |
| Nereididae | 7.32 | 0.78 |
| *Prionospio aucklandica* | 5.16 | 2.70 |
| *Scoloplos cylindifera* | 4.03 | 0.50 |
| *Aonides trifida* | 3.70 | 0.73 |
| Lysianassidae | 1.24 | 0.21 |
| *Microspio maori* | 0.70 | 0.25 |
| *Heteromastus filiformis* | 0.57 | 0.27 |
| *Zeacumantus lutulentus* | 0.43 | 0.16 |
| *Anthopleura aureoradiata* | 0.41 | 0.13 |
| Oligochaeta | 0.30 | 0.20 |
| Edwardsia sp | 0.27 | 0.07 |
| *Cominella glandiformis* | 0.27 | 0.08 |
| *Nucula hartvigiana* | 0.19 | 0.14 |
| *Paradoneis lyra* | 0.16 | 0.16 |
| *Arthritica bifurca* | 0.14 | 0.09 |
| *Haminoea zelandiae* | 0.14 | 0.07 |
| Nemertea | 0.11 | 0.05 |
| *Orbina papillosa* | 0.11 | 0.06 |
| *Colurostylis lemurum* | 0.11 | 0.05 |
| *Halicarcinus whitei* | 0.08 | 0.05 |
| *Scolecolepides benhami* | 0.03 | 0.03 |
| *Paracalliope novizealandiae* | 0.03 | 0.03 |
| *Magelona dakini* | 0.03 | 0.03 |
| Sipuncula | 0.03 | 0.03 |
| *Hemigrapsus crenulatus* | 0.03 | 0.03 |
| *Trochodata dendyi* | 0.03 | 0.03 |