Effects of macrofauna diversity on porewater nutrient concentrations following enrichment

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Frontispiece

"Sometimes in the waves of change we find our true direction" – Unknown.

Abstract

Macrofauna play a key role in the functioning of soft sediment intertidal ecosystems via bioturbation and feeding habits which modify sediment biogeochemistry and influence nutrient cycling. Eutrophication is a naturally occurring phenomenon; however, enrichment due to anthropogenic inputs has increased in frequency with largely unknown consequences to benthic macrofaunal assemblages. A decline in infaunal biodiversity is thought to result in the loss of ecosystem function due to increased disturbance in the form of enhanced nutrient addition. This is increasingly important to estuarine habitats, as benthic macrofauna play an important role in controlling sediment porewater nutrient concentrations, nutrient flux to the overlying water column and ultimately ecosystem function. Sediment modification by macrofauna behaviours (e.g. bioturbation) stimulate nutrient regeneration and influence denitrification rate. Thus understanding the responses of macrofauna to enhanced nutrient levels is vital for the understanding and subsequent management of benthic assemblages to estuarine eutrophication. The aim of this thesis was to stress the intertidal sediment of the Kaipara Harbour by the addition of slow-release fertiliser in order to identify key macrofaunal diversity responses and the influence this has in the overall nutrient processing ability. I also examined whether fertiliser addition influenced primary producers, microphytobenthos biomass and the percent coverage of seagrass.

To gain a better understanding of porewater nutrient elevation and subsequent impacts to macrofaunal diversity, 28 site locations were selected based on high and low functional macrofaunal diversity and abundance characteristics previously identified within Tapora Bank, Kaipara Harbour (Greenfield 2013). Known amounts (1400g m^{-2} (high treatment) and 600g m^{-2} (medium treatment)) of 70-day slow-release fertiliser (42 % N) was added to the intertidal sediment across a gradient in macrofaunal diversity. Porewater and sediment properties were measured 28 and 47 days after enrichment, with macrofaunal diversity determined on day 47.

The fertiliser enriched plots significantly elevated porewater ammonium concentrations in both treatments in upper (0-2 cm) and lower (5-7 cm) sediment

depths. Lower sampling depths had greater concentrations of porewater ammonium than the upper sediments. This elevation resulted a decline in the overall macrofaunal abundance in both addition treatments however, only the decline in key functional species *Macomona liliana* was significant. The number of functional individuals and number of *M. liliana* were identified as significant factors controlling the variation in porewater ammonium concentration in ambient sediments. A switch was observed after fertiliser elevation where mud become the sole driver of porewater ammonium concentration in plots of high fertiliser addition. Normalisation treatment porewater ammonium concentration by the control plot values identified both the number of species and number of functional species as important drivers of porewater ammonium processing. No effect of fertiliser enrichment to seagrass percent coverage was observed.

These results demonstrate that the elevation of porewater ammonium within intertidal sediments may have implications to the diversity and the subsequent functioning of intertidal benthic communities. In particularly, our study highlights the potential loss of functioning related to the decline of key species such as *M. liliana*; given their role as ecosystem engineers, their loss could reinforce the effects of eutrophication stress on the system and lead to further degradation.

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Dedicated to my loving parents, Lyn and Mike Hines,
and my best little friend, my shadow,
Love always
♥

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Chapter 1

Introduction:

1.1 Estuaries

An estuary is defined as a partially enclosed body of water where freshwater interacts with oceanic salt water (Pritchard 1967). This complex mixing of different water bodies creates a unique ecosystem with its own distinctive flora and fauna (Meire et al. 2005). Estuarine intertidal sandflats are areas of high diversity (Gray 1997) and are some of the most productive ecosystems in the world (Schelske & Odum 1961; Nixon et al. 1986; Wilson 1990; Snelgrove 1999; Beck et al. 2001). Estuaries are therefore regions of high intrinsic value and comprise many resources which are valued ecologically, economically and culturally (Nixon et al. 1986; Ellis et al. 2000; Levin et al. 2001).

Estuarine soft sediments provide goods and services from which humans and society benefit (Pearce & Turner 1990; De Groot 1994; Costanza et al. 1997; Daily et al. 1997; Pimentel et al. 1997; Barbier et al. 2010; Cardinale et al. 2012), including primary production, nutrient cycling and fluxes of energy (Cardinale et al. 2012). Other beneficial ecosystem services include food (e.g. fisheries), leisure and recreation, raw materials, disturbance prevention, nutrient recycling, water filtration, sediment retention and cognitive benefits (education and research) (Ewel et al. 1998; De Groot et al. 2002; Beaumont et al. 2007).

The marine soft sediments present a high spatial coverage of the earth (Snelgrove 1997, 1999; Ellingsen 2002). Coastal regions comprise less than 15 % of the Earth's surface; however, over 60 % of the world's population reside at the coast (Airoldi & Beck 2007). The coastal population continues to increase, thus increasing pressure on local ecosystems (Airoldi & Beck 2007). A number of previous studies have identified the severity of coastal marine habitat loss as a result of increased human population density (Lotze 2004; Lotze et al. 2006; Valiela 2009). Estuaries are influenced by a variety of natural and human-induced stressors including enhanced sediment deposition resulting in infilling, introduction of finer sediment,

nutrient runoff which can lead to eutrophication, pollution (both in solid and liquid form), invasive species, and fisheries exploitation (Carpenter et al. 1998; van der Wal et al. 2002). Such disturbance threatens the biodiversity and thus productivity of intertidal estuarine ecosystems, and its effects are enhanced when more than one stressor is present and when frequency is increased (Rapport et al. 1985; Halpern et al. 2007; Crain et al. 2008).

1.2 Eutrophication

Because estuaries are at the junction of land and sea (Pinckney et al. 2001), the enrichment of nutrients (eutrophication) is relatively common within estuarine environments (Nixon et al. 1986). Eutrophication occurs both as a natural phenomenon (upwelling and geological weathering) and as a result of anthropogenic influences (agriculture, wastewater treatment and urban runoff) (Smith 2003), and the rate of this enrichment is increasing (Nixon 1990; Anderson et al. 2002; Bricker et al. 2008). Estuarine eutrophication occurs where sediment or fertiliser runoff from land enters an estuary and this oversupply of nutrients causes plants and algae grow rapidly, decreasing the supply of oxygen and subsequently resulting in hypoxia in the water column following the decomposition of this plant material (Smith et al. 1999). Estuarine soft sediments are often anoxic just below the sediment surface, but when this anoxia extends above the sediment water interface the structure of the community is altered (Kennish & Townsend 2007). Eutrophication is alarmingly one of the greatest threats to coastal environments (Bricker et al. 2008), although each estuary responds differently to it (Bricker et al. 1999).

Eutrophication is possibly the best-documented anthropogenic disturbance to aquatic environments. Numerous studies have identified the effect of nutrient enrichment within marine environments where even though the focus species varies the overall effects identified are similar where the increased enrichment of nutrients results in a decline in diversity (Pearson & Rosenberg 1978; Worm et al. 1999; Szmant 2002; Cardoso et al. 2004).

Sediments provide resilience to excess nitrogen through denitrification: the reduction of nitrates to nitrogen gas (N_2). Increased nutrient loads can result in

changes to function where nutrient loading is continuous for long periods of time (Kemp et al. 1990; Hagy et al. 2004). This generally results in a shift from primary production in the benthos to the water column due to accelerated growth of phytoplankton and macroalgae (Smith et al. 2006), which as a consequence decreases the oxygen available within the surface sediments (Herbert 1999). This may have negative effects on the biota of those sediments.

Microphytobenthos (MPB) and seagrass comprise the major plant biomass within intertidal sandflats. MPB are effected by sediment properties and nutrient loading (Light & Beardall 1998; MacIntyre et al. 2004; Jesus et al. 2009) and show increased production with enhanced nutrients (Menéndez et al. 2002). Increased nutrient levels can effect seagrass beds positively, where the uptake of nutrients fuels growth. However, where nutrient levels are elevated for prolonged periods of time, a shift in species (Fourqurean et al. 1995) or a decline in seagrass coverage may occur (Lewis et al. 1985; Short & Wyllie-Echeverria 1996; Duarte et al. 2004). Seagrass decline is an increasing problem globally, with most seagrass lost in the past few decades as a consequence of anthropogenic disturbance, mainly due to eutrophication (Short & Wyllie-Echeverria 1996; Cardoso et al. 2004; Burkholder et al. 2007). Enhanced nutrient loading stimulates rapid growth of phytoplankton in turn reducing the light availability and thus photosynthesis, which limits the productivity of seagrass (Lee & Dunton 2000). The growth of seagrass is dependent on sediment porewater as a nutrient source (Fourqurean et al. 1992), but nutrients in excessively high loads are toxic to seagrass (Dennison 2009).

An effect of eutrophication is elevated porewater nutrient concentrations (Lapointe & O'Connell 1989; Lapointe & Clark 1992). This occurs where organic matter is broken down in the sediments into inorganic nutrients in the porewater, which then diffuse into the overlying water column. Porewater nutrient release affects the benthos environment locally and on greater spatial scales results in changes to nutrient concentrations within the intertidal region (Billerbeck et al. 2006). Porewater nutrient concentration reflects the balance between the supply of nutrients via bacterial breakdown of organic matter, excretion by organisms, and the consumption of these within the sediment. Therefore a reflection of the enrichment can be determined by sampling sediment porewater, as porewater

nutrient concentrations increase with increased eutrophication (Van der Heide et al. 2010).

1.3 Estuarine nitrogen cycle

Estuarine sediments act as a source and a sink for nutrients (Zimmerman & Benner 1994). The productivity within these sediments is controlled by nitrogen and light (Nixon 1981; Boynton et al. 1982). Nutrient cycling is responsible for the regeneration of ammonium (NH_4^+), although this varies seasonally (Harrison 1980). Primary production is limited by the amount of nitrogen within estuarine environments, so that with elevated inorganic nitrogen (e.g. nitrate NO_3^-, and ammonium) the likelihood of eutrophication increases (Ryther & Dunstan 1971; Howarth 1988; Howarth & Marino 2006). Ammonium regeneration and nitrification from the soft sediments control the nitrogen within the water column. Nitrogen enters an estuary in the form of nutrient runoff from land, nitrogen gas (N_2) or from precipitation. Through nitrogen fixation nitrogen gas is converted into ammonium or ammonia (NH_3), ammonium through nitrification is converted into nitrite (NO_2^-) and then to nitrate within oxic sediments. In anoxic sediments nitrate reduction occurs where nitrate is converted back to nitrite; denitrification then takes place where nitrite is converted to nitrous oxide (N_20) and nitrogen gas, where it re-enters the water column (Herbert 1999). Thus denitrification is responsible for removing excess nitrogen from the system. Some of the nitrite, however, will be further reduced to ammonium and locked within clay particles or undergo burial as organic nitrogen (Figure 1). The excretion from organisms, and the death and decomposition of phytoplankton and higher plants, and detritus entering the water column results in ammonification where the dissolved organic nitrogen is converted back into ammonium (Thamdrup & Dalsgaard 2002). Ammonium in large concentrations is toxic to soft sediment organisms and is a common measure for the indication of estuarine nutrient levels (Whiteman et al. 1996; Hyne & Everett 1998).

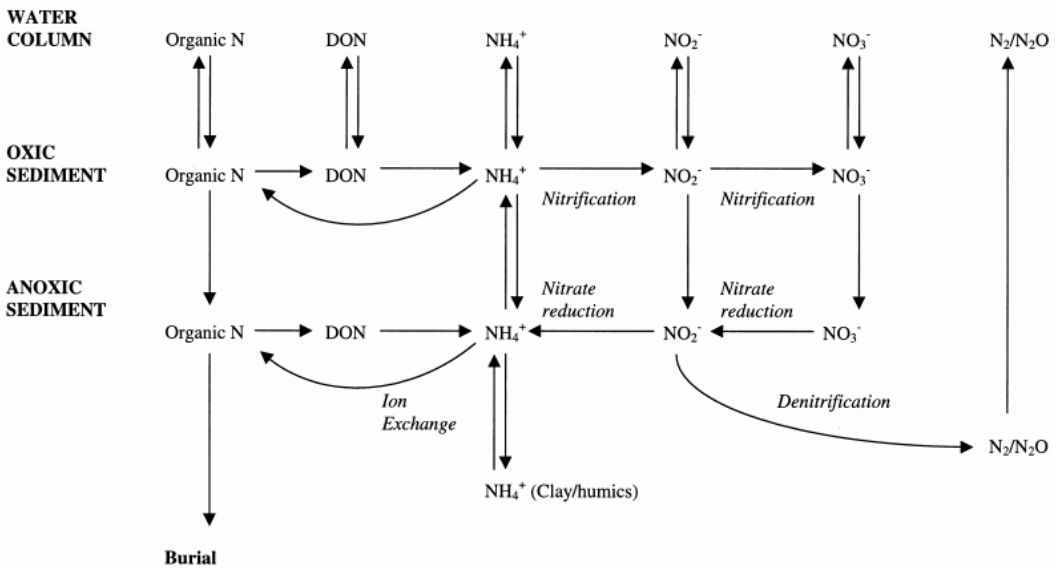

Figure 1: Nitrogen cycling within coastal sediments (Herbert 1999).

Nutrient cycling is an important process within estuarine environments as estuaries have continuous nutrient inputs derived from land. Nutrient cycling can be regulated by changes in macrofaunal density (Austen & Widdicombe 1998; Sandwell et al. 2009). The diversity of macrofauna within a sandflat can influence the rate of nutrient cycling as well as the amount of organic matter, resulting in changes to the functioning of the local ecosystem (Herman et al. 1999; Levin et al. 2001). The diversity of macrofauna can therefore provide enhanced resilience to an ecosystem (Peterson et al. 1998). The health of these systems is often represented by nitrogen processes and rates of these processes occurring within a system, which includes the remineralisation of organic matter, level of primary production and the cycling of nutrients (Klump & Martens 1981; Nixon 1981; Fisher et al. 1982; Boynton & Kemp 1985; Lohrer et al. 2004).

1.4 Biodiversity and ecosystem functioning

Biodiversity loss is a growing concern worldwide (Costanza et al. 1997; Armonies & Reise 2000), sparking much research and publication within recent years (Naeem 2002). Biodiversity is the variety of life present within an ecosystem and that variety makes a major contribution to ecosystem functioning and services (DeLong 1996; Bolam et al. 2002; Duffy 2008; Lohrer et al. 2011). A decline in biodiversity may translate to a loss of function (Walker 1992; Costanza et al. 1997; Dobson et al.

2006; Worm et al. 2006), thus biodiversity is increasingly being measured by key functional traits (Cardinale et al. 2012).

Functional traits are particular features of a species which relate to its function within an ecosystem. Grouping species by key functional traits presents a way of identifying which traits and species are thought to present patterns within the functioning of an ecosystem (Bremner et al. 2006; Norling et al. 2007). Common functional traits identified for soft sediment macrofauna include mobility, organism size, position within the sediment, trophic guild and feeding mode, because these features influence how much organisms bioturbate sediments and alter biogeochemistry and nutrient cycling. These traits have been linked to changes in organism distribution with exposure to disturbance or environmental stressors (e.g. enhanced nutrient levels) (Poore & Kudenov 1978; Beukema et al. 1999; Covich et al. 2004; Thrush et al. 2006a). The removal of important functional traits also greatly alters the community composition as well as the flux of nutrients and oxygen (Thrush et al. 2006a; Norkko et al. 2013). A number of studies have highlighted the importance of these traits, including size, which was identified as an important driver of ecosystem functioning (Thrush et al. 2006a; Norkko et al. 2013). Thus, such traits may play a crucial role in understanding the effects of eutrophication through their control on sediment porewater nutrient concentrations and flux of nutrients to the overlying water column.

While there has been considerable research into biodiversity and ecosystem functioning, little is known about changes to estuarine functioning following a disturbance (Lohrer et al. 2010). Disturbance can be physical, biological or chemical, or a combination of these and is recognised as a key driver of biodiversity loss. Often these disturbances are more readily identified as those which result in destruction of species habitat, increases in disturbance intensity, climate change, species overexploitation, eutrophication and invasion of non-native species (Gray 1997; Levin et al. 2001; Mouillot et al. 2013; Villnäs et al. 2013). These disturbances all influence the community's structure and its function through their effects on species' habitat, food and other resources.

The loss of biodiversity due to disturbance is accelerating, with its consequences and implications still being identified (Lotze et al. 2006; Worm et al. 2006). The

past two decades have seen advances in the understanding of species relationships, diversity and the processes found within ecosystems. However, identification of functionally important species and how these influence the overall functioning of an ecosystem remains the focus of much research (Loreau et al. 2001).

Both flora and fauna within soft sediment environments play key roles in primary and secondary production, providing a suite of services within the ecosystem (Levin et al. 2001; Austen et al. 2002). Some of these roles are not continuous, such that in the presence of other species some species' traits become redundant. Functional redundancy is a characteristic ecosystem trait, whereby in the absence of a species, another may be able to substitute its function by providing the same or similar service (Lawton & Brown 1994; Peterson et al. 1998; Rosenfeld 2002).

Macrofauna bioturbate and oxygenate sediments that contribute to benthic-pelagic coupling by mineralizing nutrients (Aller 1982; Kristensen & Blackburn 1987; Meysman et al. 2006). Bioturbation (biological perturbation) is a key physical function of macrofaunal species where oxygen is introduced to depths where in most cases it would be otherwise absent (Aller 1994), thus modifying sediment redox characteristics (Mortimer et al. 1999). Benthic-pelagic coupling is the exchange of both particles and solutes between the benthic sediment to the pelagic environment (Nixon et al. 1996; Marcus & Boero 1998). Therefore primary production within the pelagic zone is dependent on benthic nutrient regeneration processes (Nixon 1981).

Macrobenthic communities typically have limited movement and therefore changes in these communities often provide an indication of anthropogenic inputs and stressors and environmental change (Wass 1967; Gray 1981) within the benthos (Pearson & Rosenberg 1978; Dauer 1993; Wilson & Jeffrey 1994; Weisberg et al. 1997). Previous research has shown that macrofauna provide an important link to nutrient fluxes and their utilisation within the sediments (Biles et al. 2002; Sandwell et al. 2009; Braeckman et al. 2010; Needham et al. 2011), where changes in biodiversity subsequently alters the functioning of an ecosystem (Pratt et al. 2014). However, not all species contribute equally to functioning, and the functionality of a system may be dominated by a key species.

In New Zealand intertidal sandflats, a key species contributing to function is *Macomona liliana* a large deposit-feeder which acts as an important feedback link between nutrient regeneration and grazing activity (Thrush et al. 2006a). Another key contributor species is *Austrovenus stutchburyi*, where a decline in this species' density may have adverse effects on ecosystem function (Sandwell et al. 2009). Enhanced nutrient loading results in depletion of oxygen within the sediment, where the role of large macrofauna will be lost along with their function (Meyer-Reil & Köster 2000; Diaz & Rosenberg 2008). Thus, changes in function have been used to aid in the determination of biodiversity loss (Norkko et al. 2013).

The research presented in this thesis builds upon recent work (Greenfield 2013) that demonstrated functional group diversity within benthic sandflat communities. I intend to expand on this to determine whether different levels of functional diversity respond differently to nutrient addition in the form of slow-release fertiliser added to the intertidal sediments. This response was measured as porewater ammonium concentrations at two depth intervals on two sampling dates, providing insight as to how functional diversity contributes to ecosystem function, as the link between porewater ammonium enrichment and the impacts of this to high and low macrofaunal diversity are largely unknown within estuarine sediments.

The experimental design utilised previously identified natural gradients in species abundances and functional diversity (Greenfield 2013). By adding two different quantities of fertiliser to the sediment at different levels of functional diversity and abundance I can identify which of these (i.e. high or low diversity and abundance) is the most efficient at removing nutrients within the sediment porewater and whether there is any significant difference in porewater nutrient elevation between the two fertiliser treatments. A two way interaction is noted between macrofauna and porewater ammonium concentration, where macrofaunal diversity will influence the processing rate but will also respond to the nutrient elevation. Therefore high macrofaunal diversity may increase the processing rate of porewater nutrients; however, where porewater ammonium is in high concentrations this may cause removal of species and thus a decline in the rate of processing.

The enrichment of estuarine sandflats presents an advantage over laboratory experiments as the response of the natural system can be observed (Worm et al.

2000) and has been outlined in a number of studies (Worm et al. 2000; Lever & Valiela 2005; Posey et al. 2006). *In-situ* nutrient addition within marine sediments has been common using coated slow-release fertilisers (Worm et al. 2000). Studies on such additions allow for an understanding of how the flow-on effects alter community structure and function.

Functional diversity and abundance was ranked from high to low based on sampling from Greenfield (2013). The abundance and diversity sites were spread across the sandflat to cover a wide range of environmental conditions as well as to spread the experimental locations between areas of high and low functional diversity and abundance. Functional species are defined as those that contribute to estuarine functioning, and in the case of this research the term refers to the function of porewater nutrient processing. *A. stutchburyi* and *M. liliana* play a key role in the functioning of estuarine ecosystems in New Zealand (Hewitt et al. 1996; Tallis et al. 2004; Jones 2011), and the current study observed responses of both species' abundances to fertiliser enrichment in the form of ammonium concentration in the porewater. *M. liliana* is a surface deposit feeder: juveniles are found within the top 2 cm of sediment (Thrush et al. 2006a) while adults live within the top 5-15 cm of sediment (Hewitt et al. 1996). In contrast, *A. stutchburyi* is a suspension feeder found within the upper 2 cm of sediment (Thrush et al. 2006a), and grows to be greater than 30 mm in length (Powell 1979; Hewitt et al. 1996).

1.5 Objectives and hypotheses

To narrow the scope of this project I wanted to determine whether porewater nutrients can be elevated in treatments in both surface sediments and at depth and ultimately whether this elevation results adversely upon macrofaunal diversity, focusing largely on key functional species *A. stutchburyi* and *M. liliana*. I also wanted to determine whether the addition of fertiliser would influence primary producers, MPB biomass and the percent coverage of seagrass.

This research aims to stress the soft sediments by the addition of slow-release fertiliser in order to identify key functional diversity responses as a result of this stress. The two key functional responses examined include nutrient cycling and diversity between treatments. This research will allow identification of how the

system will cope with increased environmental pressure of nutrient enrichment in the form of fertiliser addition and whether this stays stagnant within the porewater or if it is readily utilised.

Specifically, the objective was to identify if sediment properties or functional diversity contribute to the natural variation in porewater ammonium across Tapora Bank sandflat of the Kaipara Harbour, and whether this changes with enhanced nutrient levels. This will aid in the understanding of why some regions within the same intertidal area have a better capacity to process nutrients than others. I also wanted to identify the impact of the enhanced ammonium on functional diversity and the influence this has in the overall processing ability of the sandflat, and finally to identify any changes in chlorophyll *a* concentration, an indirect measure of MPB and percent coverage of seagrass. Ammonium was used as a measure of nutrient enrichment as a direct result of fertiliser addition. It is expected the fertiliser addition will successfully enhance the ammonium concentration within the treatment plots as well as at surface and at depth.

High and low functional diversity characteristics previously identified within Tapora Bank, Kaipara Harbour were used to create four treatment combinations for the current research: (1) low functional diversity and high abundance; (2) high functional diversity and high abundance; (3) high functional diversity and low abundance; and (4) low functional diversity and low abundance. I expect that areas of high macrofaunal abundance and high functional abundance will possess greater resilience to fertiliser addition, displayed by reduced porewater ammonium concentrations as nutrient concentrations will be used within the sediment by bacteria and MPB and undergo denitrification. Bioturbation will aid in nutrient release from the sediment into the overlying water column as well as stimulate the rate of denitrification. Areas of low functional diversity and abundance will display the opposite trend, where fertiliser addition will result in the increased likelihood of eutrophication, measured by the concentration of ammonium in the sediment porewater.

A priori predictions

1. Porewater ammonium concentration will be elevated at surface and depth in both the medium and high treatments following the addition of slow-release fertiliser. This is likely to be more elevated at depth due to decreased bioturbation activity, as well as slower microbial processes and diffusion occurring at depth. It is also expected that the high treatment will display higher ammonium concentrations than the medium treatment, given the greater quantity of fertiliser added to the sediment.
2. Prior to fertiliser enrichment, ambient sediment porewater ammonium concentration is likely to be controlled by macrofaunal diversity, due to bioturbation and oxygenation of sediments that contribute to benthic-pelagic coupling by the remineralisation of nutrients. Therefore, after fertiliser addition it is expected that macrofauna will be adversely affected due to the increased ammonium concentration and a decline in available oxygen, such that they will no longer contribute as major drivers of porewater ammonium concentration within the sediment.
3. Porewater ammonium concentrations within the sandflat will vary naturally due to the composition of the macrofaunal community present. Thus with an increase in functional species it is expected that there will be a lesser effect of nutrient enrichment. This may also vary with grain size.
4. The increased porewater ammonium concentrations are likely to result in a decline of macrofaunal species, individuals, functional species and functional individuals. Thus key functional species *M. liliana* and *A. stutchburyi* are predicted to decline. This decline is expected to be greater in the high fertiliser treatment than the medium treatment.
5. The effect of enhanced nutrients on both seagrass and chlorophyll *a* will result in increased MPB and increased percent coverage of seagrass.

Chapter 2

Materials and Methods

2.1 Study site

Kaipara Harbour is New Zealand's largest estuary covering an area of 974 square kilometres and is located on the north-western side of New Zealand's North Island (36° 39' S, 174° 29' E) (Figure 2). A large scale experiment based at Tapora Bank (Figure 2) was established in January 2014 and covered an intertidal area of 300 m x 1000 m (300,000 m²), extending from the low to high tide mark. The sample site had patchy regions of seagrass (*Zostera muelleri*) as well as regions of sand and shell hash, with habitats ranging from seagrass-dominated to sand-dominated (Hewitt & Funnell 2005; Hailes et al. 2010; De Juan & Hewitt 2011).

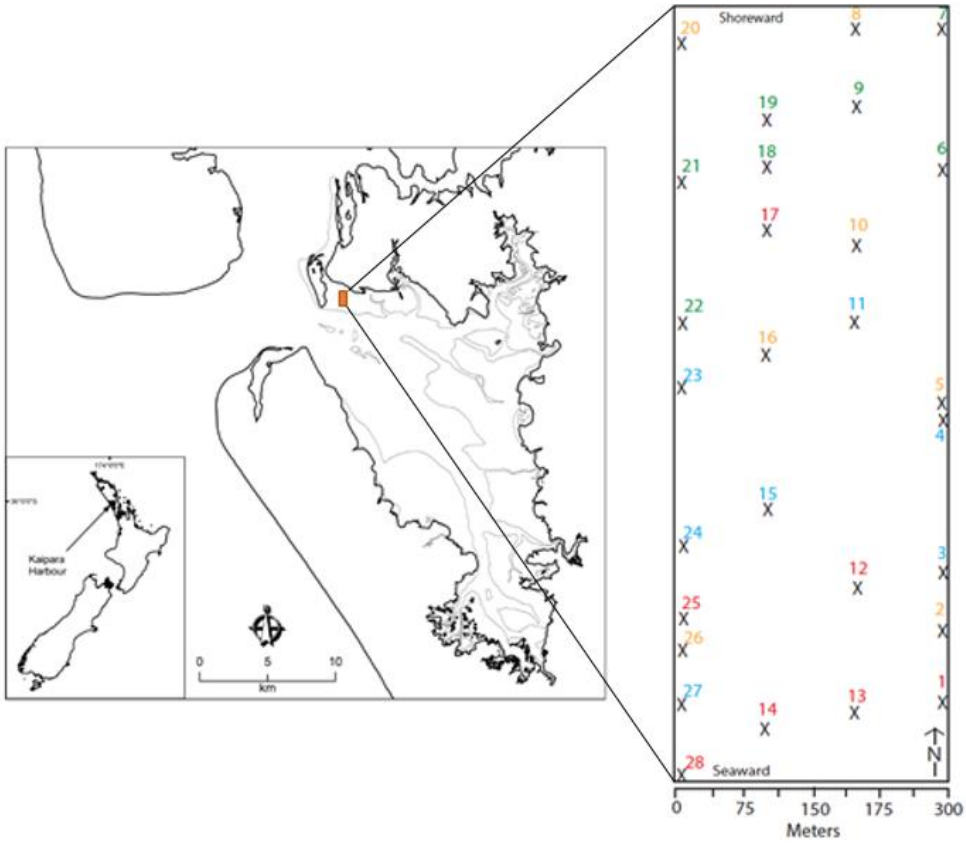

Figure 2: Study site location (orange) at Tapora Bank, Kaipara Harbour, alongside, a site diagram indicates relative site positions and high/low functional diversity and abundance measures where red represents sites of high abundance and high diversity, green represents high abundance and low diversity, yellow represents low abundance and low diversity and blue represents low abundance and high diversity.

2.2 Experimental treatments

This work was part of a larger experiment funded by a New Zealand Royal Society Marsden grant awarded to Simon Thrush, Carsten Dormann and Casper Kraan, and so experimental design was predetermined. I assisted with the experimental set-up and both collected and analysed sediment properties, porewater nutrient samples, and assisted in sorting macrofaunal samples for identification.

Experimental set-up began on January 29th 2014 (mid-late summer). GPS coordinates for each site (see Appendix 1) were predetermined based upon functional diversity attributes of macrofauna collected by Greenfield (2013). Known quantities of Nutricote 70-day slow-release fertiliser (42 % N (42:0:0), no P or trace elements) was added via coring at a depth of 10 cm to 1 m^2 plots designated as medium (168 N g/m², 400 g/m² fertiliser) and high (588 N g/m², 1400 g/m² fertiliser). A total of 20 cores were taken at each plot for the addition of fertiliser addition: after a sediment core (10 cm depth) was removed, the fertiliser was then added before being covered with a plug of sediment (2-3 cm) at the surface to prevent removal of the fertiliser into the water column (Figure 3). This coring technique is new to fertiliser addition experiments and is an improvement to previous methods. Adding fertiliser to these cores represented nutrient enrichment throughout the sediment column. Coated slow-release fertiliser pellets were used as these provide gradual enrichment over time (Heck et al. 2000; Worm et al. 2000). The control plots underwent the same coring technique with gravel (similar size to fertiliser granules) added to the sediment in place of the fertiliser.

Figure 3: Left, addition of fertiliser via coring to intertidal sediment. Right, a treatment plot following fertiliser addition.

Dose rates were determined based upon findings within the literature: 23 research papers were surveyed, then narrowed to the 12 most relevant to this study. These studies gave a range in dose rates (low of 5.7 N g/m^2 and a high of 720 N g/m^2) (Figure 4). An average was calculated based on fertiliser at 42 % N g/m^2. This average was then calculated against the number of additions we would have per plot (40 multiplied by how much fertiliser could fit into a centrifuge tube representing our field scoops (10 g (15 mL tube) for medium and 35 g (50 mL tube) for high)). That value was then multiplied by the nitrogen content of the slow-release fertiliser (42 %) giving a total of 588 N g/m^2 for high treatment plots and 168 N g/m^2 for medium treatment plots.

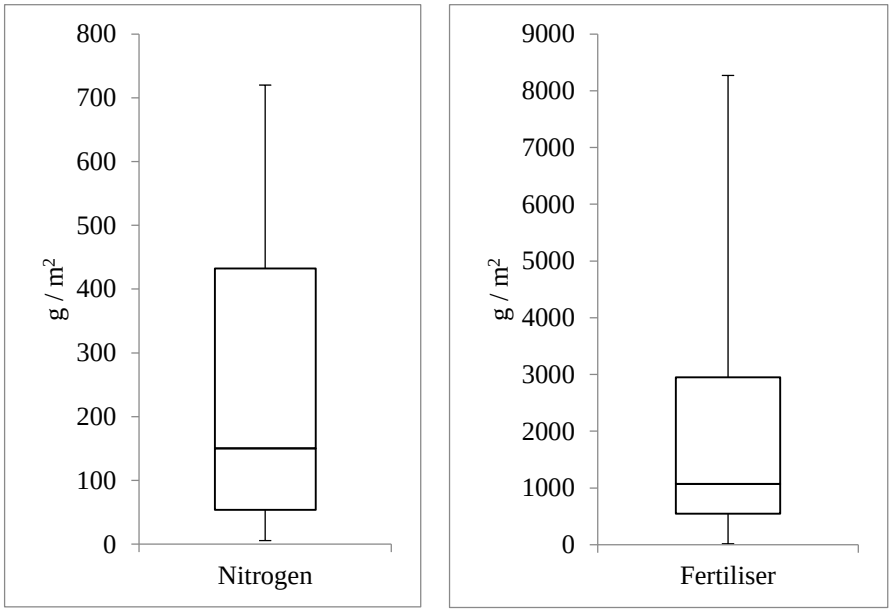

Figure 4: Box and whisker plots from the literature surveyed identified nitrogen content and fertiliser dose rates (g/m^2). Boxes identify the upper and lower quartile while the centre line highlights the median of literature surveyed, the whiskers depict the highest and lowest amount of nitrogen and fertiliser added in published studies.

A high fertiliser dose was used in addition to the medium to compare the effect of further enhanced nutrient addition to the benthos. Dose rates were trialled at Tuapiro Point, Tauranga prior to the Kaipara experiment to confirm porewater nutrient elevation (see Appendix 2). Twenty-eight sites, each of three 1 m^2 plots, were established and assigned one of three treatments (control, medium or high fertiliser dose rate). Functional diversity and abundance were ranked from high to low based on sampling from Greenfield (2013). The 28 sites encompassed four conditions, (1) high functional diversity and high abundance, (2) low functional

diversity and high abundance, (3) high functional diversity and low abundance and (4) low functional diversity and low abundance (Figure 2). These points were spread across the sandflat to cover a wide range of environmental conditions and to spread the experimental locations between areas of previously recorded high and low functional diversity and abundance.

These sites were then left for four weeks until the first sampling on February 26th (late summer). Each of the treatment and control plots of the 28 sites were sampled for sediment properties (chlorophyll *a* (chl-*a*), organic matter content (OC (Table 1)), grain size distributions (median grain size (MGS) and % fractions) and porewater nutrients at randomly selected points within the plots. Four sediment syringe cores (3 cm diameter, 2 cm depth) were collected per plot and pooled. All sediment cores were kept in cold and dark conditions following collection before being frozen to await laboratory analysis. Four syringe cores sectioned at 0-2 and 5-7 cm were collected and pooled at each of the 28 sites, at the control, medium and high level treatment plots, and stored in 50 mL centrifuge tubes in dark and cold conditions until reaching the laboratory for immediate removal of porewater. The two section depths were selected to identify any nutrient accumulation at depth and thus can potentially link to surface (e.g. *A. stutchburyi*) and deep dwelling (e.g. *M. liliana*) macrofaunal species' functional traits. The depths sampled also covered both oxic and anoxic sediments which may influence nutrient processing differently.

The second and final sampling (March 17th) took place nearly three weeks after the first sampling. Sediment properties, porewater and macrofaunal cores were sampled to determine the abundance and diversity of species across the sandflat and how these varied across the plot treatments. Two macrofaunal cores (13 cm diameter, 15 cm depth) were collected from the centre of each plot, then sieved over 500 µm mesh and preserved within 70 % isopropyl alcohol (IPA).

Prior to sediment sampling disturbance the 1 m² plot surface structure of the sediment was captured by digital photographs at each site, from both the first and second sampling dates (hereafter as D_{28} and D_{47}) to quantify surface features (shell hash, sand and seagrass coverage) using Corel Point Count with extensions (CPCe) (Kohler & Gill 2006).

2.3 Laboratory procedure

Within 24 hours of collection 3 mL of de-ionised water was added to each porewater sediment sample (to give enough sample for analysis), vortexed to homogenise and left to stand for an hour before vortexing and centrifugation (2000 rpm for 10 minutes) (Lohrer et al. 2010). After centrifugation, the porewater from the sediment surface was extracted via pipette and filtered through glass filter paper (0.45 µm), before being frozen to await laboratory analysis. Porewater nutrient samples (n=336) were analysed on a Lachat Flow Injection Analyser (FIA) for ammonium (NH_4^+) using standardised procedures (Zellweger Analytics 2000). Ammonium is a form of inorganic nitrogen and is therefore a product derived from consumer and decomposer nitrogen, which can lead to eutrophication following diffusion and break-down from fertiliser. Thus porewater ammonium provides an indication of nutrient enrichment.

Porewater ammonium concentrations following instrument analysis were corrected for de-ionised water dilution. Percent porewater was calculated (Equation 1) where the volumes were multiplied by the water density. This then allowed for the determination of the volume of porewater within the initial sample (Equation 2). The percent dilution could then be identified (Equation 3), and finally the correction calculation for ammonium concentration within the final samples could be undertaken (Equation 4).

$$\frac{\left(\frac{Ww}{(Wv \times wd)}\right) - \left(\frac{Dw}{(Wv \times wd)}\right)}{\frac{Ww}{(V \times wd)}} = \text{Percent porewater} \qquad \text{Eq. 1}$$

Ww is wet weight of the sample (g), Dw is dry weight (g) of the sample after oven drying (60 °C) to a constant weight, Wv wet volume (mL) is the volume of the sample prior to drying, wd water density (g/ml) and V volume (mL).

$$I_{nSV} \times PPW = \text{Porewater volume of initial sample} \qquad \text{Eq. 2}$$

I_{nSV} is the initial sample volume of 49 mL and PPW is the percent porewater derived from Equation 1.

$$\frac{Dv}{Ipv + Dv} \quad = \text{Percent dilution} \qquad \text{Eq. 3}$$

Dv is the dilution volume (3 mL) and Ipv is the initial porewater volume (mL) derived from Equation 2.

$$\frac{[NH_4^+]}{(100 - pd\ (/100))} \quad = \text{Corrected ammonium value} \qquad \text{Eq. 4}$$

$[NH_4^+]$ is the concentration of porewater ammonium (mg/L) and pd is the percent dilution derived from Equation 3.

One hundred and sixty-eight sediment samples were defrosted, homogenised and subsampled to forego analysis of sediment properties, namely: chl-*a*, OC and sediment grain size distributions. Chl-*a* analysis was undertaken within four weeks of sample collection. Approximately 0.1 g of freeze dried sediment was extracted in 90 % buffered acetone in dark and cold (4 °C) conditions for 24 hours. Samples were then centrifuged (3000 rpm for 10 minutes), before absorbance was measured flurometrically on a Turner 10-AU Flurometer to determine both the chl-*a* and phaeophytin (following the addition of 0.1N HCl for acidification) concentrations (Arar & Collins 1997). Sediment used for OC was weighed into pre-weighed foil pans and dried at 60 °C until reaching a constant weight, and then combusted at 550 °C for 4 hours. Sediment OC was calculated by the percent weight loss of the dried sediments following furnace combustion (Christie et al. 2000). Grain size of the sediment was determined using the Malvern Mastersizer 2000 instrument which gives a particle size range of 0.05-2000 µm. Sediments underwent digestion in 10 % hydrogen peroxide (for removal of organic matter), until reaction ceased (~ 2 weeks) (Singer et al. 1988).

Rose Bengal solution was used to stain macrofaunal samples (n=168) before fauna were separated and identified under a stereo microscope. Fauna were identified as number of individuals (N) (all individuals per core, regardless of function); number of species (S) (number of species per core); species identified as contributors to biogeochemical processing and therefore influence changes to porewater nutrients were grouped (see Appendix 1) as number of functional species (F_S) (number of species belonging to the functional group from each core location); number of functional individuals (F_N) (count of species that were defined to be part of the

functional groups); number of *A. stutchburyi* (adults and juveniles); and number of *M. liliana* (adults and juveniles) per core. Both *A. stutchburyi* and *M. liliana* are key species within estuarine sandflats due to their sizes and key functional roles (Thrush et al. 2006a; Jones et al. 2011; Norkko et al. 2013).

Digital photographs (n=168) collected during the two sampling trips underwent CPCe software analysis where 75 randomly assigned data points determined the percent coverage of sand, sea grass (*Zostera muelleri*) and shell hash on the surface of each plot.

2.4 Data Analysis

Locations were selected based on high and low functional diversity characteristics previously identified within Tapora Bank, Kaipara Harbour (Greenfield 2013) to give a wide range of macrofauna functional diversity and abundance within the sandflat. Macrofauna diversity and abundance measures comprised (1) low functional diversity and low abundance, (2) high functional diversity and low abundance, (3) low functional diversity and high abundance and (4) high functional diversity and high abundance. It was not expected that the treatments would be the same as Greenfield (2013) observed; however, the experimental design provided a way to ensure coverage for a range of macrofaunal diversity. Because the categorical data (high and low functional diversity and abundance) used for the experimental set up had changed I no longer assigned these abundance and diversity measures. A categorical approach could still be undertaken using unbalanced designs due to a change in the number of replicates, however because the diversity and abundance measures were different across the sandflat regression analysis was undertaken for data analysis.

Distance based linear models (DistLM) were conducted using PRIMER 6 (v 6.1.15) to identify the predictor variables contributing to the natural variability across the sandflat in the control, medium and high treatment plots. Treatment plots were kept separate for analysis, in order to investigate relationships between treatments and whether relationships changed with the level of fertiliser added to the soft sediment. Sampling data from D_{47} were put though DistLM analysis for all predictor and response variables, as macrofauna samples were only collected during D_{47}. Data were transformed to improve the distribution (Anderson 2001): fourth root

(macrofauna), square root (sediment properties and percent coverage of seagrass, shell hash and sand) and log (porewater ammonium) transformations presented a lesser effect of any outliers based on distributions observed within draftsman's plots. Step-wise distance-based linear models were then performed using the PRIMER PERMANOVA add-on (Anderson et al. 2008).

DistLM models generated p values allowing any significance to be identified in the predictor variables and individually. Importance of variables was assessed using marginal tests. Sequential tests identified the best fit of variables based on adjusted R^2 values (the amount of variation explained by each model), and those that explained the most variation were included. The step-wise function allowed for improvement of the selection criteria at each step, a function of this tested whether excluding variables improved the final model. The relative quality of the statistical model produced was measured by the Akaike information criterion (AIC) and the R^2 value. Similarity matrixes of Euclidean distance were created where p values for predictor variables were identified (9999 permutations to reduce the effect of non-normality). Predictor variables run within each DistLM model identified a corresponding p value, individual R^2 as well as a cumulative R^2 value. Pearson's correlation on predictor variables meant multi-collinearity could be identified and avoided by removing relationships (r >0.8) prior to DistLM analysis.

STATISTICA (v11) was used to perform three-way analysis of variance (ANOVA) to test for significant differences ($p < 0.05$) (Wonnacott & Wonnacott 1972) in sampling depths (0-2 cm and 5-7 cm), sampling day (D_{28} and D_{47}) and treatment (control, medium and high).

Sampling depth would identify whether fertiliser addition at depth or surface (or both) were significant, where significance was identified as an elevation in porewater ammonium greater than the control. Sampling day identified any significant differences in porewater nutrient enrichment between D_{28} and D_{47}, while treatment identified whether the treatments significantly elevated the porewater ammonium concentration relative to the ambient sediment. One-way ANOVA was performed to test for differences within the macrofauna community abundance between treatment and control plots. Post-hoc Tukey honest significant difference

(HSD) tests were run following ANOVA analysis to determine which of the groups tested differed from the others. All data were left transformed as per DistLM models.

Medium and high treatment porewater ammonium values were normalised by dividing by the controls for both D_{28} and D_{47} to correct for background variation. To identify any nutrient impact on chl-*a* and seagrass, both were also normalised by the control data. T-tests were performed on chl-*a* and seagrass data to identify statistical significance between the means of the medium and high treatment plots once normalised by controls. Paired t-tests were also performed to identify any significance between the means of the groups sampled.

An average rate of porewater ammonium processing/accumulation was determined by correcting by the control plots and dividing these with the nutrient values in both the medium and high treatment plots (Equation 5). Normalising by the control aids in correcting variation in background porewater concentration due to variations in sediment properties and macrofaunal communities. Outliers were removed based on extremely high values where it was likely field sampling collected some fertiliser pellets, thus elevating the porewater ammonium concentrations prior to analysis. Negative numbers demonstrated an accumulation of porewater ammonium while positive numbers indicated a removal between sampling dates. Two-way ANOVA was used to test for significance between the normalised rate of porewater ammonium against treatment and sampling depth.

$$\left(\frac{[NH_4{}^+]_1}{C_1} - \frac{[NH_4{}^+]_2}{C_2}\right) \quad = \text{Average rate of porewater } [NH_4{}^+] \text{ processing/accumulation} \qquad \text{Eq. 5}$$

Where $[NH_4{}^+]$ is the porewater ammonium concentration (mg/L) from D_{28} (1) and D_{47} (2) from the medium/high plot, and where C is the corresponding control value from D_{28} and D_{47} performed for both upper and lower sediment depths.

Table 1: Abbreviations used throughout this thesis.

Variables in full	**Abbreviation**
Chlorophyll *a*	Chl-*a*
Pheaopigment	Pheao
Organic content	OC
Median grain size	MGS
Mud content	Mud
Porewater ammonium concentration upper sediment depth	PW $[NH_4^+]_u$
Porewater ammonium concentration lower sediment depth	PW $[NH_4^+]_l$
Upper sediment depth first sampling	U-1
Upper sediment depth second sampling	U-2
Lower sediment depth first sampling	L-1
Lower sediment depth second sampling	L-2
Number of species per core	S
Number of individuals per core	N
Number of functional species per core	F_S
Number of functional individuals per core	F_N
Number *Austrovenus stutchburyi* per core	*A. stutchburyi*
Number *Macomona liliana* per core	*M. liliana*
First sampling	D_{28}
Second sampling	D_{47}

Chapter 3

Results

3.1 Site description

Fine sand (125-250 µm) was the dominant sand grain size within the sampling area with a mean of 216.3 µm. Of the 84 plots sampled (control, medium and high) 55 plots contained seagrass, of these only 12 had seagrass coverage greater than 50 %. Sediment properties for the control plots varied between D_{28} and D_{47} (Table 2), for instance the average amount of chl-*a* doubled between D_{28} and D_{47}. Changes were also observed in porewater ammonium concentrations, where the average of the lower sampling depth of 5-7 cm was more than double the amount of $D_{28;}$ however, little change was observed within the upper sediment depth of 0-2 cm. A minimum of 18 S were found per core within the control plots with a maximum of 58, while N ranged from 38 to 838 per core. The maximum number of *A. stutchburyi* per core was much greater (225) than that of *M. liliana* (56).

Table 2: The range, mean and standard deviation (SD) of control plot environmental values for D_{28} and D_{47}, with macrofaunal composition ranges for D_{47}.

	26 Feb Range	Mean	SD	17 March Range	Mean	SD
Chl-*a* (µg/g)	0-11.9	4.6	3.1	3.6-23.2	10.0	4.7
Pheao (µg/g)	0.8-7	2.9	1.9	1.5-17.9	6.4	5.3
OC (%)	0.4-2.7	1.0	0.6	0.5-2.5	1.1	0.6
MGS (µm)	181.5-242.8	216.3	15.8	176.6-240.6	211.6	17.8
Mud (%)	0-19.6	2.0	4.0	0-14.5	3.9	4.8
PW $[NH_4^+]_u$	0.04-9.6	0.7	1.8	0.003-3.6	0.9	1.0
PW $[NH_4^+]_l$	0.12-3.2	1.2	0.8	0.3-20.3	3.5	4.8
Seagrass coverage (%)	0-96	26.9	33.1	0-92	32.5	29.2
Shell hash coverage (%)	0-13	2.8	3.2	0-16	3.1	4.9
Sand coverage (%)	1-100	70.3	33.1	7-100	64.4	30.8
S				18-58	39.1	12.3
N				38-838	262.4	190.8
F_S				11-31	19.9	6.2
F_N				29-752	165	149.1
***A. stutchburyi* core^{-1}**				0-225	22.5	42.4
***M. liliana* core^{-1}**				1-56	20.0	16.2

Significant correlations were identified in a number of the control plot environmental variables (Table 3). Pheaopigment (phaeo) was correlated with both OC and chl-*a*. MGS correlations were identified between S and N. While *A. stutchburyi* was correlated significantly with chl-*a* and pheao. S correlated significantly with mud content (mud), MGS and F_S.

Table 3: Pearson's correlation coefficients (*r*) for control environmental variables from D_{47}. Multi-collinearity was identified for values >0.8.

	OC	**Chl-*a***	**Phaeo**	**MGS**	**Mud**	**S**	**N**	**F_N**	**F_S**	***A. stutchburyi***	***M. liliana***
OC											
Chl-*a*	0.56										
Phaeo	0.89*	0.74**									
MGS	-0.74	-0.48	-0.77								
Mud	0.80	0.35	0.76	-0.80							
S	0.72	0.36	0.69	-0.85**	0.82						
N	0.57	0.20	0.52	-0.53*	0.69	0.79**					
F_N	0.34	0.05	0.35	-0.45	0.55	0.67**	0.92***				
F_S	0.55	0.24	0.58	-0.83	0.72	0.89**	0.63**	0.64**			
A. stutchburyi	-0.51	-0.35*	-0.65**	0.45	-0.50	-0.28	-0.18	-0.15	-0.30		
M. liliana	-0.42	-0.26	-0.35	0.15	-0.32	-0.13	0.01	0.24	0.02	0.36	

Environmental variables: OC, organic content (%); Chl-*a*, chlorophyll *a* (µg/g); Pheao, pheaopigment (µg/g); MGS, median grain size (µm); Mud content (%) (<63µm); S, total number of species in sample (ind. $core^{-1}$); N, total number of individuals in sample (ind. $core^{-1}$); F_N, total number of functional individuals in sample (ind. $core^{-1}$); F_S, total number of functional species in sample (ind. $core^{-1}$); *A. stutchburyi*, total number of *Austrovenus stutchburyi* individuals (ind. $core^{-1}$); *M. liliana*, total of *Macomona liliana* individuals (ind. $core^{-1}$). *p <0.05, ** p <0.01, ***p <0.00.

The best model for the upper sediment depth in the control plots identified F_N as significant ($p < 0.05$), while phaeo was outlined as a marginally significant variable (Table 4). Sequential tests identified both F_N and *M. liliana* as significantly correlated with porewater ammonium concentration (Table 5), where F_N was positively correlated and explained 26 percent of the variation in porewater ammonium. DistLM models displayed a reduced set of predictor variables as these were removed based on multi-collinearity ($r > 0.80$) to find the best statistical fit based on AIC and R^2 values. DistLM models run on the control plot lower sediment depth (5-7 cm) demonstrated *M. liliana* as marginally significant (Table 6); however, no overall significance was observed in marginal or sequential tests (Table 6 and 7). *M. liliana* was negatively correlated with porewater ammonium within the upper sediment but a positive correlation was observed within the lower sediment depth.

Table 4: Distance based linear model marginal test step wise analysis between environmental predictors and porewater ammonium concentration for control treatment plots within the upper sediment depth: results of the stepwise section procedure. SS (trace) = portion of sum of squares relative to the analysed predictor variables (sediment properties and macrofaunal variables); pseudo-F ratio statistic; P = level of statistical significance ($p < 0.05$ in bold indicates significance); Prop = the proportion of variation explained by the model. Correlation directions are indicated for significant and marginally significant p-values (+ or -) for both marginal and sequential tests.

	SS(trace)	Pseudo-F	P	Prop.
Chl-*a*	1.240	1.135	0.305	0.043
Sand	0.615	0.550	0.468	0.021
Shell hash	0.061	0.053	0.819	0.002
Phaeo	3.449	3.437	0.076	0.129 (+)
Mud	2.306	2.198	0.152	0.080
S	2.633	2.541	0.120	0.092
F_N	7.532	8.964	**0.006**	0.263 (+)
M. liliana	1.044	0.949	0.335	0.036 (-)

Table 5: Distance based linear model sequential test between environmental predictors and porewater ammonium concentration for control treatment plots within the upper sediment depth: results of the stepwise section procedure. AIC = degree of support for the model; SS (trace) = portion of sum of squares relative to the analysed predictor variables (sediment properties and macrofaunal variables); pseudo-F ratio statistic; P = level of statistical significance (p <0.05 in bold indicates significance); Prop = the proportion of variation explained by the model; Cumul = cumulative variation explained; res.df = residual degrees of freedom.

	AIC	SS(trace)	Pseudo-F	P	Prop.	Cumul.	res.df
F_N	-2.778	7.532	8.964	**0.007**	0.264	0.264	25
M. liliana	-4.929	2.994	3.989	**0.053**	0.105	0.369	24
Shell hash	-5.691	1.752	2.477	0.127	0.061	0.430	23
Mud	-7.567	2.174	3.395	0.081	0.076	0.506	22

Table 6: Distance based linear model marginal test step wise analysis between environmental predictors and porewater ammonium concentration for control treatment plots within the lower sediment depth: results of the stepwise section procedure. SS (trace) = portion of sum of squares relative to the analysed predictor variables (sediment properties and macrofaunal variables); pseudo-F ratio statistic; P = level of statistical significance (p <0.05 in bold indicates significance); Prop = the proportion of variation explained by the model. Correlation directions are indicated for marginally significant p-values (+ or -) for both marginal and sequential tests.

	SS(trace)	Pseudo-F	P	Prop.
Chl-*a*	40.404	1.784	0.182	0.066
F_N	0.061	0.002	0.954	0.0001
A. stutchburyi	19.686	0.838	0.373	0.032
M. liliana	74.109	3.479	0.077	0.122 (+)
Shell hash	0.971	0.040	0.859	0.002
OC	11.387	0.478	0.508	0.019
N	0.271	0.011	0.910	0.0004
Seagrass	15.849	0.671	0.438	0.026

Table 7: Distance based linear model sequential test between environmental predictors and porewater ammonium concentration for control treatment plots within the lower sediment depth: results of the stepwise section procedure. AIC = degree of support for the model; SS (trace) = portion of sum of squares relative to the analysed predictor variables (sediment properties and macrofaunal variables); pseudo-F ratio statistic; P = level of statistical significance (p <0.05 in bold indicates significance); Prop = the proportion of variation explained by the model; Cumul = cumulative variation explained; res.df = residual degrees of freedom.

	AIC	SS(trace)	Pseudo-F	P	Prop.	Cumul.	res.df
M. liliana	84.513	74.109	3.479	0.077	0.122	0.122	25

3.2 Nutrient addition treatments

The addition of Nutricote 70-day slow-release fertiliser to the medium and high treatment plots elevated porewater ammonium in the upper (0-2 cm) and lower (5-7 cm) sediment depths for both D_{28} and D_{47} (Figure 5). The medium (24.5 mg/L) and high (74.1 mg/L) treatments in the upper sediment depth on D_{28} identified higher ammonium concentrations than those of the ambient sediment in the control plots (0.9 mg/L). The lower sediment depth displayed a greater accumulation of nutrients in both the medium (30.42 mg/L) and high (96.29 mg/L) treatment plots. D_{47} showed greater enrichment than D_{28} in all but the medium plot in the upper sediment depth. Differences between sediment depth, treatment and sampling date were assessed further via three-way ANOVA where all three variables were identified as significant ($p < 0.05$) (Table 8). Subsequent post-hoc testing (Tukey's honest significant difference (HSD)) demonstrated significance in porewater ammonium concentration, this was greater than the control in both the medium and high treatments, as well as at depth (5-7 cm).

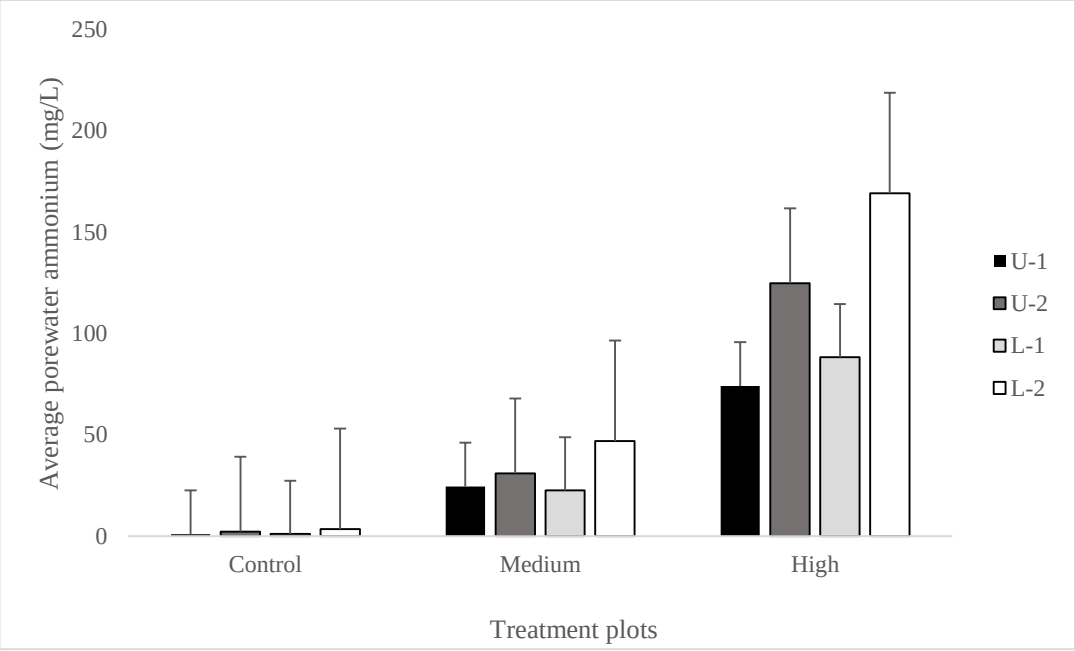

Figure 5: Average porewater ammonium for control, medium and high treatment plots for upper (0-2 cm) and lower (5-7 cm) sediment depths for both D_{28} and D_{47} ± SE.

Table 8: Three way ANOVA analysis of the effect of treatment (control, medium and high), sediment depth (0-2 and 5-7 cm) and sampling date (D_{28} and D_{47}) for porewater ammonium. Significant p values (<0.05) are indicated in bold. Differences identified were determined using Tukey honest significant difference (HSD) test**.** Data were log transformed to satisfy test assumptions.

	Sum of squares	**Degrees of freedom**	**Mean squares**	**F value**	**P value**	**HSD**
Treatment	197.917	2	98.958	334.771	**<0.001**	**C<M<H**
Sediment depth	16.678	1	16.678	56.419	**<0.001**	**U<L**
Sampling date	2.541	1	2.541	8.595	**0.003**	**D_{28}<D_{47}**
Treatment*Sediment depth	0.371	2	0.185	0.627	0.535	
Treatment*Sampling date	0.081	2	0.040	0.137	0.872	
Sediment depth*Sampling date	0.481	1	0.481	1.627	0.203	
Sediment*Sediment depth*Sampling date	0.151	2	0.075	0.255	0.775	
Error	93.410	316	0.296			

When normalised by the control, the high treatment displayed a greater average of porewater ammonium than the medium treatment for both upper and lower sediment depths (Figure 6). The high treatment displayed a greater concentration of ammonium within the surface sediments (0-2 cm) than at depth (5-7 cm) on both D_{28} and D_{47}. The medium treatment showed little difference between sampling depth and sampling date when normalised by controls. Three-way ANOVA of the effect of treatment, sediment depth and sampling day determined only treatment as significant (Table 9).

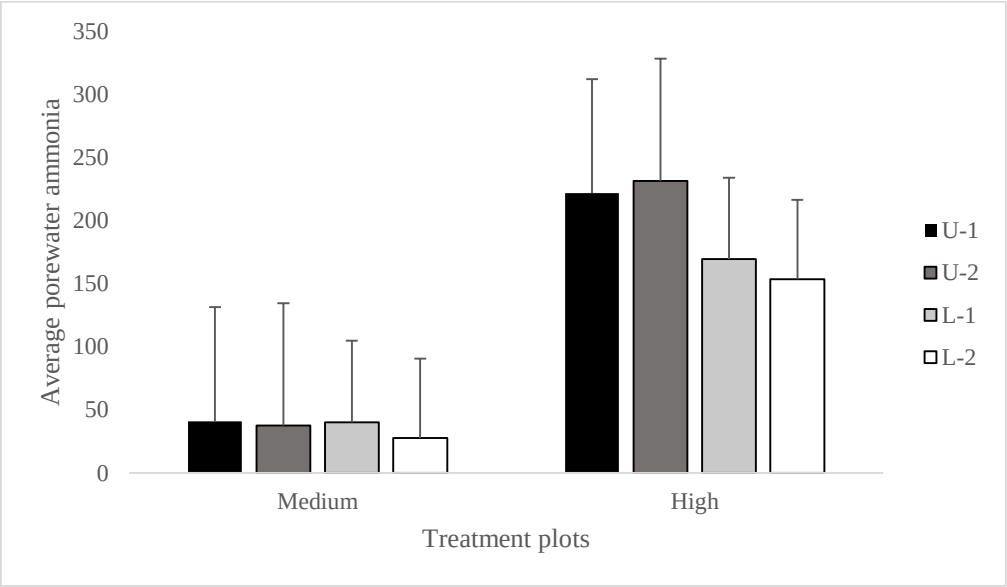

Figure 6: Average porewater ammonium for medium and high treatments normalised by control plots for both upper and lower sediment depths on D_{28} and D_{47} ± SE.

Table 9: Three way ANOVA analysis of the effect of treatment (medium and high), sediment depth (0-2 and 5-7 cm) and sampling date (D_{28} and D_{47}) for porewater ammonium normalised by controls. Significant p values (<0.05) are indicated in bold.

	Sum of Squares	Degrees of freedom	Mean Squares	F value	P value
Treatment	1340920	1	1340920	27.897	**<0.001**
Sampling depth	67189	1	67189	1.398	0.238
Sampling date	1647	1	1647	0.034	0.853
Treatment*Sampling depth	48449	1	48449	1.008	0.317
Treatment*Sampling date	298	1	298	0.006	0.937
Sampling depth* Sampling date	4061	1	4061	0.084	0.772
Treatment*Sampling depth *Sampling date	910	1	910	0.019	0.891
Error	10046092	209	48067		

3.3 Nutrient processing and influence of macrofauna and sediment properties

A total of 18850 N were identified from the macrofaunal cores taken on D_{47}. The number of N in the control (7346) was greater than that of the medium treatment (6228) and the high treatment (5276). F_N showed the same trend, where the total number of F_N calculated in the high treatment was less (475) than the medium (533) and control (556). The average number of macrofauna per core found within the high and medium treatments displayed less N and S on average than those within the control (Figure 7). The high treatment had the least abundance of both S and N while the medium identified an abundance greater than that of the high but fewer than the control. *A. stutchburyi* and *M. liliana* also readily displayed this decline (Figure 8), however the decline in *M. liliana* was greater. One-way ANOVA was performed on each of the macrofaunal community variables against treatment, of these only *M. liliana* displayed a significant decline (Table 10). *A. stutchburyi* showed no significance (Table 11) (see Appendix 3 for S, N, F_N and F_S).

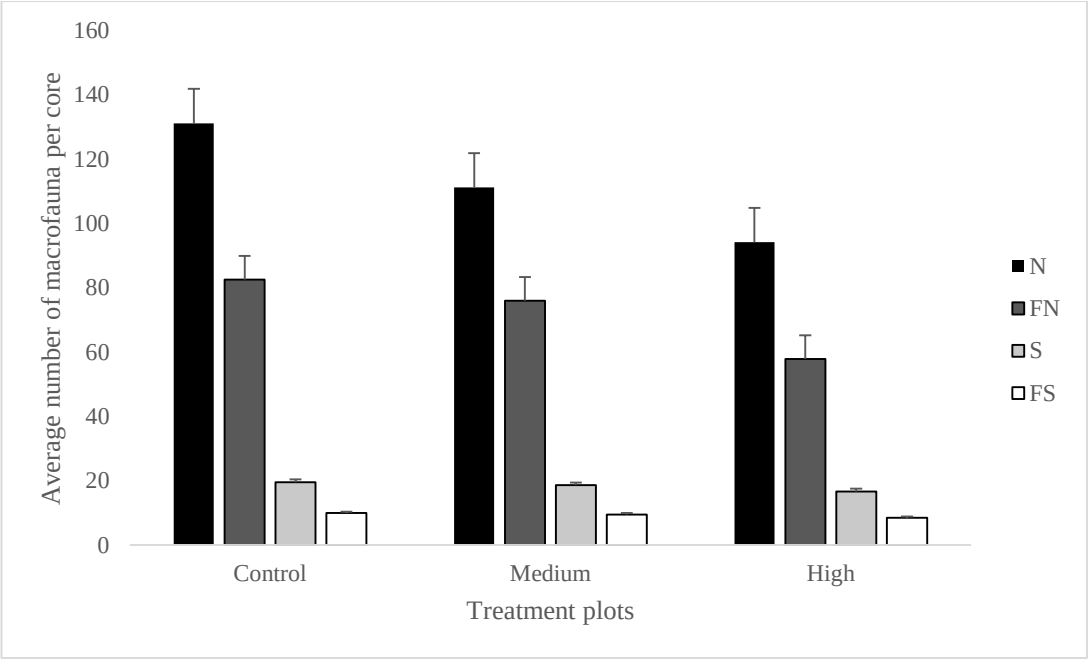

Figure 7: The average number of N, F_N, S and F_S per core in control, medium and high treatment plots for D_{47} ± SE.

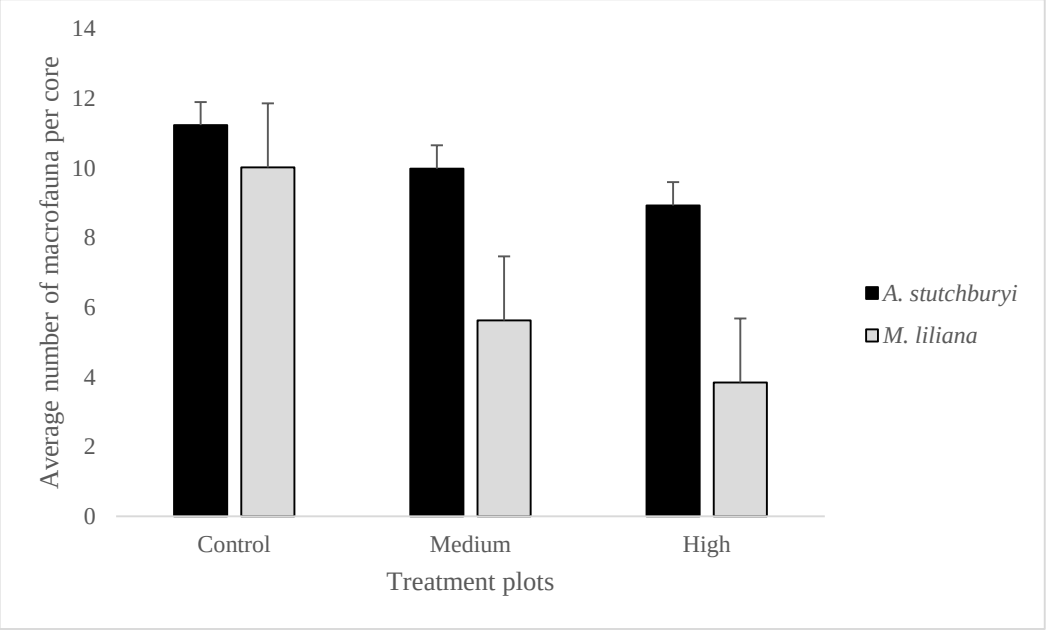

Figure 8: Average number of *A. stutchburyi* and *M. liliana* per core in control, medium and high treatment plots from D_{47} ± SE.

Table 10: One-way ANOVA testing whether *M. liliana* abundance differed per core among the treatments (control, medium and high). Significant p values (<0.05) are indicated in bold. Post hoc testing using Tukey honest significant difference (HSD).

	Sum of squares	Degrees of freedom	Mean Squares	F value	P value	HSD
Treatment	2.394	2	1.197	8.093	**0.0006**	**M=H<C**
Error	11.683	79	0.148			

Table 11: One-way ANOVA testing whether *A. stutchburyi* abundance differed per core among treatment (control, medium and high). Significant p values (<0.05) are indicated in bold.

	Sum of squares	Degrees of freedom	Mean Squares	F value	P value
Treatment	0.154	2	0.077	0.106	0.900
Error	57.557	79	0.729		

Pearson's correlations were performed on the medium and high environmental and macrofaunal variables (Table 12 and 13). Correlations were identified between S, N and F_N (Table 12 and 13). Few correlations were identified in the medium treatment for both sediment properties and macrofaunal variables compared to that of the control (Table 3) and high treatment. Macrofaunal variables in the medium treatment were correlated with each other but were not correlated in general with environmental variables. A similar trend was observed within the high treatment however, MGS was correlated with all macrofaunal variables except F_S.

Neither the upper or lower porewater ammonium concentration was correlated with environmental or macrofaunal variables in the medium fertiliser treatment (see Appendix 3). Mud was identified as the only significant environmental factor contributing to the response of porewater ammonium in the high treatment for upper sediment depth (0-2 cm) (Table 14). Like the high treatment lower sampling depth, mud was also outlined as the most significant environmental factor in the sequential tests identifying 13 percent of variation in porewater ammonium (Table 15). Sequential tests for the high treatment plot upper sediment depth also identified chl-*a* as marginally significant. Mud was found to be negatively correlated with both upper and lower porewater ammonium concentrations.

Table 12: Pearson's correlation coefficients (*r*) for the medium treatment plot environmental variables from D_{47}. Multi-collinearity was identified for values >0.8.

	OC	**Chl-*a***	**Phaeo**	**MGS**	**Mud**	**S**	**N**	**F_N**	**F_S**	***A. stutchburyi***	***M. liliana***
OC											
Chl-a	0.53										
Phaeo	0.60	0.69*									
MGS	-0.68	-0.51	-0.70								
Mud	0.89	0.45	0.63	-0.76							
S	0.74	0.49	0.62	-0.55	0.76						
N	0.70	0.46	0.47	-0.42	0.65	0.89**					
F_N	0.59	0.34	0.36	-0.34	0.60	0.81*	0.95***				
F_S	0.69	0.30	0.55	-0.53	0.76	0.91***	0.73*	0.71			
A. stutchburyi	-0.13	-0.05	-0.42	0.35	-0.30	-0.03	0.08	0.04	-0.15		
M. liliana	-0.14	-0.08	-0.06	0.21	-0.20	0.10	-0.01	0.05	0.12	0.28	

Environmental variables: OC, organic content (%); Chl-*a*, chlorophyll *a* (µg/g); Pheao, pheaopigment (µg/g); MGS, median grain size (µm); Mud content (%) (<63µm); S, total number of species in sample (ind. $core^{-1}$); N, total number of individuals in sample (ind. $core^{-1}$); F_N, total number of functional individuals in sample (ind. $core^{-1}$); F_S, total number of functional species in sample (ind. $core^{-1}$); *A. stutchburyi*, total number of *Austrovenus stutchburyi* individuals (ind. $core^{-1}$); *M. liliana*, total of *Macomona liliana* individuals (ind. $core^{-1}$). *p <0.05, ** p <0.01, ***p <0.00.

Table 13: Pearson's correlation coefficients (*r*) for the high treatment plot environmental variables from D_{47}. Multi-collinearity was identified for values >0.8.

	OC	**Chl-*a***	**Phaeo**	**MGS**	**Mud**	**S**	**N**	**F_N**	**F_S**	***A. stutchburyi***	***M. liliana***
OC											
Chl-*a*	0.30										
Phaeo	0.91**	0.38									
MGS	-0.76	-0.32	-0.80								
Mud	0.94*	0.26	0.89	-0.81							
S	0.82	0.13	0.75	-0.79**	0.82						
N	0.84	0.24	0.81	-0.75*	0.81	0.91***					
F_N	0.78	0.17	0.77	-0.74*	0.78	0.85**	0.94***				
F_S	0.80	0.09	0.76	-0.77	0.83	0.93**	0.85*	0.87**			
A. stutchburyi	-0.01	0.13	-0.16	0.28	-0.15	-0.03	0.06	-0.05	-0.12		
M. liliana	0.31	-0.06	0.42*	-0.43	0.29	0.48	0.39	0.44	0.51	-0.20	

Environmental variables: OC, organic content (%); Chl-*a*, chlorophyll *a* (µg/g); Pheao, pheaopigment (µg/g); MGS, median grain size (µm); Mud content (%) (<63µm); S, total number of species in sample (ind. $core^{-1}$); N, total number of individuals in sample (ind. $core^{-1}$); F_N, total number of functional individuals in sample (ind. $core^{-1}$); F_S, total number of functional species in sample (ind. $core^{-1}$); *A. stutchburyi*, total number of *Austrovenus stutchburyi* individuals (ind. $core^{-1}$); *M. liliana*, total of *Macomona liliana* individuals (ind. $core^{-1}$). *p <0.05, ** p <0.01, ***p <0.00.

Table 14: Distance based linear model marginal test between environmental predictors and porewater ammonium concentration for high treatment plots within the upper sediment depth: results of the stepwise section procedure. SS (trace) = portion of sum of squares relative to the analysed predictor variables (sediment properties and macrofaunal variables); pseudo-F ratio statistic; P = level of statistical significance ($p < 0.05$ in bold indicates significance); Prop = the proportion of variation explained by the model. Correlation directions are indicated for significant p-values (+ or -).

	SS(trace)	Pseudo-F	P	Prop.
Chl-*a*	12613	1.282	0.269	0.047
MGS	13161	1.341	0.264	0.049
Mud	35955	4.023	**0.050**	0.134 (-)
N	22771	2.411	0.136	0.085
F_N	26085	2.800	0.100	0.097
F_S	18002	1.870	0.180	0.067
A. stutchburyi	16087	1.658	0.210	0.060
Seagrass	8052	0.804	0.383	0.030

Table 15: Distance based linear model sequential test between environmental predictors and porewater ammonium concentration for high treatment plots within the upper sediment depth: results of the stepwise section procedure. AIC = degree of support for the model; SS (trace) = portion of sum of squares relative to the analysed predictor variables (sediment properties and macrofaunal variables); pseudo-F ratio statistic; P = level of statistical significance ($p < 0.05$ in bold indicates significance); Prop = the proportion of variation explained by the model; Cumul = cumulative variation explained; res.df = residual degrees of freedom.

	AIC	SS(trace)	Pseudo-F	P	Prop.	Cumul.	res.df
Mud	256.67	35955	4.023	**0.052**	0.134	0.134	26
Chl-*a*	255.13	27623	3.373	0.080	0.103	0.237	

High treatments for lower sediment depth (5-7 cm) identified S, F_N, F_S, OC and mud as significant environmental factors contributing to the response in porewater ammonium (Table 16). This model identified mud as being the most important environmental factor within the lower sediment depth for the high treatment plot explaining 23 percent of the variation in porewater ammonium (Table 17).

Table 16: Distance based linear model marginal test between environmental predictors and porewater ammonium concentration for high treatment plots within the lower sediment depth: results of the stepwise section procedure. SS (trace) = portion of sum of squares relative to the analysed predictor variables (sediment properties and macrofaunal variables); pseudo-F ratio statistic; P = level of statistical significance (p <0.05 in bold indicates significance); Prop = the proportion of variation explained by the model. Correlation directions are indicated for significant p-values (+ or -).

	SS(trace)	Pseudo-F	P	Prop.
Chl-*a*	14615	0.456	0.491	0.017
S	181030	7.059	**0.009**	0.214 (-)
F_N	125470	4.516	**0.039**	0.148 (-)
F_S	163000	6.189	**0.014**	0.192 (-)
M. liliana	6282	0.194	0.665	0.007
Seagrass	35459	1.135	0.294	0.042
OC	114590	4.064	**0.050**	0.135 (-)
Mud	202080	8.138	**0.004**	0.238 (-)

Table 17: Distance based linear model sequential test between environmental predictors and porewater ammonium concentration for high treatment plots within the lower sediment depth: results of the stepwise section procedure. AIC = degree of support for the model; SS (trace) = portion of sum of squares relative to the analysed predictor variables (sediment properties and macrofaunal variables); pseudo-F ratio statistic; P = level of statistical significance (p <0.05 in bold indicates significance); Prop = the proportion of variation explained by the model; Cumul = cumulative variation explained; res.df = residual degrees of freedom.

	AIC	SS(trace)	Pseudo-F	P	Prop.	Cumul.	res.df
Mud	285.28	202000	8.138	**0.007**	0.238	0.238	26
OC	284.5	61086	2.612	0.112	0.072	0.310	25
Chl-*a*	284.43	41746	1.846	0.169	0.049	0.360	24

Correlation graphs were plotted to identify any relationships between treatment and controls before normalisation by the controls was undertaken. This allowed for identification of background levels as a high background of ammonium within the control may have generated a high concentration in the high treatment. The relationship between both medium and high treatment and the control in the upper sediment depth displayed weak negative correlations (Figure 9). The reverse was observed in the medium and high treatments in the lower sediment depth where a weak positive correlation was identified (Figure 10).

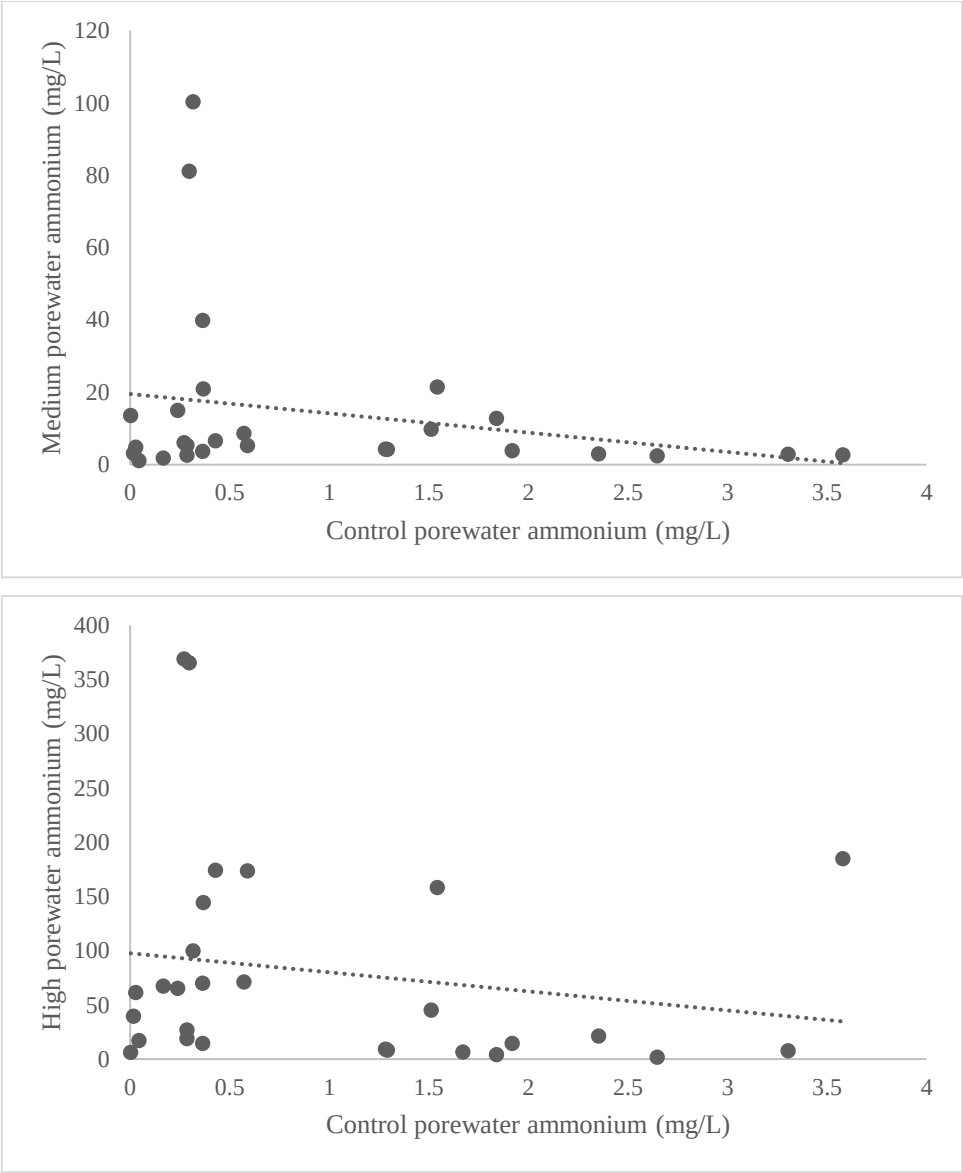

Figure 9: Top: Control and medium treatment porewater ammonium concentration correlation within the upper sediment depth (0-2 cm). Bottom: Control and high treatment porewater ammonium concentration correlation within the upper sediment depth.

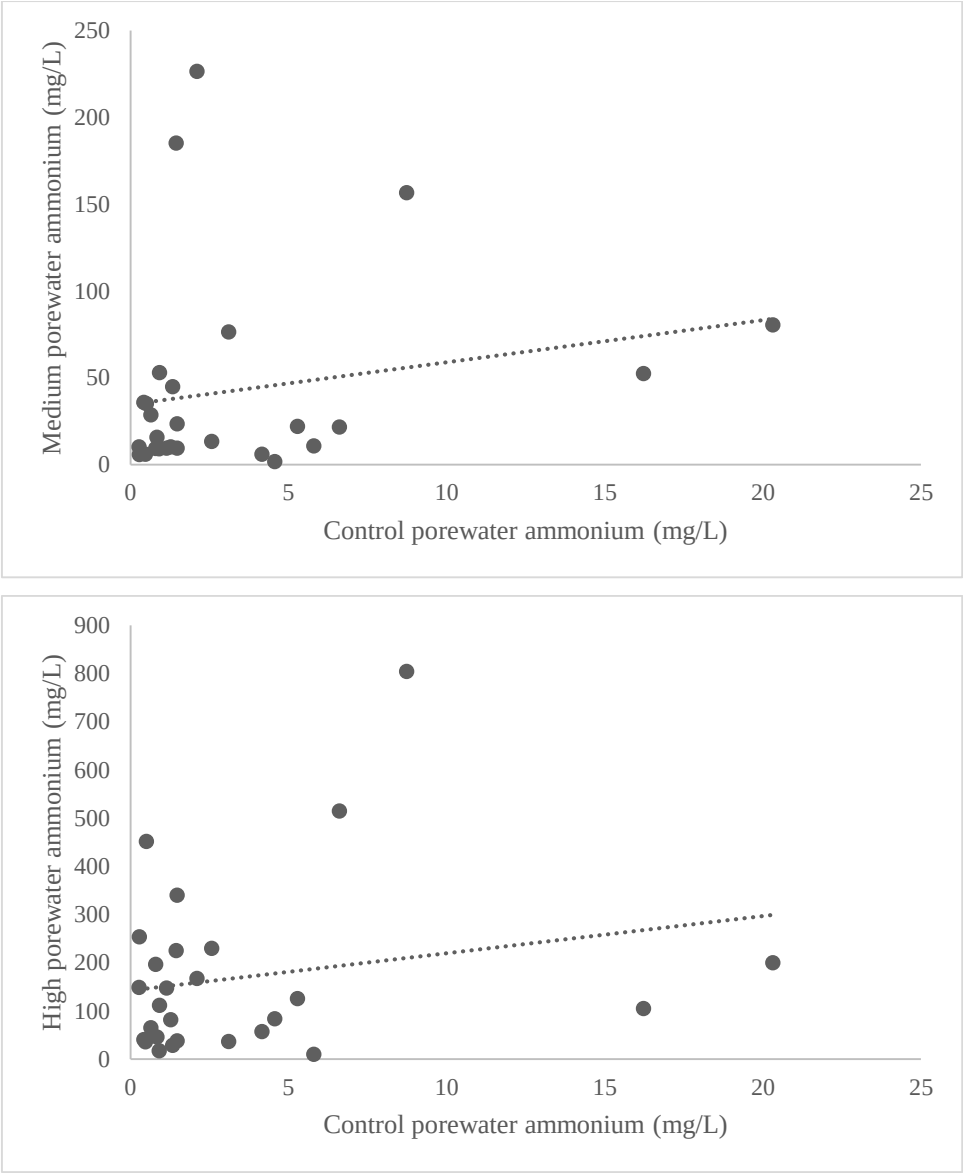

Figure 10: Top: Control and medium treatment porewater ammonium concentration correlation within the lower sediment depth (5-7 cm). Bottom: Control and high treatment porewater ammonium concentration correlation within the lower sediment depth.

After normalisation by the control plots to give a rate of nutrient processing/accumulation (Equation 5) much variation in the processing of porewater nutrients was observed across the sandflat. The medium treatment had accumulated ammonium (negative values) within the porewater in the upper sediment (Figure 11). The medium and high treatments displayed a similar rate of processing, these were less than the accumulation rates. Two-way ANOVA for normalised rate data displayed no significance between treatment ($p = 0.314$) or sampling depth ($p = 0.149$) (see Appendix 3).

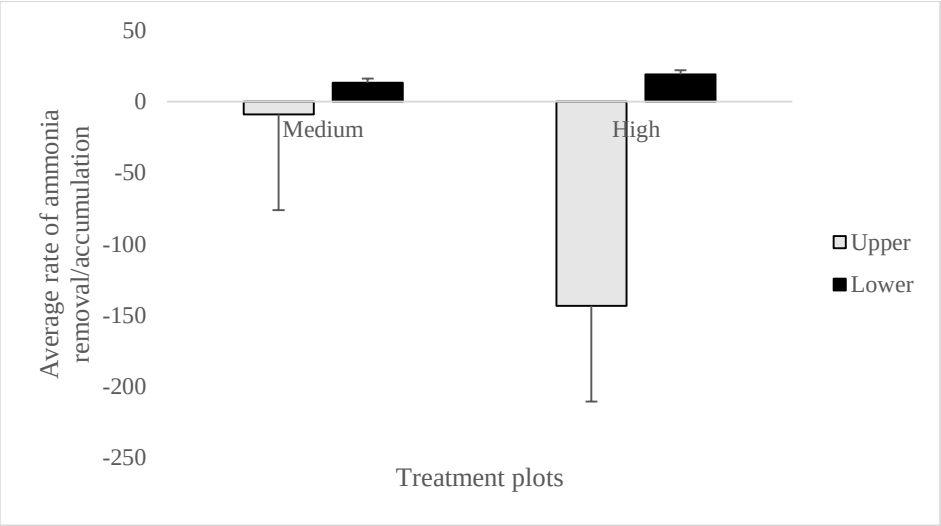

Figure 11: Average rate of ammonium processing and accumulation for upper (0-2 cm) and lower (5-7 cm) sediment depths derived from D_{47} control and nutrient data from medium and high treatments (see equation 5) ± SE.

Neither the medium or high treatments in the lower sediment were correlated with porewater ammonium processing rates (see Appendix 3). However, marginal significance was observed in the medium upper treatment for *M. liliana* and shell hash (Table 18), while sequential tests identified *M. liliana* and *A. stutchburyi* as marginally significant (Table 19). *M. liliana* was negatively correlated with porewater ammonium processing in the medium treatment upper sediment depth, while *A. stutchburyi* was positively correlated. S was correlated with the high treatment plot upper sediment depth ammonium processing rates (Table 20). Sequential tests further identified S and F_S as significant (Table 21), where 16 percent of the variation in porewater ammonium was explained by S. Both S and F_S were positively correlated with porewater ammonium processing.

Table 18: Normalised distance based linear model marginal test between environmental predictors and porewater ammonium processing for medium treatment within the upper sediment depth: results of the stepwise section procedure. SS (trace) = portion of sum of squares relative to the analysed predictor variables (sediment properties and macrofaunal variables); pseudo-F ratio statistic; P = level of statistical significance (p <0.05 in bold indicates significance); Prop = the proportion of variation explained by the model. Correlation directions are indicated for marginally significant p-values (+ or -) for both marginal and sequential tests.

	SS(trace)	**Pseudo-F**	**P**	**Prop.**
Chl-*a*	19.19	0.013	0.912	0.0005
Phaeo	281.02	0.187	0.683	0.008
MGS	1709.10	1.182	0.286	0.047
Mud	1349.30	0.924	0.348	0.037
F_N	214.20	0.142	0.703	0.006
F_S	907.80	0.614	0.450	0.025
A. stutchburyi	1665.20	1.150	0.290	0.046 (+)
M. liliana	5024.80	3.843	0.059	0.138 (-)
Seagrass	2137.90	1.497	0.233	0.059
Sand	0.36	0.0004	0.988	0.0009
Shell hash	4930.60	3.760	0.069	0.135

Table 19: Normalised distance based linear model sequential test between environmental predictors and porewater ammonium processing for medium treatment within the upper sediment depth: results of the stepwise section procedure. AIC = degree of support for the model; SS (trace) = portion of sum of squares relative to the analysed predictor variables (sediment properties and macrofaunal variables); pseudo-F ratio statistic; P = level of statistical significance (p <0.05 in bold indicates significance); Prop = the proportion of variation explained by the model; Cumul = cumulative variation explained; res.df = residual degrees of freedom.

	AIC	**SS(trace)**	**Pseudo-F**	**P**	**Prop.**	**Cumul.**	**res.df**
M. liliana	188.49	5024.8	3.843	0.060	0.138	0.138	24
A. stutchburyi	186.93	4015.9	3.375	0.078	0.110	0.248	23
F_S	186.04	2882	2.590	0.121	0.079	0.328	22

Table 20: Normalised distance based linear model marginal test between environmental predictors and porewater ammonium processing for high treatment within the upper sediment depth: results of the stepwise section procedure. SS (trace) = portion of sum of squares relative to the analysed predictor variables (sediment properties and macrofaunal variables); pseudo-F ratio statistic; P = level of statistical significance (p <0.05 in bold indicates significance); Prop = the proportion of variation explained by the model. Correlation directions are indicated for significant p-values (+ or -) for both marginal and sequential tests.

	SS(trace)	Pseudo-F	P	Prop.
F_S	481000	1.590	0.220	0.060 (+)
Mud	640000	2.161	0.152	0.080
N	1030000	3.676	0.065	0.128
F_N	545000	1.816	0.184	0.068
Phaeo	157000	0.499	0.495	0.020
S	1290000	4.761	**0.037**	0.160 (+)
M. liliana	74409	0.234	0.635	0.009
Sand	642	0.002	0.973	0.0007
Shell hash	78259	0.246	0.628	0.010

Table 21: Normalised distance based linear model sequential test between environmental predictors and porewater ammonium processing for high treatment within the upper sediment depth: results of the stepwise section procedure. AIC = degree of support for the model; SS (trace) = portion of sum of squares relative to the analysed predictor variables (sediment properties and macrofaunal variables); pseudo-F ratio statistic; P = level of statistical significance (p <0.05 in bold indicates significance); Prop = the proportion of variation explained by the model; Cumul = cumulative variation explained; res.df = residual degrees of freedom.

	AIC	SS(trace)	Pseudo-F	P	Prop.	Cumul.	res.df
S	339.61	1290000	4.761	**0.038**	0.160	0.160	25
F_S	336.83	1100000	4.644	**0.041**	0.136	0.296	24
M. liliana	336.19	5280000	2.366	0.139	0.066	0.362	23

3.4 Impact of porewater nutrient elevation on chlorophyll *a* and seagrass

Medium and high treatment plots for chl-*a* and seagrass coverage data from D_{47} were normalised by control data (Figure 12). When the medium and high treatments were plotted against the average chl-*a* accumulation, chl-*a* displayed an increase within the medium plot, at an average of 1.28, while the high plot displayed a slightly smaller value of 1.24. T-tests performed on the normalised data for average chl-*a* accumulation identified the increase in chl-*a* within the medium treatment as significant (p = 0.01), however no significant increase was identified in the high

treatment (see Appendix 3). Paired t-tests for chl-*a* were not significant (p = 0.834) in medium or high treatments.

The average seagrass coverage plotted against treatment displayed little difference between the high (0.9) and medium (1.06) treatment. T-tests performed using normalised data on the average seagrass coverage identified no significant growth or decline in both high and medium treatment plots (see Appendix 3). Paired t-tests for seagrass were not significant (p = 0.429) in medium and high treatments.

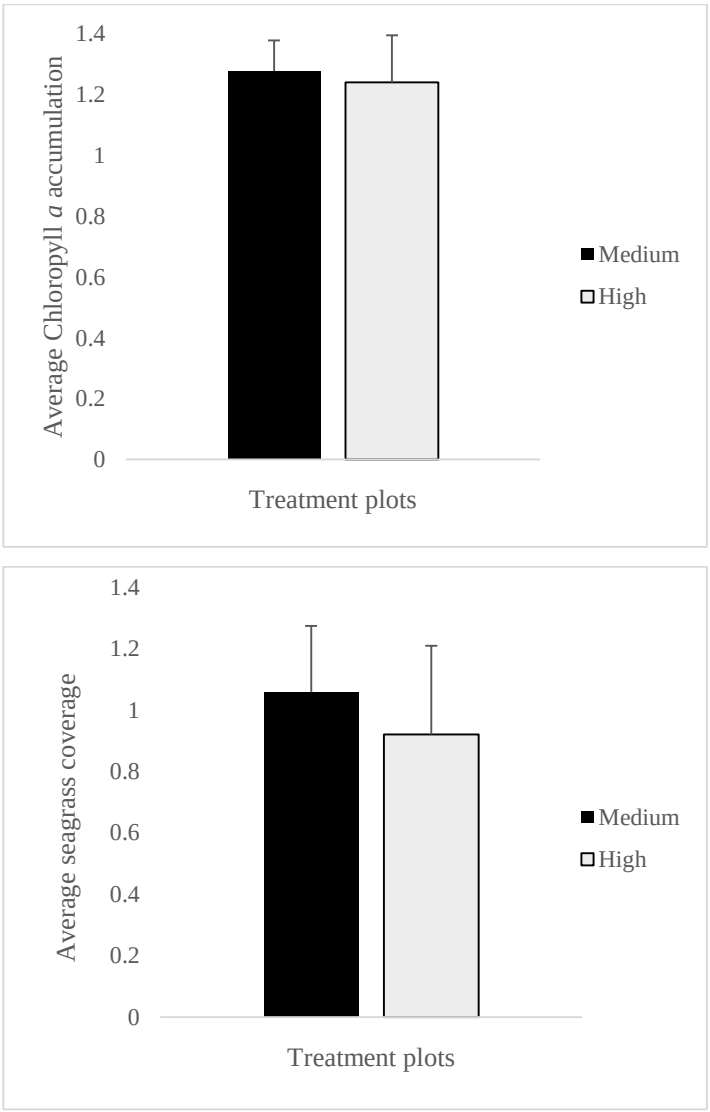

Figure 12: Top: Average chl-*a* accumulation in medium and high treatment normalised by control data from D_{28} and D_{47} ± SE. Bottom: Average seagrass coverage increase within medium and high treatment normalised by control data from D_{28} and D_{47} ± SE.

Chapter 4

Discussion

This was the first time a fertiliser addition experiment of this size had been undertaken in intertidal sediment to determine effects of enhanced nutrients in areas of high and low functional diversity and abundance. The goal of this research was to address effects of enhanced fertiliser loading to benthic intertidal sediments and provide a link to functional diversity. Factors controlling porewater ammonium concentration in ambient and enriched sediment were also sought. Two fertiliser addition treatments (medium and high) were selected alongside a control of ambient sediment to compare the effects of enhanced ammonium concentration in the sediment porewater and whether this varied with depth. Porewater ammonium concentration was significantly elevated at surface (0-2 cm) and depth (5-7 cm) in both the medium and high treatments. A trend was observed in the decline of macrofaunal diversity within fertiliser treatments, however only the decline in *M. liliana* was significant. The number of F_N and the number of *M. liliana* were identified as significant factors controlling the variation in porewater ammonium concentration in the ambient upper sediment depth. However, a switch was observed from macrofaunal to sediment properties where mud showed a significant correlation with porewater ammonium concentration in regions of high nutrient addition. When normalised by the controls significance was observed within the upper sediment depth, both S and F_S were positively correlated with porewater ammonium processing rate in the high treatment, with marginal significance identified for key species *M. liliana* and *A. stutchburyi* in the medium treatment.

4.1 Porewater ammonium elevation

The addition of fertiliser to intertidal sediments is not new to research as others have shown nutrient elevations in sediment porewater as a result of fertiliser addition (Worm et al. 2000; Morris & Keough 2003b; Armitage et al. 2005; Lever & Valiela 2005; O'Brien et al. 2009). Therefore it was expected the addition of slow-release fertiliser to the intertidal sediment of the Kaipara Harbour would result in increased porewater ammonium concentration in both treatments. Unlike many

previous studies the fertiliser selected for the current study contained no P or trace elements, therefore the response observed between the current study and previous studies may have resulted in a different level of macrofauna and porewater response.

Previous fertiliser addition research has studied additions as shallow as 0.75 cm (Udy & Dennison 1997) with deeper additions of up to 12 cm (Erftemeijer et al. 1994). By adding fertiliser via coring to a depth of 10 cm with a plug (2-3 cm) of sediment to prevent removal of fertiliser into the water column, enrichment throughout the sediment column could be achieved. As far as I am aware this is the first *in-situ* study to look at two porewater sediment depths (0-2 and 5-7 cm) to cover both oxic and anoxic sediments from a single enrichment experiment, as porewater samples in previous research have commonly looked at porewater elevation at a single depth. As anticipated porewater ammonium concentration results indicated a difference in nutrient processing with depth, where the lower sampling depth displayed an increased concentration of porewater ammonium than the upper sediments. Falcão & Vale (1995) undertook laboratory experiments which demonstrated the removal of ammonium from the upper 2 cm of sediment via bioturbation, thus with increased bioturbation the nutrient removal from the sediment increased. Therefore, the removal of ammonium within the surface sediments is linked to the process of bioturbation, a key physical function of macrofauna which decreases with depth (Boudreau 1998), similarly a decline in oxygen occurs with increased sediment depth (Andersen & Helder 1987). Therefore nutrient elevation at depth is likely as the macrofaunal contribution to benthic-pelagic coupling through bioturbation and oxygenation is decreased.

A switch from macrofaunal to sediment properties was observed in correlations against porewater ammonium concentration from ambient to enriched sediments. Significant correlations of porewater ammonium concentration in the upper control plot sediments were identified as F_N and the number of *M. liliana,* one of the key functional species (Thrush et al. 2006a), while the sole significant correlation of porewater ammonium in areas of high fertiliser enrichment in both upper and lower sediment depths was mud. F_N displayed a positive correlation in ambient sediment porewater ammonium concentration where porewater ammonium concentration increased with increased F_N. This may be linked to the increased density of individuals where excretion from these organisms is also contributing to the

ammonium elevation within the porewater. Excretion from benthic organisms may be increasingly elevated during low tide when porewater sampling was undertaken, thus concentrating ammonium within the porewater until high tide where the action of bioturbation will aid in the release of ammonium into the overlying water column (Falcão & Vale 1995). A negative correlation was observed between *M. liliana* and porewater ammonium concentration, thus with increased *M. liliana* abundance the porewater ammonium concentration was shown to decrease. When porewater ammonium was elevated in both the medium and high treatments the abundance of *M. liliana* declined, thus in ambient sediment where conditions are seemingly more suited, *M. liliana* may be able to further contribute to ecosystem functioning in the form of nutrient cycling. However, it must be noted that one of the major limitations of the correlative approach adopted here is that we cannot deduce causative mechanisms underlying relationships between macrofauna diversity and changes in porewater ammonium concentrations.

After fertiliser enrichment the high treatment plots displayed a negative correlation between mud and porewater ammonium concentration, therefore porewater ammonium concentration decreased with increased mud content. This was contrary to expectations, given that muddy sediments are often resultant from high rates of deposition, thus tend to be organically rich, with often much higher concentrations reported than their sandy sediment counterparts (e.g. Erftemeijer & Middelburg 1993). The addition of fertiliser to the sediment and the resulting porewater ammonium increase elevated ammonium more than what would be expected from organic material surrounding mud particles. The greater porosity of sand particles and therefore porewater space may allow greater diffusion from the fertiliser pellets resulting in a greater build-up of nutrients. In the absence of bioturbation, the sandy sediment porewater ammonium concentration may be somewhat trapped in the deeper layers for longer time periods meaning a greater ammonium concentration is observed. Thus, the negative effect of mud on porewater ammonium concentration could be due to slower release and a build-up of nutrients.

When porewater ammonium data was normalised by the controls (Equation 5) to give a rate of porewater ammonium processing, models for the upper sediment depth within the medium treatment identified marginal significance in both *M. liliana* and *A. stutchburyi*. *M. liliana* was positively correlated with porewater

ammonium processing thus an increase in *M. liliana* abundance correlated with an increased rate of porewater ammonium processing. Although *M. liliana* live at depths greater than the 0-2 cm sampled for porewater in the upper sediment (Hewitt et al. 1996; Thrush et al. 2006a), results suggest they may in fact be important contributors to nutrient cycling of the upper sediment. *A. stutchburyi* displayed the reverse of this, where in increased abundance the rate of porewater ammonium processing decreased. Thus, with increased abundance of *A. stutchburyi* the rate of processing may be influenced by other functions such as bioturbation of the sediment, where not all species contribute equally to functioning as the functionality of a system may be dominated by a key species. *A. stutchburyi* is an important contributor to the processing of nutrients within the soft sediments (Sandwell et al. 2009), therefore with a decline in this key species, a decline in porewater processing is expected. *A. stutchburyi* may be able to tolerate enhanced levels of ammonium, contrary to this they may have responded adversely to this increase in ammonium where *A. stutchburyi* may have become somewhat dormant thus decreasing nutrient processing within the sediments.

Both S and F_S were positively correlated with the rate of porewater ammonium processing, therefore increased S or F_S abundance correlated with lower porewater ammonium concentrations. Increased S and F_S supports the idea of increased ammonium processing with increased diversity, therefore increasing the processing stability of the soft sediments where there is a constant feedback from the sediment to the overlying water column. Previous research has shown that increased abundance has led to increased stability of an ecosystem in other assemblages (Tilman 2001); however, instead of increased biomass an increase in nutrient cycling associated with species diversity may be observed within the soft sediment systems. The increased number of species therefore provides an increased number of functions; enhanced bioturbation increases oxygen availability within the sediment surface; which has a knock-on effect on nutrient cycling by increasing the nutrient remineralisation rates (Aller 1982; Kristensen & Blackburn 1987; Aller 1994; Meysman et al. 2006). These processes are collectively important for controlling porewater nutrient concentrations within the sediment.

4.2 Macrofaunal response

There was an obvious trend in the number of N and S including those of functional significance for the processing of porewater nutrients, where a decline was observed with increased porewater ammonium in the medium and high treatment. This decline was not significant and the testing of this was beyond the scope of this study. *M. liliana* did however decline significantly with increased porewater ammonium concentration. This decline may be due to sensitivity to ammonium as it is known high levels of ammonium can be toxic to benthic macrofauna (Gray et al. 2002), hence it was expected the addition of fertiliser and the subsequent increase in porewater ammonium concentration would potentially result in adverse effects (O'Brien et al. 2009). A two way interaction is noted between porewater ammonium concentration and macrofaunal diversity; with increased diversity macrofauna influence the concentration of ammonium in the porewater, while with increased porewater ammonium concentration the macrofaunal diversity declines.

Benthic macrofauna along with external physical factors (porewater advection and sediment re-suspension) influence the rate of nutrient exchange (Hansen & Kristensen 1997; McGlathery et al. 2004). Adult *M. liliana* live in the top 5- 15 cm of sediment (Hewitt et al. 1996; Thrush et al. 2006a), thus the depth *M. liliana* live may play a key role here as with increased depth the role of diffusion is slower, and the amount of oxygen may be increasingly limited due to the enhanced nutrient levels and the subsequent increase in respiration. Contrasting to *M. liliana*, *A. stutchburyi* live at shallower depths of 0- 2 cm (Thrush et al. 2006a) where due to bioturbation oxygen levels are increased within the upper sediment and decrease nutrients as increase nutrient flux from the benthos, which may have led to the lower porewater ammonium concentrations observed in the surface sediments. Therefore it is possible *A. stutchburyi* showed no significant decline in medium and high treatments due to the lower porewater ammonium concentration in the surface sediments as a result of such processes.

Large deposit feeders such as *M. liliana* provide an important feedback link to both nutrient regeneration and grazing (Thrush et al. 2006b). Thus enhanced ammonium enrichment of porewater such as that within the high and medium treatments results in depletion of oxygen where the role of large macrofauna is lost, subsequently so is their function (Meyer-Reil & Köster 2000; Diaz & Rosenberg 2008).

Performance of an ecosystem is modified due to changes in community structure, identified by the significant decline of *M. liliana* and the decline in F_N and F_S. Ecosystem functions are used as measures of biodiversity loss (Norkko et al. 2013) therefore the removal of traits belonging to organisms such as *M. liliana* not only alters the community composition but alters the flux of nutrients and oxygen (Thrush et al. 2006a; Norkko et al. 2013), resulting in increased porewater ammonium concentration at depth.

The theory of functional redundancy links to species loss or decline in regards to the functioning of an ecosystem (Rosenfeld 2002). It would appear this was somewhat absent in the lower sediment depths where *M. liliana* was removed with enhanced porewater ammonium concentration, as the porewater nutrient concentrations exceeded those of the upper sediment. However due to the macrofaunal sampling technique the abundance of organisms in the lower sediment depth is unknown so comparisons between the upper and lower depths cannot be determined.

Contrary to expectations the addition of fertiliser and subsequent elevation in porewater nutrients did not result in significant removal of macrofauna within the high or medium treatments. Therefore it is likely the ammonium concentration within the porewater was enough to stress macrofauna to the point where a trend in the decline was observed; however, this decline was not of any significance. The trend between the treatments and the macrofaunal community, although not statistically significant it may be possible that due to the decline in macrofaunal species the community response variables are no longer significant, thus sediment properties (i.e. grain size) displayed a significant correlation against porewater nutrients in their absence. I suspect a longer enrichment period may have resulted in the significant macrofaunal decline hypothesized.

Much research has identified the response of seagrass to nutrient addition within coastal regions (e.g. Orth, 1977; Bulthuis & Woelkerling, 1981; Erftemeije et al. 1994; van Lent et al. 1995; Udy & Dennison, 1997b), however little research has identified the influence or the response of sediment macrofauna. O'Brien et al. (2010) enriched sediments with different sources of nutrients including fertiliser, this resulted in an elevation of porewater nutrients however no obvious changes

were observed in the macrofaunal community. Other enrichment experiments where similar levels of slow-release fertiliser had been used identified increased abundance of some species and a decline in others (Morris & Keough 2003a), however the duration of the current study was shorter.

The addition of fertiliser to the intertidal sediments may have decreased the available oxygen, particularly within the deeper sediments where available oxygen is scare, thus adverse effects of this are likely to impact macrofaunal abundance and diversity (Diaz & Rosenberg 1995; Kelaher & Levinton 2003; O'Brien et al. 2010). The increase in nutrients and temperature given the season both sampling and experimentation occurred it is possible increased oxygen consumption occurred within the sediments ultimately resulting in the removal of macrofaunal species from within the enrichment plots (Fitch & Crowe 2011).

4.3 Microphytobenthos

Both the medium and high treatments showed an increase in the average chl-*a* concentration after normalisation by the controls; however, only the increase within the medium was statistically significant. Nutrient enrichment, sediment properties and macrofaunal interaction affect the production of MPB (Guarini et al. 1998; Chapman et al. 2009), measured as chl-*a* concentration within the sediment. MPB is an important source of organic material for macrofauna (Underwood & Kromkamp 1999; Middelburg et al. 2000), therefore due to macrofaunal abundance decline or absence it is expected the concentration of this would increase.

Previous experiments give supporting evidence for the link of nitrogen enrichment and the effect of increased growth of benthic microalgae (Posey et al. 1995). Earlier sediment fertiliser addition experiments run over similar time periods of several weeks found increases in MPB as a result of enhanced porewater nutrient levels (Flothmann & Werner 1992; Morris & Keough 2003a; Posey et al. 2006). Lever and Valiela (2005) found with grazer exclusion the amount chlorophyll *a* increased, however this varied with the amount of nitrogen loading and therefore varied between the estuaries they sampled. In the current experiment by adding two treatment levels, the nitrogen difference between the medium and high treatment may have somewhat contributed to the difference observed. Alike to the high

treatment of the current study O'Brien et al. (2010 & 2009) observed no detectable effect within chl-*a*, even though the elevation of porewater nutrients was achieved. This may be due to rapid turnover where the grazing community consumes and subsequently removes this biomass (Hillebrand et al. 2000; Levinton & Kelaher 2004).

4.4 Seagrass

The percent coverage of seagrass was used as a measure of growth as a result of elevated porewater nutrient concentrations. Both the medium and high treatments showed no significant effect of enhanced porewater ammonium, thus no increase or decrease in growth was determined as a result of the fertiliser addition. The time period (D_{28} to D_{47}) may not have been long enough for seagrass growth to significantly occur. It is also possible the amount of fertiliser added may have stunted the growth of the seagrass as elevated ammonium concentrations have been found to have an adverse response to seagrass (e.g. *Zostera noltii*) (Brun et al. 2002), yet a decline in growth was not observed. It has been noted that season plays an important role in such observations (Brun et al. 2002), for example seagrass *Posidonia oceanica* obtains maximum growth in spring and minimum growth in late-summer (Invers et al. 2004), where experimentation of the current study was undertaken mid-late summer. Dennison et al. (1987) also identified a lack of seagrass response with fertiliser addition, however this was identified to be a result of failed enrichment, which is not the case of this experiment. Contrary to the current results Kelaher et al. (2013) found nutrient enrichment within the sediment increased seagrass biomass, however the duration of the experiment was longer, where fertiliser was replaced every two months. The measures of growth however are also different to the current study, this study undertook measures based solely on the percentage coverage of the plot surface normalised by the control plots, which are not common indicators of seagrass growth (Short & Duarte 2001).

4.5 Limitations

Monitoring nutrient enrichments through time may provide a better understanding of benthic responses (Worm et al. 2000). Longer addition periods and increased sampling dates would have provided a greater data set and therefore potentially a greater understanding of the controls on porewater ammonium concentration.

This was a large study with many samples for laboratory analyses, this resulted in an increased cost of processing and analysing, hence nitrite, nitrate and phosphorus porewater concentrations were not analysed.

4.6 Summary of major findings

Changes to porewater ammonium concentration were analysed from within a soft sediment estuarine environment after the addition of slow-release fertiliser. This experiment compared medium and high fertiliser additions as well as porewater ammonium elevation at surface (0-2 cm) and depth (5-7 cm) to ambient sediment in areas of both high/low functional diversity and high/low abundance.

The major findings of the experiment were:

1. Porewater ammonium concentration was significantly elevated in both medium and high fertiliser addition treatments, this was greater on D_{47} than $D_{28.}$ As expected the porewater ammonium concentration was greater in the high treatment than the medium treatment.
2. The addition of fertiliser increased the ammonium concentration significantly within surface (0-2 cm) sediments and at depth (5-7 cm). The concentration of ammonium was greater at depth than in the surface sediments.
3. Correlations of porewater ammonium concentration within the upper sediment depth of the ambient sediment identified F_N and key functional species *M. liliana* as significant. No correlations were observed between macrofaunal or sediment properties in the lower sediment depth.
4. With enhanced porewater ammonium concentration a switch was observed from macrofauna to sediment properties, where mud displayed a significant correlation with porewater ammonium concentration in high treatments of both the upper and lower sediment depth.
5. Only the upper sediment depth when normalised by the control plots displayed significant correlations. The medium plot highlighted both key functional species *M. liliana* and *A. stutchburyi* as marginally significant.

While the high treatment identified both S and F_S as significant when correlated against the rate of porewater ammonium processing.

6. With enhanced porewater ammonium concentration a decline in S, F_S, N, F_N and *A. stutchburyi* was observed, however only the decline in *M. liliana* was significant.

4.7 Suggestions for future research

Through complex biochemical interactions macrofauna influence the processing of nutrients within the sediment, an essential link for benthic-pelagic coupling and the cycling of nutrients. The functioning of estuaries is complex given such interactions which vary on spatial scales. This research looked at a single estuary over the New Zealand summer where two sampling dates provided sediment properties and macrofauna samples for analysis. A trend was observed in the decline of S, N, F_S, F_N and *A. stutchburyi* with enhanced porewater ammonium concentration, although not significant, further research may be able to identify the impact or severity of this decline, particularly if it were to continue in the same trajectory. Identification of this would involve longer field experiments to further determine implications of enhanced porewater ammonium concentration. It may be beneficial to look at rare species as well as those common (e.g. *A. stutchburyi* and *M. liliana*) as previous research has identified rare species also play key roles in functional biodiversity (Ellingsen et al. 2007). If we can grasp a greater understanding of the marine intertidal sandflats and how the associated communities are structured and function we can begin to mitigate biodiversity loss and minimise disturbance.

Where sediment porewater sampling is undertaken in future I suggest sectioning of macrofauna, chl-*a*, grain size and OC sample cores into upper and lower sediment depths as this could aid in the identification of controls on porewater nutrients at surface and at depth. A comparison of two different estuaries may also provide some insight into whether estuaries have the same or similar controlling factors.

In-situ studies to determine what level of porewater ammonium elevation macrofauna abundance and diversity begins to decline may also be beneficial. A maximum level tolerated could be identified before the system becomes anoxic and linked to sediment properties. This would also identify which species are more

tolerant to nutrient elevation, which may be somewhat linked to different ranges in sediment properties. Determination of how far macrofaunal species migrate away from addition zones may also be of interest.

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Appendices

Appendix 1

Table A.1. 1: GPS coordinates for the 28 sites across the Kaipara Harbour sandflat.

Site No.	Easting	Northing	Latitude WGS84	Longitude WGS84
	NZTM	NZTM		
1	1715904	5971943	36 23 26.42860 S	174 17 32.53337 E
2	1715908	5971771	36 23 32.00775 S	174 17 32.78630 E
3	1715908	5971577	36 23 38.30246 S	174 17 32.89052 E
4	1715904	5971494	36 23 40.99730 S	174 17 32.77458 E
5	1715921	5971296	36 23 47.41441 S	174 17 33.56325 E
6	1715921	5971196	36 23 50.65911 S	174 17 33.61699 E
7	1715922	5971171	36 23 51.46985 S	174 17 33.67056 E
8	1715923	5971091	36 23 54.06517 S	174 17 33.75369 E
9	1715922	5971015	36 23 56.53157 S	174 17 33.75440 E
10	1716025	5970999	36 23 57.00595 S	174 17 37.89705 E
11	1716004	5971315	36 23 46.76185 S	174 17 36.88424 E
12	1715991	5971518	36 23 40.18077 S	174 17 36.25334 E
13	1715985	5971672	36 23 35.18654 S	174 17 35.92974 E
14	1715978	5971755	36 23 32.49649 S	174 17 35.60420 E
15	1715977	5971813	36 23 30.61499 S	174 17 35.53289 E
16	1716072	5971935	36 23 26.61516 S	174 17 39.27986 E
17	1716072	5971835	36 23 29.85986 S	174 17 39.33365 E
18	1716075	5971656	36 23 35.66656 S	174 17 39.55035 E
19	1716079	5971559	36 23 38.81218 S	174 17 39.76307 E
20	1716111	5971224	36 23 49.66798 S	174 17 41.22766 E
21	1716120	5971055	36 23 55.14759 S	174 17 41.67986 E
22	1716225	5971094	36 23 53.83644 S	174 17 45.87313 E
23	1716216	5971177	36 23 51.14727 S	174 17 45.46719 E
24	1716205	5971253	36 23 48.68610 S	174 17 44.98476 E
25	1716172	5971446	36 23 42.43821 S	174 17 43.55636 E
26	1716167	5971463	36 23 41.88879 S	174 17 43.34654 E
27	1716143	5971774	36 23 31.80823 S	174 17 42.21589 E
28	1716127	5971955	36 23 25.94230 S	174 17 41.47635 E

Table A.1. 2: Species and functional group contributors to biogeochemical processing.

Taxonomic ID	Functional group
Aglaophamus macroura	Soft-bodied Pred.Scav Below surface. Deep Freely motile No habitat structure
Alpheus sp.	Rigid Pred.Scav Below surface Freely motile Large burrow former
Asychis sp.	Soft-bodied Below surface Tube structure
Austrohelice crassa	Rigid Pred.Scav Below surface Freely motile Large burrow former
Austrovenus stutchburyi	Calcified Suspension feeding Top 2 cm Freely motile
Boccardia syrtis	Soft-bodied Suspension feeding Tube structure
Callianassa sp.	Rigid Pred.Scav Below surface Freely motile Large burrow former
Ceratonereis sp.	Soft-bodied Pred.Scav Below surface. Deep Freely motile No habitat structure
Euchone sp.	Soft-bodied Suspension feeding Tube structure
Glycera americana	Soft-bodied Pred.Scav Below surface. Deep Freely motile No habitat structure
Glycinde grahami	Soft-bodied Pred.Scav Below surface. Deep Freely motile No habitat structure
Glycinde trifida	Soft-bodied Pred.Scav Below surface. Deep Freely motile No habitat structure
Hemiplax hirtipes	Rigid Pred.Scav Below surface Freely motile Large burrow former
Lepidonotinae	Soft-bodied Pred.Scav Below surface. Deep Freely motile No habitat structure
Lumbrineridae	Soft-bodied Pred.Scav Below surface. Deep Freely motile No habitat structure
Macomona liliana	Calcified Deposit feeding Deep Limited mobility No habitat structure Large
Macroclymenella stewartensis	Soft-bodied Below surface Tube structure
Magelona dakini	Soft-bodied Deposit feeding Below surface Limited mobility
Musculista senhousia	Calcified Suspension feeding Top 2 cm Sedentary
Nemertean sp.	Soft-bodied Pred.Scav Below surface. Deep Freely motile No habitat structure
Nicon aestuariensis	Soft-bodied Pred.Scav Below surface. Deep Freely motile No habitat structure
Notomastus sp.	Soft-bodied Deposit feeding Deep
Nucula hartvigiana	Calcified Deposit feeding Top 2 cm Limited mobility
Orbinia papillosa	Soft-bodied Deposit feeding Below surface Freely motile
Owenia petersonae	Soft-bodied Below surface Tube structure
Paphies australis	Calcified Suspension feeding Top 2 cm Freely motile
Pectinaria australis	Soft-bodied Below surface Tube structure
Perinereis vallata	Soft-bodied Pred.Scav Below surface. Deep Freely motile No habitat structure
Philocheras australis	Rigid Pred.Scav Below surface Freely motile Large burrow former
Phoronis sp.	Soft-bodied Suspension feeding Tube structure
Platynereis australis	Soft-bodied Pred.Scav Below surface. Deep Freely motile No habitat structure
Pseudopolydora FAT	Soft-bodied Below surface Tube structure
Pseudopolydora THIN	Soft-bodied Suspension feeding Tube structure
Scalibregmatidae	Soft-bodied Deposit feeding Deep
Scolecolepides benhami	Soft-bodied Deposit feeding Below surface Freely motile

Taxonomic ID	Functional group
Scoloplos cylindrifer	Soft-bodied Deposit feeding Below surface Freely motile
Solemya parkinsoni	Calcified Deposit feeding Top 2 cm Limited mobility
Soletellina siliqua	Calcified Suspension feeding Top 2 cm Limited mobility
Squilla armata	Rigid Pred.Scav Below surface Freely motile Large burrow former
Travisia olens	Soft-bodied Deposit feeding Top 2 cm Freely motile
Trochodota dendyi	Soft-bodied Deposit feeding Below surface Freely motile

Appendix 2

Nutrient addition field trial Tauranga Harbour, Tuapiro point

The aim of this trial was to determine whether the nutrient concentrations selected (based on findings in the literature) would elevate porewater nutrients, and if the addition of nutrients would disperse outside of the plot area elevating porewater ammonium in the ambient sediment.

Study site

Tuapiro point is located in the northern region of the Tauranga Harbour (Figure A.2.1) on the east coast of New Zealand. This was a sand-dominated site edged by mangroves with an absence of seagrass.

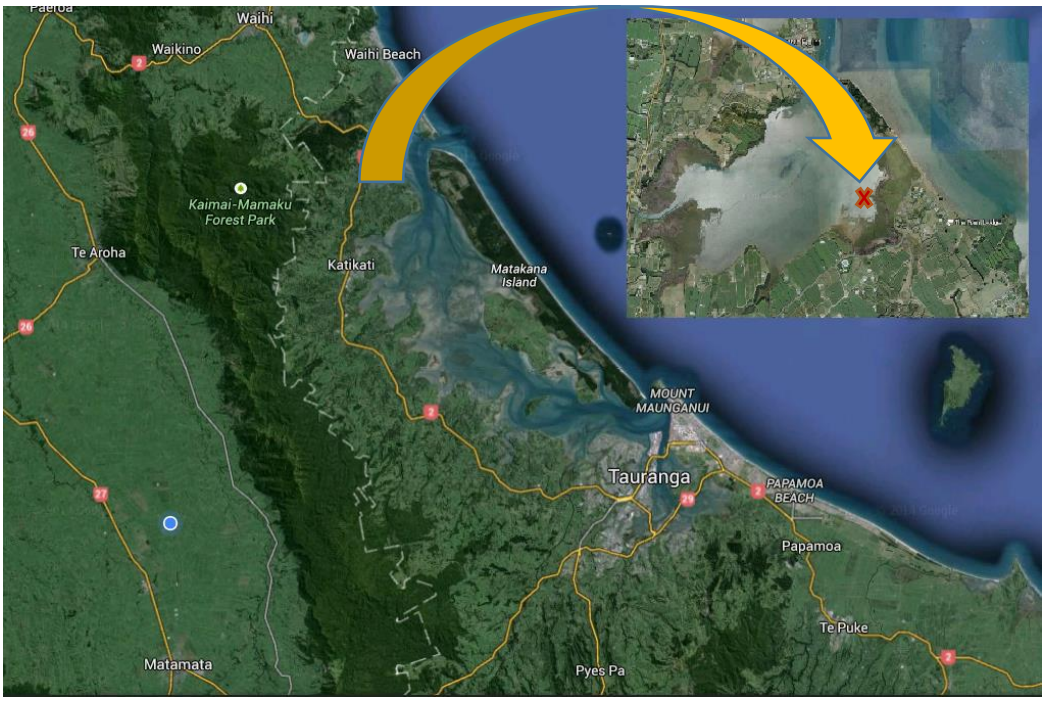

Figure A.2. 1: Location of Tuapiro point sampling site, East Coast of New Zealand.

Experimental treatments

Treatment and control plots were established on the 18th of December 2013 at low tide on the intertidal sandflat. 50 mL centrifuge tubes with 1.5 mm holes drilled in the bottom, sides and lid were filled with 70-day slow-release Nutricote fertiliser (42 % Nitrogen (42:0:0), no P or trace elements). Holes were large enough to allow

for nutrient dispersal into the sediment yet small enough to contain the pellets within the tubes. Tubes were added via sediment cores at a depth ~30 cm to the sediment. Five 1 m^2 plots were selected randomly at Tuapiro point with GPS coordinates recorded (Table A.2. 1). These five treatments were: (1) a procedural control (50 mL centrifuge tube containing only gravel); (2) low treatment (5 g fertiliser amongst gravel in 50 mL centrifuge tube); (3) medium treatment (10 g fertiliser amongst gravel in 50 mL centrifuge tube); (4) high treatment (35 g fertiliser, no gravel in 50 mL centrifuge tube); and (5) a control (Table A.2. 2). The low and medium treatment addition tubes contained gravel to spread the fertiliser throughout the tube and therefore provide a nutrient gradient throughout the sediment column.

Table A.2. 1: GPS locations for each trial plot.

	GPS locations
High	S 37 ˚29.418’ E 175 ˚57.121’
Medium	S 37 ˚29.422’ E 175 ˚57.121’
Low	S 37 ˚29.420’ E 175 ˚57.123’
Procedural Control	S 37 ˚29.422’E 175 ˚57.125’
Control	S 37 ˚29.418’ E 175 ˚57.124

Table A.2. 2: Treatment levels for each of the five plots.

	Tubes	**Fertiliser/tube (g)**	**Nitrogen (g/m^2)**	**Fertiliser (g/m^2)**
High	40	35	588	1400
Medium	40	10	168	400
Low	40	5	84	200
Procedural Control	40	0	0	0
Control	0	0	0	0

The 50 mL centrifuge tubes containing fertiliser/gravel were added to the treatment plots in a gird arrangement (Figure A.2. 2). Each plot surface was broken into 20 squares, within each of these squares two 50 mL centrifuge tubes were added one on top of the other beneath the first 4-5 cm of sediment to give a gradient throughout the porewater space, giving a total of 40 tubes per plot. The centrifuge tubes were centred within each of the squares (Figure A.2. 3).

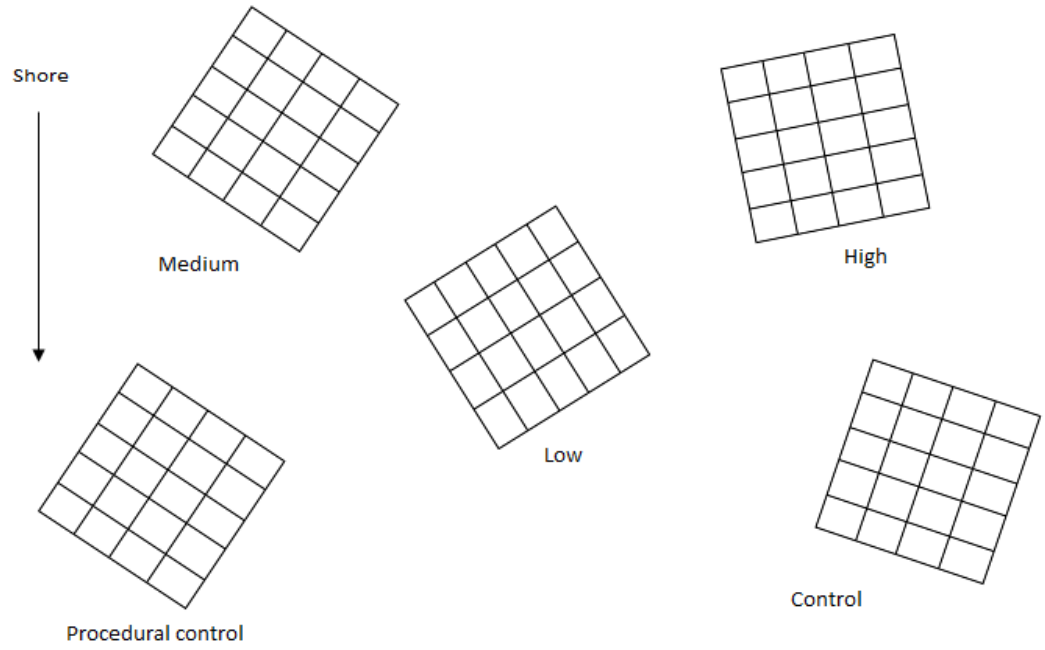

Figure A.2. 2: Plot treatment arrangement.

Figure A.2. 3: Left, polyvinyl chloride (PVC) pipe is used to remove cores of sediment from the centre of each section, following this two centrifuge tubes of gravel/fertiliser are added top to tail. Right, plot after the tubes have been added to the sediment and covered with 4-5 cm of sediment.

Sampling procedure

Plots were checked three weeks after fertiliser addition (7th of January 2014) visually at low tide and photographed. The first sampling was undertaken a week later on January 14th. Syringe cores (2.5 cm diameter) were used to collect sediment samples from each of the five treatment plots at 0-2 cm depth for determination of sediment properties (Chlorophyll *a*, organic matter content and grain size). Four sediment cores were taken per plot and pooled. All sediment cores were kept in cold

and dark conditions following collection before being frozen to await laboratory analysis.

Four syringe cores sectioned at 0−2 and 5−7 cm were collected and pooled for each of the five experimental plots, these were stored in 50 mL centrifuge tubes in dark and cold conditions until reaching the laboratory for immediate removal of porewater. Two section depths would allow for the identification of porewater nutrient enrichment differences at both surface (0-2 cm) and depth (5-7 cm). The second and final sampling occurred on the 11th of February, where both sediment properties and porewater samples were taken.

Laboratory analysis

Chlorophyll *a*, organic matter content and grain size were analysed following the methods described in the main body of the thesis.

Porewater nutrient samples were centrifuged (2000 rpm for ten minutes) immediately upon arrival to the laboratory (Lohrer et al. 2010). Following centrifugation the sediment porewater was removed via pipette, filtered through glass fibre filter paper (0.45 µm) where they were then combined with those of the same location within each respective plot (indicated by matching colours Figure A.2. 4) before being frozen to await nutrient analysis. This gave a total of 8 samples per plot, four within the upper sediment depth (0-2 cm) and four within the lower sediment depth (5-7 cm). Porewater nutrient samples were analysed on a Lachat Flow Injection Analyser for ammonium (NH_4^+) using standardised procedures (Zellweger Analytics 2000).

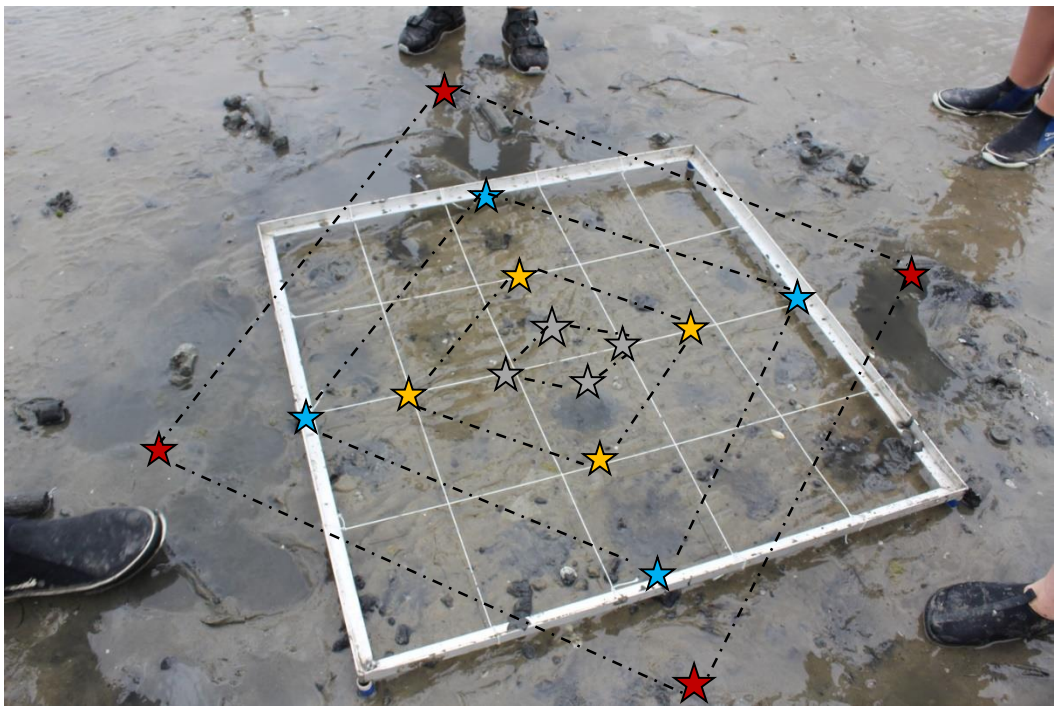

Figure A.2. 4: Stars represent core locations. The distance between the red and green stars is a total 1 m. Stars within the plot are 25 cm apart while the red and blue stars = a distance of 50 cm (not to scale). Grey stars = 1, yellow 2, Blue 3 and Red 4.

General results

Sediment properties and porewater nutrient concentrations from the first and second sampling were identified for the five trial plots (Table A.2. 3 and A.2. 4). Fine sand was the dominant grain size at each plot for both first and second sampling. In the first sampling an overall decline in chlorophyll *a* was observed from positions 1 to 4 in high, medium and low treatments, thus the greater concentrations are observed within the centre of the plots. The second sampling also demonstrated this decline in chlorophyll *a* concentration towards the plot edges, however this was only observed in the medium and high treatment, as the low treatment had increased in concentration. Porewater ammonium concentration was in most cases greater in positions 1, 2 and 3 than in position 4.

Table A.2. 3: Sediment properties and porewater ammonium concentration from the first sampling. Position identifies the position within the plot the sample was taken (see Figure A.2. 4). Some porewater ammonium values are absent due to lack of porewater volume for analysis. Environmental variables: MGS, median grain size; Chl-a, chlorophyll *a*; Mud, mud content; Pheao, phaeopigment; OC, organic matter content; PW [NH_4^+], porewater ammonium concentration.

Treatment	Position	MGS (µm)	Chl-*a* (µg/g)	Mud (%)	Pheao (µg/g)	OC (%)	PW [NH_4^+]
High	1	196	11.42	3.92	2.45	1.53	15.2
High	2	185.6	10.67	3.67	2.24	1.44	
High	3	191.4	8.11	1.25	3.27	1.88	20.6
High	4	201.2	5.88	3.28	4	1.48	
Medium	1	202.7	11.41	2.74	3.27	1.5	212
Medium	2	212.2	9.82	2.22	4.09	1.6	
Medium	3	196.2	8.03	3.24	2.31	1.5	15.6
Medium	4	192	7.73	2.88	2.25	1.54	0.8
Low	1	197.1	9.01	2.57	2.26	1.51	20.3
Low	2	199.7	11.08	4.28	2.75	1.63	
Low	3	181.4	14.16	3.62	2.79	1.42	6.62
Low	4	199.8	5.52	3.19	1.78	1.42	0.33
Procedural control	1	189.2	6.95	3.72	1.89	1.49	0.21
Procedural control	2	214.1	8.065	3.17	1.97	1.52	
Procedural control	3	204.2	6.12	3.08	3.77	1.39	0.15
Procedural control	4	206	6.39	2.6	1.63	1.51	0.1
Control	1	184.5	4.93	2.5	2.88	1.53	
Control	2	202.3	6.41	2.26	1.55	1.5	
Control	3	191.4	6.88	2.95	1.42	1.46	
Control	4	212.1	8.26	2.98	2.55	1.5	

Table A.2. 4: Sediment properties and porewater ammonium concentration from the second sampling. Position identifies the position within the plot the sample was taken (see Figure A.2. 4). Environmental variables: MGS, median grain size; Chl-a, chlorophyll *a*; Mud, mud content; Pheao, phaeopigment; OC, organic matter content; PW [NH_4^+], porewater ammonium concentration.

Treatment	Position	MGS (µm)	Chl-*a* (µg/g)	Mud (%)	Pheao µg/g	OC (%)	PW [NH_4^+]
High	1	194.7	6.83	2.78	1.87	1.51	15.1
High	2	203.2	6.38	2.80	1.30	1.52	8.55
High	3	186.7	6.35	3.19	2.02	1.40	11.85
High	4	202.8	6.59	2.28	1.35	1.44	0.06
Medium	1	216.0	7.37	2.93	2.91	1.58	0.17
Medium	2	200.5	8.80	3.35	1.42	1.61	6.08
Medium	3	221.3	7.80	1.93	1.01	1.54	0.72
Medium	4	209.8	7.34	3.03	1.41	1.47	0.02
Low	1	200.0	12.18	3.26	3.94	1.64	3.01
Low	2	197.5	12.84	2.23	2.47	1.66	0.17
Low	3	204.9	9.69	2.21	2.16	1.61	0.38
Low	4	193.3	6.64	2.76	2.03	1.41	0.03
Procedural control	1	226.3	9.73	2.25	2.78	1.58	0.01
Procedural control	2	225.6	11.65	2.71	3.30	1.51	0.04
Procedural control	3	210.3	12.13	2.72	2.33	1.59	0.01
Procedural control	4	203.4	7.74	2.97	3.36	1.48	0.21
Control	1	193.0	11.53	2.77	2.41	1.44	0.01
Control	2	189.2	13.71	2.35	2.70	1.48	1.40
Control	3	201.2	15.83	2.34	2.05	1.51	0.03
Control	4	201.9	2.97	1.16	0.12	1.59	0.01

Photographs from the visual check in early January showed some interesting surface features. Grazing activity upon the plots of fertiliser addition displayed an increase compared to those of the control and procedural control. Grazing was especially prominent within the high (Figure A.2. 5) and medium (Figure A .2. 6) treatment plots.

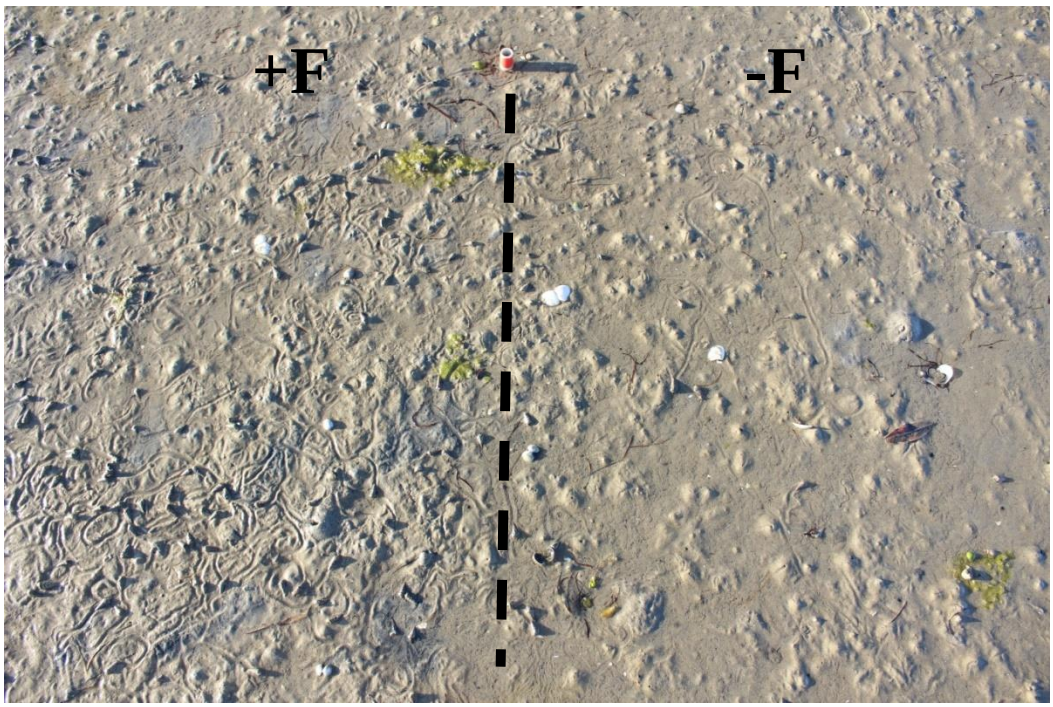

Figure A.2. 5: Photo illustrating high fertiliser plot surface (left) with a greater amount of grazing than the surrounding sediment (right). +F highlights the sediment surface above the fertiliser addition, while -F identifies the ambient sediment surface outside of the treatment plot.

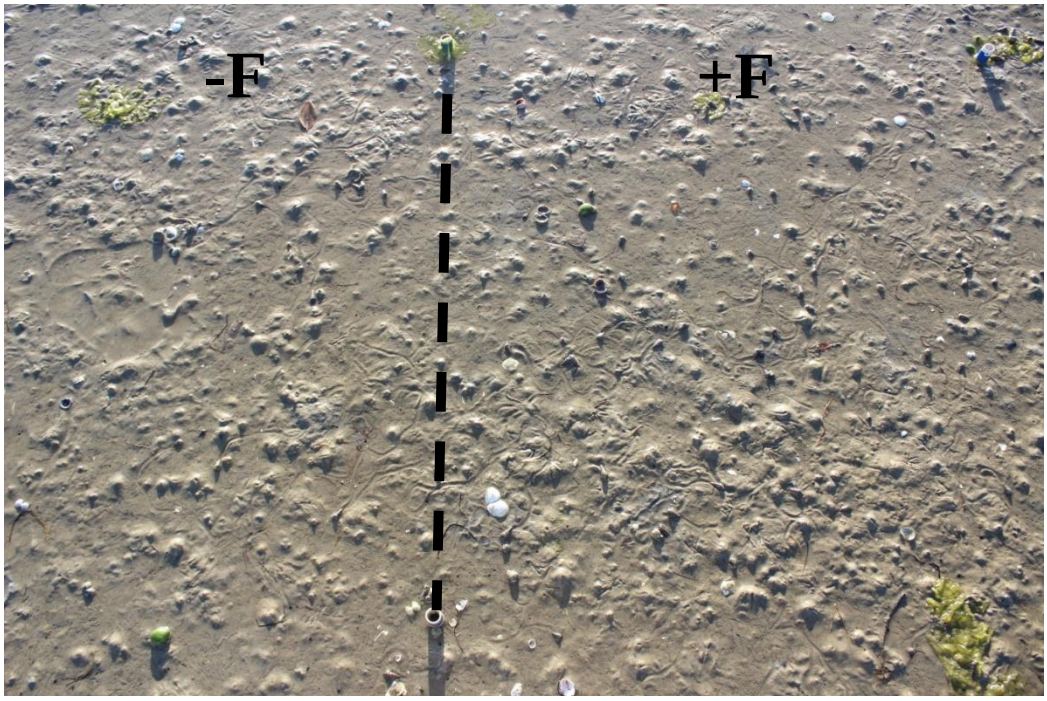

Figure A.2. 6: Photo illustrating medium fertiliser level plot (right) with more grazing than the surrounding sediment (left). +F highlights the sediment surface above the fertiliser addition, while -F identifies the ambient sediment surface outside of the treatment plot.

Findings from this trial indicated an elevation in porewater ammonium concentration within treatment plots (low, medium and high). The addition of slow-release fertiliser to the treatment plots did not elevate the ambient sediments surrounding the plots, thus diffusion of ammonium outside of the plot area was not observed in this trial.

Appendix 3

Statistical results not reported in the main text.

Table A.3. 1: Distance based linear model marginal test between environmental predictors and porewater ammonium concentration for medium treatment plots within the lower sediment depth: results of the stepwise section procedure. SS (trace) = portion of sum of squares relative to the analysed predictor variables (sediment properties and macrofaunal variables); pseudo-F ratio statistic; P = level of statistical significance; Prop = the proportion of variation explained by the model. No significance is observed.

	SS(trace)	Pseudo-F	P	Prop.
Chl-*a*	2622.6	0.695	0.421	0.027
Phaeo	5675.1	1.554	0.221	0.059
MGS	911.2	0.237	0.634	0.009
Mud	3082.2	0.821	0.378	0.032
F_N	832.9	0.217	0.653	0.009
F_S	8314.7	2.345	0.139	0.086
A. stutchburyi	7423.4	2.073	0.159	0.077
M. liliana	2925.1	0.778	0.390	0.030
Sand	834.5	0.217	0.654	0.009
Shell hash	407.8	0.106	0.755	0.004
OC	1235.4	0.323	0.587	0.013
S	5553.3	1.519	0.224	0.057
N	1019.7	0.266	0.618	0.011
Seagrass	2981.5	0.793	0.393	0.031

Table A.3. 2: Distance based linear model marginal test between environmental predictors and porewater ammonium concentration for medium treatment plots within the upper sediment depth: results of the stepwise section procedure. SS (trace) = portion of sum of squares relative to the analysed predictor variables (sediment properties and macrofaunal variables); pseudo-F ratio statistic; P = level of statistical significance; Prop = the proportion of variation explained by the model. No significance is observed.

	SS(trace)	Pseudo-F	P	Prop.
Chl-*a*	75.78	0.130	0.738	0.005
Phaeo	135.55	0.234	0.656	0.009
MGS	804.46	1.458	0.242	0.055
Mud	1067.40	1.972	0.181	0.073
F_N	109.20	0.188	0.670	0.008
F_S	907.73	1.657	0.215	0.062
A. stutchburyi	246.93	0.430	0.522	0.017
M. liliana	14.96	0.026	0.880	0.001
Sand	7.51	0.013	0.899	0.0005
Shell hash	38.89	0.067	0.850	0.003
OC	819.40	1.486	0.262	0.056
S	533.04	0.947	0.352	0.037
N	207.08	0.360	0.559	0.014
Seagrass	54.16	0.093	0.777	0.004

Table A.3. 3: Normalised distance based linear model marginal test between environmental predictors and porewater ammonium concentration for medium treatment plots within the lower sediment depth: results of the stepwise section procedure. SS (trace) = portion of sum of squares relative to the analysed predictor variables (sediment properties and macrofaunal variables); pseudo-F ratio statistic; P = level of statistical significance; Prop = the proportion of variation explained by the model. No significance is observed.

	SS(trace)	Pseudo-F	P	Prop.
Chl-*a*	108.04	0.024	0.869	0.0009
MGS	1160.30	0.254	0.639	0.010
OC	977.48	0.214	0.681	0.009
Mud	775.14	0.169	0.720	0.007
A. stutchburyi	183.23	0.040	0.845	0.002
Seagrass	11540	2.783	0.109	0.100
F_N	1494.90	0.329	0.525	0.013
F_S	904.82	0.198	0.682	0.008

Table A.3. 4: Normalised distance based linear model marginal test between environmental predictors and porewater ammonium concentration for high treatment plots within the lower sediment depth: results of the stepwise section procedure. SS (trace) = portion of sum of squares relative to the analysed predictor variables (sediment properties and macrofaunal variables); pseudo-F ratio statistic; P = level of statistical significance; Prop = the proportion of variation explained by the model. No significance is observed.

	SS(trace)	Pseudo-F	P	Prop.
Chl-*a*	54408	0.469	0.489	0.018
MGS	1967	0.017	0.905	0.0006
Fs	18570	0.158	0.703	0.006
A. stutchburyi	50777	0.437	0.491	0.017
M. liliana	2031	0.017	0.904	0.0006
Sand	994	0.008	0.897	0.0003
Shell hash	26107	0.223	0.641	0.009

Table A.3. 5: One-way ANOVA testing whether the number of N differed among the treatments (control, medium and high). No significance is observed.

	Sum of squares	Degrees of freedom	Mean Squares	F value	P value
Treatment	92.17	2	46.08	1.374	0.259
Error	2650.68	79	33.55		

Table A.3. 6: One-way ANOVA testing whether the number of F_S differed among the treatments (control, medium and high). No significance is observed.

	Sum of squares	Degrees of freedom	Mean Squares	F value	P value
Treatment	2.359	2	1.179	1.734	0.183
Error	53.720	79	0.680		

Table A.3. 7: One-way ANOVA testing whether the number of S differed among the treatments (control, medium and high). No significance is observed.

	Sum of squares	Degrees of freedom	Mean Squares	F value	P value
Treatment	4.210	2	2.105	1.554	0.218
Error	106.973	79	1.354		

Table A.3. 8: One-way ANOVA testing whether the number of F_N differed among the treatments (control, medium and high). No significance is observed.

	Sum of squares	Degrees of freedom	Mean Squares	F value	P value
Treatment	78.418	2	39.209	1.441	0.243
Error	2149.806	79	27.213		

Table A.3. 9: Two-way ANOVA testing the average rate of ammonium accumulation and removal against treatment (medium and high) and sampling depth (upper and lower). No significance is observed.

	Sum of squares	Degrees of freedom	Mean Squares	F value	P value
Treatment	110906	1	110905.7	1.025	0.314
Sampling depth	228899	1	228899.4	2.115	0.149
Treatment* Sampling depth	131465	1	131465.0	1.215	0.273
Error	11146490	103	108218.3		

Table A.3. 10: Single sample t-test for chlorophyll *a*. M = mean, SD = standard deviation. SE= standard error. Significant p values (<0.05) are identified in bold.

	Mean	SD	N	SE	Reference Constant	t-value	df	p
Medium	1.276	0.533	28	0.1008	1.000000	**2.7445**	27	**0.0106**
High	1.239	0.817	28	0.1544	1.000000	1.5518	27	0.1324

Table A.3. 11: Single sample t-test for seagrass. Significant p values (<0.05) are identified in bold. M = mean, SD = standard deviation. SE= standard error. No significance is observed.

	M	SD	N	SE	Reference Constant	t-value	df	p
Medium	1.1	1.1389	28	0.2152	1.000000	0.271	27	0.788
High	0.9	1.5264	28	0.2885	1.000000	-0.276	27	0.784

Pearson's correlations for the average rate of porewater processing and accumulation of porewater ammonium.

Table A.3.12: Pearson's correlation for the average rate of porewater processing and accumulation (Equation 5) for medium treatment upper sediment depth. Multi-collinearity was identified for values >0.8.

	OC	**Chl-*a***	**Phaeo**	**MGS**	**Mud**	**S**	**N**	**F_S**	**F_N**	***A. stutchburyi***	***M. liliana***
OC											
Chl-*a*	0.52										
Phaeo	0.59	0.68									
MGS	-0.67	-0.50	-0.70								
Mud	0.89	0.44	0.62	-0.75							
S	0.73	0.47	0.61	-0.53	0.75						
N	0.69	0.45	0.47	-0.40	0.64	0.88					
F_S	0.58	0.33	0.35	-0.33	0.59	0.81	0.95				
F_N	0.67	0.28	0.55	-0.51	0.75	0.91	0.72	0.71			
A. stutchburyi	-0.13	-0.05	-0.42	0.36	-0.30	-0.03	0.08	0.05	-0.15		
M. liliana	-0.13	-0.08	-0.06	0.21	-0.20	0.11	0.00	0.05	0.13	0.28	

Environmental variables: OC, organic content (%); Chl-*a*, chlorophyll a (µg/g); Pheao, pheaopigment (µg/g); MGS, median grain size (µm); Mud content (%) (<63µm); S, total number of species in sample (ind. core^{-1}); N, total number of individuals in sample (ind. core^{-1}); F_N, total number of functional individuals in sample (ind. core^{-1}); F_S, total number of functional species in sample (ind. core^{-1}); *A. stutchburyi*, total number of *Austrovenus stutchburyi* individuals (ind. core^{-1}); *M. liliana*, total of *Macomona liliana* individuals (ind. core^{-1}). *p <0.05, ** p <0.01, ***p <0.00.

Table A.3. 13: Pearson's correlation for the average rate of porewater processing and accumulation (Equation 5) for medium treatment lower sediment depth. Multi-collinearity was identified for values >0.8.

	OC	**Chl-*a***	**Phaeo**	**MGS**	**Mud**	**S**	**N**	**F_S**	**F_N**	***A. stutchburyi***	***M. liliana***
OC											
Chl-*a*	0.53										
Phaeo	0.60	0.69									
MGS	-0.68	-0.51	-0.70								
Mud	0.89	0.45	0.63	-0.76							
S	0.74	0.49	0.62	-0.55	0.76						
N	0.70	0.46	0.47	-0.42	0.65	0.89					
F_S	0.59	0.34	0.36	-0.34	0.60	0.81	0.95				
F_N	0.69	0.30	0.55	-0.53	0.76	0.91	0.73	0.71			
A. stutchburyi	-0.13	-0.05	-0.42	0.35	-0.30	-0.03	0.08	0.04	-0.15		
M. liliana	-0.14	-0.08	-0.06	0.21	-0.20	0.10	-0.01	0.05	0.12	0.28	

Environmental variables: OC, organic content (%); Chl-*a*, chlorophyll a (µg/g); Pheao, pheaopigment (µg/g); MGS, median grain size (µm); Mud content (%) (<63µm); S, total number of species in sample (ind. core^{-1}); N, total number of individuals in sample (ind. core^{-1}); F_N, total number of functional individuals in sample (ind. core^{-1}); F_S, total number of functional species in sample (ind. core^{-1}); *A. stutchburyi*, total number of *Austrovenus stutchburyi* individuals (ind. core^{-1}); *M. liliana*, total of *Macomona liliana* individuals (ind. core^{-1}). *p <0.05, ** p <0.01, ***p <0.00.

Table 4.3. 14: Pearson's correlation for the average rate of porewater processing and accumulation (Equation 5) for high treatment upper sediment depth. Multi-collinearity was identified for values >0.8.

	OC	**Chl-*a***	**Phaeo**	**MGS**	**Mud**	**S**	**N**	**F_S**	**F_N**	***A. stutchburyi***	***M. liliana***
OC											
Chl-*a*	0.29										
Phaeo	0.91	0.37									
MGS	-0.76	-0.31	-0.81								
Mud	0.94	0.24	0.89	-0.81							
S	0.82	0.11	0.74	-0.79	0.81						
N	0.83	0.23	0.81	-0.74	0.81	0.90					
F_S	0.78	0.16	0.77	-0.73	0.78	0.84	0.94				
F_N	0.80	0.07	0.74	-0.78	0.82	0.94	0.85	0.87			
A. stutchburyi	0.01	0.14	-0.13	0.27	-0.13	-0.01	0.09	-0.03	-0.10		
M. liliana	0.28	-0.09	0.38	-0.42	0.26	0.46	0.36	0.42	0.48	-0.18	

Environmental variables: OC, organic content (%); Chl-*a*, chlorophyll a (µg/g); Pheao, pheaopigment (µg/g); MGS, median grain size (µm); Mud content (%) (<63µm); S, total number of species in sample (ind. core^{-1}); N, total number of individuals in sample (ind. core^{-1}); F_N, total number of functional individuals in sample (ind. core^{-1}); F_S, total number of functional species in sample (ind. core^{-1}); *A. stutchburyi*, total number of *Austrovenus stutchburyi* individuals (ind. core^{-1}); *M. liliana*, total of *Macomona liliana* individuals (ind. core^{-1}). *p <0.05, ** p <0.01, ***p <0.00.

Table A.3.15: Pearson's correlation for the average rate of porewater processing and accumulation (Equation 5) for high treatment lower sediment depth. Multi-collinearity was identified for values >0.8.

	OC	**Chl-*a***	**Phaeo**	**MGS**	**Mud**	**S**	**N**	**F_S**	**F_N**	***A. stutchburyi***	***M. liliana***
OC											
Chl-*a*	0.24										
Phaeo	0.91	0.35									
MGS	-0.76	-0.30	-0.80								
Mud	0.94	0.21	0.88	-0.81							
S	0.82	0.09	0.74	-0.79	0.81						
N	0.82	0.19	0.80	-0.74	0.80	0.91					
F_S	0.77	0.12	0.76	-0.73	0.77	0.84	0.94				
F_N	0.81	0.06	0.76	-0.77	0.83	0.93	0.86	0.87			
A. stutchburyi	-0.20	0.02	-0.31	0.41	-0.34	-0.14	-0.10	-0.20	-0.22		
M. liliana	0.31	-0.07	0.42	-0.43	0.29	0.48	0.39	0.44	0.51	-0.25	

Environmental variables: OC, organic content (%); Chl-*a*, chlorophyll a (µg/g); Pheao, pheaopigment (µg/g); MGS, median grain size (µm); Mud content (%) (<63µm); S, total number of species in sample (ind. core^{-1}); N, total number of individuals in sample (ind. core^{-1}); F_N, total number of functional individuals in sample (ind. core^{-1}); F_S, total number of functional species in sample (ind. core^{-1}); *A. stutchburyi*, total number of *Austrovenus stutchburyi* individuals (ind. core^{-1}); *M. liliana*, total of *Macomona liliana* individuals (ind. core^{-1}). *p <0.05, ** p <0.01, ***p <0.00.

Appendix 4

Table A.4. 1: Macrofauna composition per plot for D_{47}.

Site #	Treatment	S	N	F_N	F_S	*A. stutchburyi*	*M. liliana*
1	Control	58	450	166	22	43	7
2	Control	38	183	114	19	47	38
3	Control	51	663	294	18	225	7
4	Control	37	214	64	14	1	1
5	Control	57	347	279	31	2	7
6	Control	43	273	215	27	17	26
7	Control	25	94	50	15	2	2
8	Control	37	143	97	21	15	14
9	Control	52	259	174	31	19	24
10	Control	55	679	497	27	2	9
11	Control	47	838	752	24	1	30
12	Control	36	252	105	15	37	3
13	Control	38	221	131	20	37	33
14	Control	28	105	59	14	21	11
15	Control	27	179	122	15	40	28
16	Control	20	157	119	11	4	53
17	Control	33	254	105	12	8	11
18	Control	33	173	122	18	26	56
19	Control	28	108	89	15	15	49
20	Control	49	215	120	23	0	9
21	Control	55	284	156	26	12	21
22	Control	48	271	121	25	0	11
23	Control	53	405	248	27	0	13
24	Control	50	176	111	28	0	11
25	Control	34	190	146	19	30	47
26	Control	24	76	60	15	17	14
27	Control	22	99	76	11	5	21
28	Control	18	38	29	13	3	5

Site #	Treatment	S	D	F_N	F_S	*A. stutchburyi*	*M. liliana*
1	High	30	189	76	15	32	2
2	High	27	119	60	12	29	17
3	High	47	471	236	21	170	7
4	High	52	290	68	23	5	2
5	High	59	353	265	32	2	10
6	High	42	156	95	24	14	16
7	High	10	20	19	9	3	1
8	High	22	38	24	12	7	2
9	High	41	122	68	20	25	9
10	High	52	690	602	31	4	7
11	High	35	148	117	23	1	9
12	High	57	457	202	24	86	4
13	High	25	91	48	16	15	12
14	High	21	114	27	9	15	5
15	High	15	36	25	9	12	3
16	High	24	101	67	13	2	20
17	High	15	93	34	6	4	3
18	High	23	51	36	13	8	11
19	High	26	60	26	11	13	2
20	High	59	485	407	32	1	8
21	High	44	306	211	23	7	14
22	High	48	351	236	23	0	9
23	High	54	277	138	27	1	19
24	High	38	95	46	16	0	11
25	High	23	55	30	9	16	3
26	High	18	45	32	7	18	2
27	High	15	47	31	7	8	4
28	High	10	16	14	8	2	3

Site #	Treatment	S	N	F_N	F_S	*A. stutchburyi*	*M. liliana*
1	Medium	47	199	76	25	11	13
2	Medium	28	154	78	12	39	24
3	Medium	48	569	369	20	226	4
4	Medium	46	263	69	21	2	2
5	Medium	54	381	320	30	2	8
6	Medium	36	134	103	20	27	23
7	Medium	22	52	41	16	5	4
8	Medium	24	72	56	12	8	1
9	Medium	48	210	153	28	6	22
10	Medium	53	437	309	25	0	4
11	Medium	65	1147	1038	30	1	10
12	Medium	50	475	191	22	68	11
13	Medium	31	124	77	15	27	22
14	Medium	31	90	45	11	19	11
15	Medium	24	73	32	12	11	6
16	Medium	26	111	87	15	2	16
17	Medium	26	112	65	11	5	2
18	Medium	34	125	90	16	34	32
19	Medium	34	76	50	20	8	17
20	Medium	54	383	263	29	0	8
21	Medium	47	366	293	27	6	13
22	Medium	44	158	97	23	1	7
23	Medium	34	121	79	21	0	2
24	Medium	38	94	46	20	0	14
25	Medium	33	123	103	19	34	14
26	Medium	24	59	37	13	11	6
27	Medium	23	77	62	12	6	11
28	Medium	17	43	24	8	0	8

Table A.4. 2: Sediment properties for $D_{28.}$

Site Number	Treatment	OC	Chl-*a*	Phaeo	MGS	Mud
1	Control	2.435	8.124	6.717	198.693	19.639
2	Control	0.572	8.015	1.512	221.983	0
3	Control	2.721	11.894	7.04	211.006	5.231
4	Control	0.942	5.305	2.582	204.62	0
5	Control	1.301	4.177	3.111	193.698	4.647
6	Control	0.964	5.848	3.543	199.305	1.597
7	Control	0.583	4.87	0.979	239.289	0
8	Control	0.54	0.006	0.801	234.832	0
9	Control	0.855	0.006	1.474	220.906	0
10	Control	1.645	6.132	4.565	205.687	4.316
11	Control	0.888	4.127	2.8	225.43	1.185
12	Control	1.669	5.973	5.713	219.541	3.692
13	Control	0.686	9.312	2.555	221.523	0
14	Control	0.723	7.958	2.465	227.263	0
15	Control	0.703	7.188	1.717	214	0
16	Control	0.817	2.58	1.287	242.834	0
17	Control	0.594	6.349	1.448	234.52	0
18	Control	0.672	6.355	1.819	221.187	0
19	Control	0.605	4.631	1.064	219.088	0
20	Control	1.205	0.007	5.078	197.616	5.189
21	Control	1.063	3.923	2.05	195.371	0
22	Control	1.273	4.629	5.615	205.361	4.815
23	Control	1.498	0.007	5.071	208.645	4.113
24	Control	0.941	4.303	2.62	181.503	1.599
25	Control	0.674	3.606	1.055	214.182	0
26	Control	0.655	3.204	1.196	226.318	0
27	Control	0.44	0.005	1.038	232.89	0
28	Control	0.544	0.006	3.35	239.467	0
1	High	1.391	11.456	5.284	232.573	1.11
2	High	0.599	5.55	2.058	219.889	0
3	High	1.884	10.228	4.667	207.962	6.533
4	High	0.454	3.894	3.423	203.398	0
5	High	1.196	5.595	3.569	190.529	7.164
6	High	0.753	4.669	1.539	213.381	2.753
7	High	0.543	4.236	0.844	239.047	0
8	High	0.586	3.069	2.002	240.907	0
9	High	0.921	3.625	1.365	217.976	0
10	High	1.297	7.083	3.653	206.786	1.236
11	High	0.695	4.725	3.921	222.218	0.684
12	High	1.672	5.118	5.326	217.699	3.581
13	High	0.693	0.006	2.311	229.598	0
14	High	0.645	8.712	1.12	224.604	0
15	High	0.646	7.285	1.271	217.996	0

Site number	Treatment	OC	Chl-*a*	Phaeo	MGS	Mud
16	High	0.578	0.006	1.174	234.199	0
17	High	0.564	6.103	1.876	231.945	0
18	High	0.639	3.142	1.605	224.801	0
19	High	0.72	5.694	1.763	219.341	0
20	High	1.158	0.007	7.123	200.717	4.114
21	High	1.193	0.006	2.788	190.91	2.346
22	High	1.053	5.954	3.432	198.026	5.101
23	High	1.465	0.006	7.514	199.555	4.418
24	High	0.961	0.007	4.152	205.503	0.627
25	High	0.49	4.24	1.155	228.732	0
26	High	0.233	4.42	0.925	224.657	0
27	High	0.521	7.135	0.79	230.087	0
28	High	0.589	9.698	2.195	235.755	0
1	Medium	0.92	8.947	4.778	234.147	1.035
2	Medium	0.754	6.936	2.135	217.833	0
3	Medium	1.226	13.464	4.555	217.811	1.254
4	Medium	1.227	4.955	3.99	207.109	0.679
5	Medium	0.85	5.278	3.159	183.546	4.473
6	Medium	1.273	5.891	2.607	215.932	1.483
7	Medium	0.47	3.303	1.528	230.892	0
8	Medium	0.601	3.599	0.885	242.621	0
9	Medium	0.826	0.006	2.463	229.021	0
10	Medium	1.196	6.341	4.324	220.231	0.848
11	Medium	1.485	4.19	3.802	208.699	8.299
12	Medium	1.01	0.006	5.615	231.238	0
13	Medium	0.851	0.006	2.144	225.218	0
14	Medium	0.65	9.33	1.379	227.06	0
15	Medium	0.862	0.006	3.308	212.279	0
16	Medium	0.527	0.006	0.91	241.488	0
17	Medium	0.669	5.081	3.497	231.943	0
18	Medium	0.52	8.169	1.229	222.384	0
19	Medium	0.544	5.176	1.429	222.514	0
20	Medium	2.385	11.744	12.279	201.14	8.712
21	Medium	0.985	4.748	4.561	197.822	2.444
22	Medium	1.444	6.861	4.334	197.777	4.229
23	Medium	1.001	6.768	5.776	203.386	4.934
24	Medium	1.208	6.026	2.767	198.412	3.533
25	Medium	0.457	4.052	0.666	228.93	0
26	Medium	0.568	4.015	1.532	231.157	0
27	Medium	0.5	7.619	1.084	234.66	0
28	Medium	0.694	0.005	1.543	245.668	0

Table A.4. 3: Sediment properties for D_{47}.

Site number	Treatment	OC	Chl-*a*	Phaeo	MGS	Mud
1	Control	2.136	13.161	9.673	209.860	10.038
2	Control	0.729	12.743	1.592	215.845	0.000
3	Control	1.749	14.626	11.247	207.024	7.251
4	Control	2.044	12.473	9.338	209.377	1.074
5	Control	1.144	5.959	5.197	187.047	9.856
6	Control	1.476	7.853	6.384	191.475	4.932
7	Control	0.452	6.089	1.474	236.602	0.000
8	Control	0.735	5.500	2.106	223.186	0.000
9	Control	1.089	7.001	4.868	196.089	13.579
10	Control	1.596	23.239	16.878	194.843	8.664
11	Control	0.974	3.572	3.836	217.838	4.132
12	Control	1.230	7.829	5.811	210.958	4.091
13	Control	0.712	9.792	4.880	223.463	0.000
14	Control	0.733	12.147	3.833	224.630	0.000
15	Control	0.747	11.658	3.237	215.756	0.000
16	Control	0.567	5.337	2.198	240.565	0.000
17	Control	0.713	8.570	3.180	227.029	3.482
18	Control	0.694	10.331	3.761	214.159	0.000
19	Control	0.602	5.925	2.154	223.077	0.000
20	Control	1.835	14.496	13.716	176.580	14.496
21	Control	1.057	7.164	4.368	189.365	2.494
22	Control	2.502	13.723	17.902	194.052	9.185
23	Control	1.892	12.846	15.247	194.843	12.195
24	Control	1.371	20.898	17.467	182.204	4.504
25	Control	0.660	5.678	2.277	221.794	0.000
26	Control	0.584	4.171	2.218	227.313	0.000
27	Control	0.697	9.636	1.988	232.075	0.000
28	Control	0.647	8.894	2.330	238.724	0.000

Site number	Treatment	OC	Chl-*a*	Phaeo	MGS	Mud
1	High	1.644	17.400	9.108	209.677	9.758
2	High	0.540	8.818	4.433	218.365	0.000
3	High	1.950	16.295	9.869	203.038	8.606
4	High	1.302	8.716	7.743	200.722	4.532
5	High	1.142	5.515	3.984	193.782	4.085
6	High	1.208	6.751	3.430	219.876	3.347
7	High	0.487	5.661	1.826	236.889	0.000
8	High	0.581	5.276	1.677	250.474	0.000
9	High	1.004	5.865	3.303	212.650	0.838
10	High	1.526	16.769	12.106	193.408	8.391
11	High	1.229	9.587	8.322	206.060	7.879
12	High	1.956	9.925	7.674	220.699	4.306
13	High	0.760	15.464	4.821	226.523	0.000
14	High	0.646	13.946	3.164	221.436	0.000
15	High	0.669	13.080	2.864	219.015	0.000
16	High	0.581	6.855	2.668	235.373	0.000
17	High	0.746	9.009	3.044	238.814	0.000
18	High	0.752	9.375	3.117	220.945	0.000
19	High	0.601	28.316	1.840	218.088	0.000
20	High	1.967	12.100	12.530	193.727	8.809
21	High	1.348	9.271	9.849	189.992	2.185
22	High	1.515	12.230	9.916	195.101	6.694
23	High	2.349	15.001	18.753	190.233	12.029
24	High	1.280	10.115	6.921	191.399	5.441
25	High	0.584	5.684	1.863	229.859	0.000
26	High	0.632	8.473	1.062	223.758	0.000
27	High	0.648	8.769	2.866	232.484	0.000
28	High	0.724	10.765	2.406	229.931	0.000
1	Medium	1.693	12.071	8.984	225.950	8.397
2	Medium	0.679	9.756	3.598	219.897	0.000
3	Medium	2.432	31.914	4.296	211.127	7.244

Site number	Treatment	OC	Chl-*a*	Phaeo	MGS	Mud
4	Medium	1.363	25.844	21.518	196.175	1.919
5	Medium	1.219	6.217	9.207	189.984	3.841
6	Medium	1.328	8.811	3.573	209.302	2.728
7	Medium	0.506	5.598	1.579	229.436	0.000
8	Medium	0.562	4.633	1.609	239.603	0.000
9	Medium	1.069	9.929	6.366	233.139	1.241
10	Medium	1.407	31.175	21.611	201.010	8.161
11	Medium	1.577	9.329	10.291	210.300	7.888
12	Medium	2.254	9.217	7.652	220.831	2.145
13	Medium	0.759	14.931	18.388	214.899	0.000
14	Medium	0.685	11.036	4.509	225.268	0.000
15	Medium	0.713	10.098	3.178	213.166	0.000
16	Medium	0.607	4.847	2.565	241.615	0.000
17	Medium	0.680	9.660	3.898	223.438	0.000
18	Medium	0.752	25.878	10.649	231.378	0.000
19	Medium	0.637	5.020	3.051	225.377	0.000
20	Medium	1.890	16.366	14.334	200.023	5.215
21	Medium	2.055	14.214	10.824	193.654	7.040
22	Medium	1.580	11.309	10.586	181.659	13.169
23	Medium	2.325	12.789	20.163	194.062	13.911
24	Medium	1.452	18.075	12.816	199.625	2.855
25	Medium	0.596	5.816	2.013	230.423	0.000
26	Medium	0.581	7.130	2.365	225.595	0.000
27	Medium	0.588	8.185	3.963	231.893	0.000
28	Medium	0.978	11.248	3.034	231.178	0.000

Table A.4. 5: Percent coverage of seagrass, sand and shell hash for D_{28}.

Site number	Treatment	Seagrass (%)	Sand (%)	Shell hash (%)
1	Control	68	21	11
2	Control	0	92	8
3	Control	68	25	7
4	Control	93	7	0
5	Control	0	96	4
6	Control	5	91	4
7	Control	0	99	1
8	Control	17	83	0
9	Control	64	33	3
10	Control	65	35	0
11	Control	1	96	3
12	Control	77	18	4
13	Control	0	97	3
14	Control	0	100	0
15	Control	0	96	4
16	Control	0	96	4
17	Control	0	99	1
18	Control	16	81	3
19	Control	0	96	4
20	Control	77	23	0
21	Control	29	71	0
22	Control	0	100	0
23	Control	51	48	1
24	Control	75	25	0
25	Control	23	72	5
26	Control	0	99	1
27	Control	1	96	3
28	Control	0	93	7
1	High	61	27	12
2	High	5	83	12
3	High	59	28	13
4	High	77	20	3
5	High	0	97	3
6	High	31	68	1
7	High	0	99	1
8	High	21	79	0
9	High	40	53	7
10	High	32	68	0
11	High	21	79	0
12	High	83	16	1
13	High	0	99	1

Site number	Treatment	Seagrass (%)	Sand (%)	Shell hash (%)
14	High	0	96	4
15	High	0	93	7
16	High	0	91	9
17	High	0	100	0
18	High	0	93	7
19	High	12	88	0
20	High	89	11	0
21	High	52	48	0
22	High	24	76	0
23	High	56	44	0
24	High	57	41	1
25	High	3	96	1
26	High	0	99	1
27	High	0	96	4
28	High	0	93	7
1	Medium	89	11	0
2	Medium	0	92	8
3	Medium	75	16	9
4	Medium	88	12	0
5	Medium	0	97	3
6	Medium	23	75	3
7	Medium	0	99	1
8	Medium	0	100	0
9	Medium	44	53	3
10	Medium	55	45	0
11	Medium	41	57	1
12	Medium	93	5	1
13	Medium	0	99	1
14	Medium	0	96	4
15	Medium	0	96	4
16	Medium	0	97	3
17	Medium	3	95	3
18	Medium	4	95	1
19	Medium	1	96	3
20	Medium	96	1	3
21	Medium	64	33	3
22	Medium	4	95	1
23	Medium	-	-	-
24	Medium	95	5	0
25	Medium	3	96	1
26	Medium	0	99	1
27	Medium	1	99	0
28	Medium	0	99	1

Table A.4. 6: Percent coverage of seagrass, sand and shell hash for D_{47}.

Site number	Treatment	Seagrass (%)	Sand (%)	Shell hash (%)
1	Control	56	36	8
2	Control	23	61	16
3	Control	75	24	1
4	Control	17	83	0
5	Control	12	83	5
6	Control	0	100	0
7	Control	48	41	11
8	Control	41	48	11
9	Control	77	17	6
10	Control	84	15	1
11	Control	72	12	16
12	Control	92	7	1
13	Control	0	100	0
14	Control	0	100	0
15	Control	0	100	0
16	Control	19	81	0
17	Control	32	68	0
18	Control	47	53	0
19	Control	0	99	1
20	Control	0	100	0
21	Control	0	97	3
22	Control	19	81	0
23	Control	21	79	0
24	Control	0	96	4
25	Control	47	53	0
26	Control	32	65	3
27	Control	48	52	0
28	Control	48	52	0
1	High	25	75	0
2	High	72	28	0
3	High	4	96	0
4	High	32	67	1
5	High	20	80	0
6	High	40	48	12
7	High	75	24	1
8	High	70	27	3
9	High	0	96	4
10	High	0	99	1
11	High	0	100	0
12	High	0	97	3
13	High	0	97	3

Site number	Treatment	Seagrass (%)	Sand (%)	Shell hash (%)
14	High	0	96	4
15	High	0	96	4
16	High	0	96	4
17	High	0	92	8
18	High	0	95	5
19	High	0	96	4
20	High	0	91	9
21	High	0	96	4
22	High	0	97	3
23	High	9	88	3
24	High	9	85	6
25	High	0	96	4
26	High	19	76	5
27	High	8	89	3
28	High	15	84	1
1	Medium	12	83	5
2	Medium	47	53	0
3	Medium	53	47	0
4	Medium	97	3	0
5	Medium	29	71	0
6	Medium	23	77	0
7	Medium	39	61	0
8	Medium	29	71	0
9	Medium	45	55	0
10	Medium	21	79	0
11	Medium	32	68	0
12	Medium	36	64	0
13	Medium	29	71	0
14	Medium	43	57	0
15	Medium	49	51	0
16	Medium	87	13	0
17	Medium	15	85	0
18	Medium	20	77	3
19	Medium	27	70	3
20	Medium	0	99	1
21	Medium	0	97	3
22	Medium	3	93	4
23	Medium	8	92	0
24	Medium	1	96	3
25	Medium	11	89	0
26	Medium	0	100	0
27	Medium	0	97	3
28	Medium	0	95	5

Table A.4. 7: Porewater ammonium concentrations for both upper and lower sediment depths on D_{28} and D_{47}.

Site number	Treatment	U-1 (mg/L)	L-1 (mg/L)	U-2 (mg/L)	L-2 (mg/L)
1	Control	0.67	2.40	0.59	5.28
2	Control	9.57	0.69	0.17	4.56
3	Control	0.75	3.29	0.57	
4	Control	0.63	1.58	1.29	4.15
5	Control	0.42	2.20	1.67	3.10
6	Control	0.23	0.95	2.35	5.80
7	Control	0.27	0.48	0.43	0.91
8	Control	0.27	1.72	1.28	1.47
9	Control	0.27	0.84	0.24	0.80
10	Control	0.22	1.34	3.30	1.14
11	Control	0.27	1.68	2.65	0.90
12	Control	0.13	1.94	1.51	1.27
13	Control	0.10	1.42	0.27	1.47
14	Control	0.16	1.35	3.58	0.28
15	Control	0.32	0.89	1.54	2.10
16	Control	0.71	1.71	0.37	20.32
17	Control	0.33	1.10	0.32	1.44
18	Control	0.96	1.49	0.30	8.73
19	Control	0.17	1.14	0.37	2.57
20	Control	1.14	0.71	0.29	0.84
21	Control	0.32	1.37	0.37	16.22
22	Control	0.98	0.79	1.84	1.33
23	Control	0.48	0.31	1.92	0.64
24	Control	0.14	0.19	0.05	0.47
25	Control	0.15	0.12	0.29	0.42
26	Control	0.34	0.27	0.02	6.61
27	Control	0.04	0.16	0.00	0.26
28	Control	0.17	0.26	0.03	0.50
1	High	95.99	111.70	173.50	125.89
2	High	43.29	40.55	67.39	83.92

Site number	Treatment	U-1 (mg/L)	L-1 (mg/L)	U-2 (mg/L)	L-2 (mg/L)
3	High	147.88	267.65	71.33	166.86
4	High	3.76	4.41	8.23	57.08
5	High	18.15	84.54	6.68	36.56
6	High	16.76	4.09	21.25	10.20
7	High	2.85	9.94	174.24	111.48
8	High	103.43	204.50	9.22	38.40
9	High	38.63	6.77	65.35	196.91
10	High	18.53	108.82	7.90	147.92
11	High	18.58	59.07	2.00	17.71
12	High	30.14	177.41	45.26	82.22
13	High	2.19	38.85	368.80	339.88
14	High	76.43	25.94	184.70	254.04
15	High	133.12	119.03	158.27	167.46
16	High	42.42	41.94	14.56	200.15
28	High	49.33	77.50	61.59	451.64
1	Medium	12.06	24.91	5.27	22.12
2	Medium	12.96	21.96	1.86	1.78
3	Medium	9.22	38.82	8.65	152.29
4	Medium	12.78	10.72	4.20	6.02
5	Medium	9.40	108.68		76.57
6	Medium	9.54	63.13	2.99	10.80
7	Medium	13.94	2.89	6.60	53.01
8	Medium	18.43	47.75	4.30	23.54
9	Medium	1.40	41.97	15.01	9.40
10	Medium	2.29	16.45	2.92	9.52
11	Medium	1.64	13.15	2.44	9.18
12	Medium	4.14	10.43	9.82	10.27
13	Medium	2.19	7.48	6.08	9.50
14	Medium	3.57	17.22	2.70	5.85
15	Medium	4.39	3.99	21.52	226.58
16	Medium	11.76	16.68	39.87	80.56
17	Medium	98.60	101.12	100.35	185.34

Site number	Treatment	U-1 (mg/L)	L-1 (mg/L)	U-2 (mg/L)	L-2 (mg/L)
18	Medium	214.30	128.04	81.18	156.81
19	Medium	0.41	2.21	3.74	13.39
20	Medium	1.08	3.20	2.66	15.82
21	Medium	3.43	8.57	21.01	52.55
22	Medium	4.35	20.95	12.85	45.00
23	Medium	4.72	11.33	3.87	28.67
24	Medium	1.01	12.82	1.15	6.06
25	Medium	0.41	1.58	5.41	35.90
26	Medium	52.44	77.83	3.20	21.80
27	Medium	0.35	17.78	13.64	10.23
28	Medium	10.44	20.11	4.83	35.24