Effects of elevated pore water nutrient concentrations on seagrass meadows (*Zostera muelleri*) and their associated macrofauna assemblages in relation to sediment characteristics

A thesis submitted in partial fulfilment

of the requirements for the degree

of

Master of Science (Research)

in Biological Sciences

at

The University of Waikato

by

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The University of Waikato

2016

KO TE TANGATA

THE UNIVERSITY OF

WAIKATO

Te Whare Wānanga o Waikato

Frontispiece

“Even if you lose in battle, if you surpass what you've done before, you have bested yourself”

- Marshal Renbu

Abstract

While seagrasses and their associated macrofaunal assemblages naturally endure eutrophication events, nutrient enrichment as a result of anthropogenic activities has subjected them to prolonged levels of elevated nutrients. The impacts of elevated nutrient concentrations vary between studies, with sediment composition, seagrass metrics, and the macrofauna that live within them often implicated as mitigating the observed responses. How meadows and their associated macrofaunal community assemblages respond individually has been the focus of many studies to date; though few examine both variables in tandem while also considering the role of the surrounding sediment. Here I attempt to discern the role that sediment characteristics have in determining how meadows and macrofauna respond to elevated nutrient levels within the Tauranga Harbour, New Zealand.

The sediment pore water ammonium (NH_4^+) concentration was elevated using a slow release fertiliser at a dose hypothesised to stimulate a response, but not mass death (200 N g/m^2) within seagrass meadows. This was conducted over 60 days during the seagrass growing season at six sites that varied across a gradient of sediment mud content. Different seagrass metrics were measured as attributes of seagrass meadows, the amalgamation of which was referred to as seagrass health. The overall community composition of macrofaunal assemblages was measured, with species richness and abundance examined as aspects.

Treatment effects were observed in all attributes of seagrass health at the sandiest site, which lost most of its elevated NH_4^+ concentration, and thus at this site sediment characteristics were deemed responsible for how seagrass attributes responded. Two muddy sites also exhibited a response in seagrass cover, and no correlations between sediment composition and responses in seagrass health were apparent. As such, it was concluded that sediment characteristics alone could not be used to predict whether meadows would respond to nutrient enrichment.

None of the sites which exhibited a response in seagrass metrics also did so in macrofaunal community assemblage. The macrofaunal assemblages of two sites, one sandy and one muddy, responded to enrichment, while also decreasing in both species richness and abundance. The sandy site lost its elevated nutrient concentration due to the combination of low mud content and fine particle size, though the thick cover of its meadows acted as a buffer to seagrass degradation. The muddy site had the highest mud content of all sites, and as such retained its sediment pore water NH_4^+ concentration in many of its treatment plots. The sensitivity of the polychaete worm *Heteromastus filiformis* to elevated NH_4^+ and bioturbation by the bivalve *Linucula hartvigiana* contributed to responses in assemblages seen at both sites. As these sites were from different sediment classes, mud content alone could not be used to predict whether macrofaunal assemblages would respond to enrichment.

The combination and interaction of site traits may explain why community assemblages responded to treatment, despite different sediment characteristics and attributes of seagrass health. As such, sediment characteristics, seagrass metrics, and the composition of macrofaunal assemblages may work in tandem to denote the magnitude and type of response evocated in community assemblages.

Acknowledgements

The past two years of study have been filled with an amalgam of amazing people and unique experiences. First of all, to Conrad Pilditch, my supervisor, I would like to express my utmost thanks for guiding my hand through my sporadic bursts of productivity and fleeting presence. Without your patience and understanding, I have no doubt that many of the setbacks I encountered would have become permanent roadblocks rather than bumps along the way. For this and everything else you have provided during my time at Waikato University, I am eternally grateful.

Thanks to all of those who have lent a hand in the lab, field, office, or in late night bouts of enthusiasm and support on social media. I would like to thank Rebecca Gladstone-Gallagher, Emily Douglas, and Clarisse Niemand for their help in the conceptualisation and realisation of my experiment, as well as helping a hopeless biologist come to terms with the many results a single PRIMER session can yield. Thanks again to these three with the addition of Dudley Bell, Catherine Kelly, Melissa Hempstead, Benjamin Johnston, Nicole Sturgess, Clare Beet, Morgan Warnock, and Katie Brasell for their time sieving, washing, and wading knee high in mud to make sure I obtained all the necessary samples and observations, while keeping me in high spirits with laughs, photographs and antics along the way.

I would like to express my gratitude to the Bay of Plenty Regional Council for providing the funding needed for field work and analyses conducted in the lab. This enabled me to make the many trips needed to my numerous study sites, and allow me to focus on the questions at hand. Thank you for your assistance.

I would also like to thank the technicians that helped me in the many different lab duties that were completely alien to me prior to this study. Thanks to Janine Ryburn for her help realising a young scientist's dream of firing lasers, even if it was just to measure sediment grain size. Thanks to Ronald Ram, Lee Labroyie and Grant Tempero for their help navigating the lab and processing my many samples. I am hugely grateful to Barry Greenfield for his time and patience double-checking countless macrofauna samples and instructing me on the delicate science that is invertebrate identification.

A very heartfelt thank you to those who saw me through the last two years and provided assistance in person and from afar. Mum for keeping me positive and thinking that that I was where I should be, and for supporting me even though going back to study for another round probably sounded a little insane. I know the last few years have been tough enough for the both of us outside of our professions, but I'll always love you for the kindness you've shown me and the potential you've seen in me – even when I felt neither were merited. Clare for sending me pictures of puppies at 3am and letting me vent even though you were going through the exact same situation. I couldn't have asked for a better friend. Lastly, and most importantly, my partner Helen. I know that biology wasn't exactly your forte, and "underwater grass" is a bit of a niche interest, but thank you for showing interest and keeping me moving this last year. I truly do not think that anything I have done would have been possible without you or your love and understanding.

Dedicated to my parents, Margaret and Davey; my loving partner, Helen; and those who suffer the daily battles of anxiety or depression.

This is proof you can achieve, despite yourself.

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Chapter One:
Introduction

1.1 Seagrasses: their state, physiology, and function

Seagrasses are widely recognised as an integral part of estuarine and coastal systems (Fitzpatrick & Kirkman, 1995), best known for their use as a nursery for the juvenile stage of numerous teleost species (Connolly, 1994) and their role in processing and subsequently reducing both sedimentation and waterborne nutrients (Newell & Koch, 2004). The highest number of seagrass species occurs in the tropics (Green & Short, 2003), with an overall distribution amongst 120 countries. The exact number of seagrass species is debated, as they are defined by their taxonomic characteristics rather than any uniform morphological traits or evolutionary history (Short et al., 2011). Between 50 and 72 species of marine angiosperms are recognised as seagrasses amongst 12 genera, with *Zostera*, *Posidonia,* and *Halophila* encompassing almost 60% of the species between them (Hemminga & Duarte, 2000).

Physically, seagrasses are comprised of above ground tissues such as leaves and shoots (vertical rhizomes), and below ground tissues such as roots and horizontal rhizomes (Short & Duarte, 2001). Reproduction almost exclusively occurs via budding of the horizontal rhizomes, though very rarely may involve the flowering of vertical rhizomes (Hemminga & Duarte, 2000). Seagrasses can be classified into different growth forms based on the replacement of their leaf tissue (Short & Coles., 2001), and these forms can be used in studies measuring growth rates and morphological changes.

Seagrasses provide ecosystem functions at both local and global levels. Seagrass meadows aid in reducing the effects of sedimentation, and act as a natural buffer to waves and coastal erosion (Heiss et al., 2000). Meadows are also home to a great variety of benthic invertebrates when compared to barren sediments (Du et al., 2012), which rely on meadows for physical protection from predators and as a food source (Nagelkerken, 2009). Despite their importance, seagrasses are in a state of global decline (Longstaff et al., 1999; Waycott et al., 2009; Rasheed &

Unsworth, 2011), with some studies suggesting that meadows are disappearing at a faster rate than tropical forests (Les et al., 1997). Seagrasses sequester carbon (C) from the surrounding water column so effectively that current levels of degradation could be responsible for the release of 300 Tg of C into the atmosphere every year (Fourqurean et al., 2012), making them important primary producers at both local and global scales in the face of climate change. Economically, seagrass beds play an important role as a nursery for the juvenile stages of several commercial fish species (Carr & Adams, 1973; Connolly, 1994), and as such a decline could have a serious effect on the long term sustainability of commercial fisheries. Based on their role in nutrient cycling alone, seagrasses are estimated to have a global worth of $20,000 per ha every year (Constanza, et al., 1997).

New Zealand's only seagrass, *Zostera muelleri,* was once distributed throughout the subtidal reaches of the country's coastline (Turner & Schwarz, 2006a). *Z. muelleri* is now almost exclusively found in the intertidal reaches – a distribution often linked to poor water quality and coastal development (Turner et al., 2006b). *Z. muelleri* can tolerate a measure of naturally occurring abiotic stresses such as exposure and variations in salinity, allowing it to photosynthesise in less than ideal temperatures and salinity levels and survive in intertidal conditions (Kohlmeier et al., 2014). Populations of *Z. muelleri* in New Zealand have been shown to have a low genetic diversity, despite being widely distributed geographically (Jones et al. 2008), suggesting that genetic exchange, although at low levels, is occurring between them. Should degradation widen the already large geographic distances between meadows, this already low exchange may be at risk.

Studies conducted on *Z. muelleri* often use the decline of meadows in the Tauranga Harbour as a benchmark, as it is one of the best studied distributions in New Zealand. The harbour has experienced an overall loss of 30% in seagrass meadows – although this may be as high as 70% at some sites (Turner et al., 2006a). Recent aerial surveys of the Harbour have suggested that the rate of loss over the last decade has slowed in comparison to previous observations – though meadows within the Harbour are still receding overall (Park, 2016). *Z. muelleri* undergoes a surge in growth during November-March (Dos Santos., 2011) and as such experiments examining physiology are often conducted during this period.

Studies within the Tauranga Harbour have examined biotic stressors, such as herbivory by invasive waterfowl (Dixon, 2009; Dos Santos, 2011) and abiotic stressors, such as sedimentation, coastal development, and nutrient enrichment (Schwarz et al., 2004). Biotic stressors such as grazing by swans affect seagrass distribution and biomass (Dos Santos et al., 2013), while abiotic stresses imposed by suspended solids and nutrient enrichment impede their photosynthetic ability (Kerr & Strother, 1985; Kohlmeier, et al., 2014) and affect its morphology (Collier et al., 2012). Therefore, seagrass decline within the harbour, as in other places, should be considered a multi-stressor issue with compounding influences.

1.2 The impacts of elevated nutrients on seagrasses

Seagrass loss has regularly been linked to instances of eutrophication (Ralph et al., 2006; McGlathery et al., 2007). Eutrophication encompasses a myriad of effects, both direct and indirect, that can lead to varying responses from seagrasses (Burkholder et al., 2007). The most commonly observed result of eutrophication reported by studies is light reduction as a result of epiphytic and algal growth (Ceccherelli & Cinelli, 1997; Hughes et al., 2004) as both are normally nutrient limited. Light reduction has notable impacts on the seagrasses ability to photosynthesise within a relatively short period of time, and is characterised by sudden shifts in abundance rather than gradual changes (Ralph et al., 2006). The recession of meadows generates a negative feedback, whereby the the physical loss of seagrass itself leads to the resuspension of solids – resulting in further turbidity within the water column (Hauxwell & Valiela, 2004). Light reduction via epiphytes and macroalgae can be mitigated by grazing macrofauna (Hughes et al., 2004), although this is a short-term palliation in the face of long-term eutrophication (Waldbusser & Marinelli, 2006).

Over extended periods of time, eutrophication can lead to the elevation of nutrients within the sediment porewater (Worm, 2000), normally the main supply of nutrients for seagrasses (Fourqurean et al., 1992). This "nutrient loading" is a phenomenon common in estuarine systems and often associated with seagrass loss

(Bostrom et al., 2002; Moore & Short, 2006). The response that seagrasses have to nutrients depends on the dosage as nutrients stimulate growth when present at moderate levels (Lapointe et al., 2004); particularly nitrogen (N) and phosphorous (P) (Udy & Dennison, 1997). Fluctuations in nutrient levels occur naturally in estuaries, particularly after seasonal weather events (Moore et al., 1997) - sometimes at concentrations found to be toxic to seagrass (van Katwijk et al., 1997). When nutrients persist at these levels for extended periods of time seagrasses must endure conditions that are not only toxic (Burkholder et al., 2007), but can make the afflicted areas almost impossible to recolonise (Armitage et al., 2006).

Studies have found that the morphological characteristics of seagrasses are an accurate indicator of their response to increases in nutrients and can be used to predict how meadows will cope with enrichment (Udy & Dennison, 1997). As they are often nutrient limited, seagrass biomass will typically increase with nutrient fluxes in an attempt to fixate more C (Lee et al., 2007) as it is required to assimilate N at high levels (Brun et al., 2002). Ammonium (NH_4^+), the most common form of N in New Zealand estuaries (Pratt et al., 2014), can cause toxicity in seagrasses upon saturation, at which point there are diminishing effects on biomass with the possibility of complete die off (van Katwijk et al., 1997; Brun et al., 2002). The sediment pore water of seagrass meadows is considered to be the main source of nutrients for seagrass growth (Fourqurean et al., 1992), and as such the below ground tissues of seagrass have a higher saturation rate in comparison to above ground tissues (Lee & Dunton, 1999). The uptake of NH_4^+ by roots can be so effective at naturally occuring levels that it can alter the NH_4^+ concentration of the sediment pore water (Hasegwa et al., 2008); regulating the exchange between sediment pore water and the water column. Physiological attributes of seagrass morphology have been used as indicators for health, such as leaf width, length, and count (Marba et al., 2013). Cover is used as an indicator of abundance at small scales (Montefalcone, 2009) and distribution at larger scales (Roelfsema et al., 2009). These attributes are collectively reffered to within the scope of this study as seagrass "health", and are used as an indicator of the overall status of the seagrass meadow in question,

While many studies observe the response of seagrass attributes to enrichment (Lapointe et al., 2004; Devlin et al., 2012), not many seek to induce elevated pore water nutrient levels in the field. Raising the nutrient levels on site is often considered too hard to maintain (van Katwijk et al., 1997; Worm, 2000) – especially in cases where seagrasses inhabit subtidal reaches. This is not to say that these ex situ studies lack scientific merit, but that this experiment, conducted entirely in the field, has novel value as it incorporates the natural processes that are occurring in the seagrass meadows in situ.

Sediment characteristics, namely mud content and grain size, may affect the level of, or even type of, response that seagrasses have to nutrient enrichment (Fonseca & Bell., 1998). The seagrasses themselves can modify the sediment, making it either sandier or muddier depending on the current state of the sediment and the density of seagrass coverage (van Katwijk et al., 2010). Sandier sediments are likely to lose nutrients from pore water at a different rate to muddier ones (Corbett, 2010), and may therefore expose meadows to varying levels of nutrient concentrations. By comparison, muddier sediments tend to be more toxic and retain nutrients due to lower interaction with the overhead water column (Rusch & Huettel, 2000; Koch, 2001) and as such generally have higher levels of porewater NH_4^+. The characteristics of the sediment these seagrasses are found in are also likely to define the natural state of these meadows (van Katwijk & Wijgergangs, 2004). Sandier sediments allow for a greater diffusion of oxygen, and may therefore inhibit fertility by constricting rhizome growth (McKenzie, 2007). As such, sediment characteristics may be responsible for how seagrasses respond to nutrient enrichment, as well as the attributes of these meadows pre-enrichment.

1.3 The macrofaunal community assemblages of seagrass meadows

Macrofaunal community assemblages found in seagrass meadows (including *Z. muelleri*) (van Houte-Howes et al., 2004) differ in composition from those found in comparatively unvegetated areas (Fredriksen et al, 2010; Du et al., 2012). These assemblages have also been shown to differ between *Z. muelleri* meadows of varying size, location, and consistency (Mills & Berkenbusch, 2009). The loss of seagrass meadows may cause hypoxia-induced species loss in associated assemblages (Middelburg & Levin, 2009). Direct exposure to high levels of NH_4^+

can be toxic to macrofauna (Gray et al., 2002), although at low NH_4^+ levels benthic macrofauna species can mitigate the pore water nutrient content (Mortimer et al., 1999; Waldbusser & Marinelli, 2006) and sediment structure (Lelieveld et al., 2010; Harris et al., 2015) of their local environment through bioturbation. Within meadows, bioturbation via benthic macrofauna can alter the nutrient exchange between the pore water and overhead water columns, providing a service to the growth of local seagrasses (Pascal et al., 2016). Declines in these species can be a result of the degradation of seagrass meadows (Pillay et al., 2010), or contribute to it (Hughes et al., 2004), depending on the stessor(s) responsible.

Thus, nutrient enrichment may not only affect these assemblages directly, but indirectly, through the degradation and removal of seagrass. How these assemblages respond to enrichment, and how these responses affect the meadows themselves is therefore of interest. For example, macrofauna that graze on epiphytic algae have been shown to have a positive effect on seagrass leaf density (Hughes et al., 2004) comparable to negative effects seen by nutrient loading. However, this grazing acts only to buffer the initial effects of nutrient enrichment, and under prolonged enrichment these positive grazing effects decline as the macrofauna become stressed nutrients themselves (McGlathery et al., 2007). The loss of these grazers allows for increases in foreign macrofauna that out-compete seagrasses for space (Burkholder et al., 2007). Therefore the presence of these macrofauna may mitigate, if not define, the level and type of effect imposed by pore water nutrient enrichment.

Sediment grain size has been shown to effect the macrofaunal assemblages of seagrass meadows, explaining some of the variation in assemblages between them (Frost et al., 1999; Berkenbusch & Rowden, 2007; Christie et al., 2009). Therefore while enrichment of the pore water within seagrass meadows may affect the attributes of the seagrass and its associated macrofaunal assemblages; the magnitude of these effects can be further discerned by the sediment grain size. Correlations between all of these factors simultaneously are hard to discern, and perhaps this is why no study to date has effectively done so with all of these factors.

1.4 Study objectives

This study aimed to address the long-term effects of elevated nutrients on seagrasses and their associated macrofaunal community assemblages by elevating the nutrient concentration of the sediment pore water within seagrass meadows. There is a gap in the literature regarding the interaction between nutrient enrichment of sediment pore water within meadows and sediment characteristics. This project was conducted with the aim of examining the potential coupled effects of these variables on seagrass meadow biota. Understanding how these variables interact may aid the accuracy of both future research and mitigation to anthropogenic stressors such as nutrient enrichment.

In reading the relevant literature, I conceptualised two hypotheses:

1. Raising the sediment pore water ammonium concentration within seagrass meadows will lead to differences in attributes of seagrass health, and these differences will vary between sandy and muddy sites

2. Raising the sediment pore water ammonium concentration within seagrass meadows will lead to differences in macrofaunal assemblages, and these differences will vary between sandy and muddy sites

To test these hypotheses, I conducted a field experiment that manipulated the sediment pore water nutrient concentrations of plots within seagrass meadows at sites of varying mud content. After a period of observation, comparisons were made between the attributes of seagrass health and macrofaunal assemblages of these meadows with control plots. This experiment was performed in the Tauranga Harbour during the seagrass growing season in December 2014, in order to make observations during the annual surge in seagrass growth.

Chapter Two:

Methodology

2.1 Study site

Meadows were sampled at sites across Tauranga Harbour (North Island, New Zealand; **Figure 2.1**) during the period of November 2014 to March 2015. Six sites containing continuous seagrass meadows of at least 100 m^2 within the mid-intertidal zone were chosen within the harbour (**Table 2.1, Figure 2.1**). Bridgman Lane (BL), the sandiest site, was comprised of several continuous meadows and was located in an area of mass mangrove removal in 2009. Otumoetai (OTU) was located in the southern basin, near the Tauranga Inlet, and was exposed to a moderate level of wave action. Tuapiro (TUA) contained meadows with continuous coverage that exhibited no patches. Athenree (ATH) contained a large continuous expanse and was located in the northern basin, near the Katikati Inlet. Matahui (MAT) was located towards the middle of the harbour, adjacent to several minor channels – and therefore submerged for longer periods than the other sites. Te Puna (TP), the muddiest site, had continuous meadows with sparse cover, and the site was located at the base of a large channel that lead inland.

In order to discern the influence of sediment characteristics on the nutrient enrichment of seagrass meadows within the harbour, two site classes were used based on mud content and grainsize – “sandy”, and “muddy”. Sites BL, OTU, and TUA were classed as “sandy”, while sites ATH, MAT, and TP were classed as “muddy” due to their comparably lower and higher mud content, respectively. To establish a uniform standard, meadows were chosen within the mid-intertidal zone, with all six having similar periods of inundation.

Table 2.1 The GPS coordinates of sites within the Tauranga Harbour (NZGD2000) as well as the size of the seagrass meadow at each site (m^2).

Site	Latitude	Longitude	Size of seagrass meadow (m^2)
BL	37º27.964’S	175º56.935’E	255
OTU	37º40.093’S	176º09.677’E	315
TUA	37º28.982’S	175º57.181’E	600
ATH	37º26.901’S	175º58.107’E	240
MAT	37º35.176’S	175º59.067’E	480
TP	37º39.680’S	176º02.685’E	930

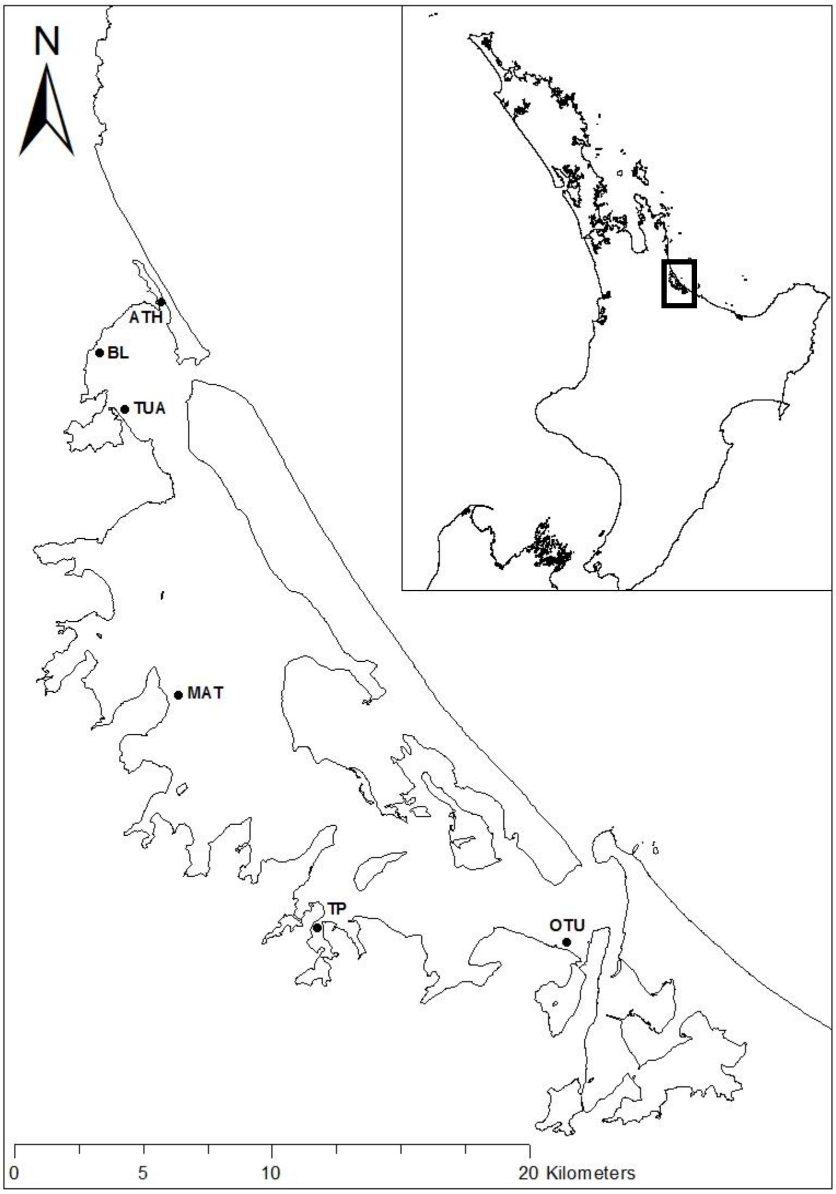

Figure 2.1. The location of study sites within the Tauranga Harbour

2.2 Field methods

A recent literature review (Douglas et al., 2016, submitted) on studies involving the response of seagrass physiology and benthic community assemblages to nutrient enrichment was used to determine how best to artificially induce enrichment. As estuarine systems are usually nitrogen limited (Howarth & Marino, 2006) a nitrogen-only fertiliser was selected. The slow-release Nutricote® N (70 day release) was used, at a dose hypothesised to stimulate a response, but not mass death (200 N g/m^2) based on median dose rates from the literature review. As urea is hydrolysed into ammonium (NH_4^+) in estuarine systems (Glibert et al., 2006) and is the most common form of nitrogen in New Zealand estuaries (Pratt et al., 2014), NH_4^+ was used as a measure of porewater nitrogen content upon analysis. As *Z. muelleri* is a mono-meristematic species which replaces its blades every 15 days (Short & Duarte, 2001), the experiment was designed to cover a 60 day period, allowing for a photographic measurement of four growth periods while the fertiliser was released from the sediment.

All sites were established over two days at the start of the seagrass growing season in December, 2014, with both experimental setup and sample gathering conducted during low tide. Plots of 1 m^2 were marked in an area of continuous seagrass cover at each site. Photographs were then taken of the undisturbed plots to be used in random point count coverage estimates. 12 plots were placed randomly within this area and were at least 2 m apart. These plots were randomly assigned as either control (contains gravel) or treatment (contains Nutricote®). This method has been shown to elevate the pore water nitrogen evenly throughout the plot with enrichment effects occurring no more than 0.5 m away (Douglas et al., 2016, submitted).

Six "time zero" cores were also taken at each site using a 13 cm diameter, 15 cm deep corer to be used as a benchmark for seagrass biomass. The seagrass was sieved (0.5 mm) and then placed between foil sheets and frozen. After a recovery period of three days the plots were photographed to establish coverage estimates post-disturbance. The plots were then photographed every 15 days until the experiment was concluded at day 60.

Final coverage photographs were taken of each plot before they were sampled in February, 2015. Three core samples were taken from each plot using a 15 cm diameter corer - two for seagrass morphometrics, one for macrofauna assemblage. Seagrass samples were sieved (0.5mm) on site and then laid flat between foil sheets for freezing. Macrofauna samples were sieved through a 500 µm mesh and then preserved in 70% IPA until identified. Three porewater samples were taken at a depth range of 3-5 cm at each plot using syringe corers with a diameter of 2 cm. Three samples were also taken at a depth of 2 cm at each plot to attain sedimentary characteristics (chlorophyll *a*, grain size, mud content, and organic matter) using syringe corers, and then pooled together for analysis.

2.3 Sample analysis

Sediment samples were kept on ice and in the dark to avoid any alteration in chlorophyll *a* (chl *a*) content. Once thawed, each sample was manually homogenised before being subsampled. Chl *a* analysis involved freeze-drying samples (~0.1 g), which were then placed in 90% buffered acetone for 24 h and then centrifuged at 3000 rpm for 10 min. The chl *a* fluorescence and phaeophytin (phaeo) levels (following acidification) of extracted material (Arar & Collins, 1997) were measured using a Turner 10-AU fluorometer. The organic matter (OM) content of the sediment was determined as % weight loss on ignition after being placed in a furnace at 450 °C for 4 h (Dos Santos, 2011). Samples for grain size and mud content analysis were digested in 10% hydrogen peroxide for a number of weeks until bubbling had stopped (Konert & Vandenberghe, 1997) followed by analysis in a Malvern Mastersizer 2000 (size range 0.05- 2000 µm).

Porewater was extracted immediately upon arriving back from the field via a centrifuge at 3000 rpm for 10 min, which was then filtered and frozen (Lohrer et al., 2010). Porewater NH_4^+ levels were analysed using a Lachat QuickChem 8000 series FIA+ (Zellweger Analytics Inc. Milwaukee, Wisconsin, 53218, USA) with standard operation procedures for flow injection.

Seagrass coverage was estimated from photographs using random point count analysis in CPCe ver. 4.1 (Kohler & Gill, 2006). Seagrass samples were separated into above (AGB) and below (BGB) ground tissues by hand in order to calculate the weight of the total, above- and below-ground biomass (Dos Santos, 2011). 10 leaves were randomly selected and measured per sample via a random number generator in order to represent the average leaf width (LW), leaf length (LL), and leaf number (L#) of each plot (Dunton et al., 2009). LW and LL were measured using digital calipers, and the epiphytic algae of each blade was removed using a razor and kept for analysis. All seagrass tissues were then dried at 60 ^{o}C in an oven until their weight remained constant. Once the leaves were measured they were submitted for particulate carbon (C) and nitrogen (N) analysis via a CHN analyser (Elementar Vario EL 111, Method 01-1090). The measurements of these attributes were used to determine a multivariate measure of overall seagrass health.

Macrofauna samples were stained with Rose Bengal dye for 24 h and then washed through a series of 4 stacked sieves ranging from 5.6 - 0.5 mm. The macrofauna were then sorted and identified to the lowest possible taxonomic level (usually species) using a dissection microscope and placed in IPA (50%) for secondary identification checks.

2.4 Data analysis

Preliminary statistics on sedimentary and seagrass variables indicated that no transformations of the data were needed. A series of dissimilarity matrices were created for environmental characteristics (Euclidean), seagrass health (Euclidean), and macrofaunal assemblages (Bray-Curtis). These matrices were then used in non-metric multi-dimensional scaling (MDS) analyses on both seagrass morphometrics and macrofaunal assemblages to see if any characteristics could attributed to any sites or treatment effects. These matrices were then used to perform a series of two-way permutational multivariate analysis of variance (PERMANOVA) to determine the significance of treatment (fixed factor, 2 levels) and site (fixed factor, 6 levels) and their interaction on sediment characteristics, seagrass health, and macrofaunal assemblages.

Post-hoc PERMANOVA pairwise tests were performed to discern which sites had treatment effects where significant interactions between treatment and site had been detected (see results). A similarity percentage (SIMPER) analysis was used to ascertain which macrofaunal taxa contributed to dissimilarities in community assemblage between treatments at each site separately. All multivariate analyses were performed using PRIMER ver. 7.0 PERMANOVA+ (Clarke & Gorley 2015).

Chapter Three:

Results

3.1 Sediment characteristics

The treatment plots at most sites exhibited NH_4^+ concentrations that reflected their mud content, and as such form a gradient, increasing in concentration from sandy to muddy sites (**Table 3.1**). OTU does not fit into this gradient, exhibiting the highest treatment NH_4^+ concentration amongst sandy sites as well as overall. BL contained the lowest NH_4^+ concentration, in both control and treatment plots. MAT had the highest control concentration, but also the most variable amongst control plots. The results of a PERMANOVA analysis revealed a variation in NH_4^+ concentration between sites, as well as between control and treatment plots (**Table 3.2**) - indicating both a natural variation in concentration between sites and the successful enrichment of treatment plots at each site. When excluding NH_4^+ concentration, sediment characteristics varied distinctively between sites (**Table 3.3**), though no treatment effects were detected. TUA had the largest OM, chl *a* and phaeo readings, illustrating the lush state of seagrass meadows at this site.

Table 3.1. Sediment characteristics of both control and treatment plots at each site (values are mean +/- standard deviation). The abbreviations featured in the column represent the following: organic matter (OM), chlorophyll *a* (chl *a*), phaeopigment (phaeo), and ammonium concentration (NH_4^+).

Site	**Mud content (%)**	**Grain size (μm)**	**OM (%)**	**Chl *a* (μg/g)**	**Phaeo (μg/g)**	**NH_4^+ (N g/m^2)**
B.L.						
Control	2.91 ± 0.40	168.7 ± 4.6	2.24 ± 0.32	33.4 ± 11.4	14.49 ± 6.25	1.08 ± 1.13
Treatment	3.06 ± 0.52	166.3 ± 3.9	2.23 ± 0.41	27.4 ± 3.8	16.05 ± 1.44	8.64 ± 5.60
Mean	2.77	167.5	1.73	16.6	7.47	1.19
OTU						
Control	3.09 ± 0.67	265.0 ± 38.1	4.06 ± 0.24	11.1 ± 1.7	7.29 ± 1.25	2.64 ± 2.34
Treatment	2.89 ± 0.79	281.4 ± 29.3	4.13 ± 0.16	11.92± 2.6	7.21 ± 3.07	381.4 ± 223.8
Mean	2.99	273	4.09	11.5	7.25	192
TUA						
Control	7.74 ± 2.77	180.4 ± 25.9	6.07 ± 1.73	42.9 ± 16.6	24.40 ± 7.47	2.10 ± 1.19
Treatment	7.91 ± 2.70	173.4 ± 6.6	5.66 ± 0.56	48.1 ± 5.2	32.53 ± 9.50	34.12 ± 13.01
Mean	7.82	176.9	5.86	45.5	28.47	6.96
ATH						
Control	11.38 ± 2.46	327.0 ± 22.7	2.39 ± 0.20	17.6 ± 8.7	8.01 ± 4.65	2.07 ± 0.92
Treatment	10.56 ± 1.09	327.1 ± 9.4	2.35 ± 0.28	20.4 ± 10.5	9.64 ± 5	162.6 ± 130.8
Mean	10.97	327	2.37	19	8.82	4.61
MAT						
Control	14.23 ± 4.24	131.5 ± 12.9	4.60 ± 1.05	20.2 ± 3.5	8.79 ± 3.35	6.72 ± 10.31
Treatment	14.82 ± 1.31	143.6 ± 4.6	4.79 ± 0.48	26.9 ± 4.6	12.46 ± 2.21	249.7 ± 37.16
Mean	14.90	137.5	4.70	23.9	10.79	32.87
T.P.						
Control	18.01 ± 6.04	374.5 ± 31.6	3.01 ± 0.27	32.2 ± 6.3	19.51 ± 3.51	1.79 ± 0.55
Treatment	18.38 ± 4.10	317.9 ± 40.7	2.78 ± 0.29	32.3 ± 7.7	22.13 ± 7.41	256.6 ± 281.5
Mean	18.19	348.8	2.89	32.3	20.82	3.97

Table 3.2. Summary of two-way PERMANOVA (Euclidean) comparing NH_4^+ concentration between sites, treatment plots, and site by treatment plot interaction. P-values < 0.05 are shown in bold.

Source	**df**	**MS**	**Pseudo-F**	**P**
Site	5	1.883	2.49	**0.0286**
Treatment	1	10.06	13.3	**0.0001**
Site *X* treatment	5	1.172	1.55	0.1772
Residual	59	0.757		

Table 3.3. Summary of two-way PERMANOVA (Euclidean) comparing sediment characteristics (NH_4^+ concentration excluded) between sites, treatment plots, and site by treatment plot interaction. P-values < 0.05 are shown in bold.

Source	**Df**	**MS**	**Pseudo-F**	**P**
Site	5	55.22	47.3	**0.0001**
Treatment	1	2.198	1.88	0.1171
Site *X* treatment	5	0.969	0.83	0.6634
Residual	59	1.167		

3.2 Seagrass health

A summary of seagrass metrics is given in **Table 3.4**, and can be used to compare the attributes of meadows in time zero, control, and treatment plots. The results of a two-way PERMANOVA analysis conducted on overall seagrass health revealed a significant difference in health between sites, as well as with treatment (**Table 3.5**). A post-hoc pair-wise test comparing control plots revealed that the overall health of each site differed significantly, apart from between ATH and TP (**Table 3.6**). When examining the individual attributes in this analysis, we see a range of differences between sites, often specific to the attribute.

No difference was seen in seagrass cover in control plots between sites (**Table 3.6**), as the cover of control plots at each site was relatively similar (**Table 3.4**) - with the exception of TUA. The high seagrass cover at TUA may explain the low (though not statistically significant) p-values seen when comparing cover from TUA to each of the other plots (**Table 3.6**). The BG and AG biomass of control plots at each site did little to reflect the seagrass cover, as there was much variation in these attributes despite the comparable level of cover between most sites (**Table 3.4**). This can be seen in the pair-wise results (**Table 3.6**), where these attributes differ significantly between varying sites.

The leaf count of control plots was more closely associated with cover, with most sites having a comparable measurement – though MAT showed a count approximately half that of sites with similar cover (**Table 3.4**). This is reflected in the pair-wise results, as the leaf count of control plots at MAT differs significantly from those seen at every other site (**Table 3.6**). TUA, the site with the highest cover, exhibited the highest leaf count by a notable margin (**Table 3.4**), explaining why the leaf count at TUA differed significantly from every other site (**Table 3.6**). The leaf width of control plots differed significantly between every site (**Table 3.6**), with the exception of OTU and TP – as both sites had a comparable average leaf width amongst control plots (**Table 3.4**). The leaf length of most sites was comparable, with only a few sites differing in this attribute (**Table 3.6**). The control plots at ATH contained the highest C% (**Table 3.4**), and differed significantly from all three sandy sites - though also with MAT (**Table 3.6**). TUA and TP also differed in C% amongst their control plots. The N% of control plots at all sites was relatively similar (**Table 3.4**), with the only differences being MAT vs ATH, and ATH vs TP (**Table 3.6**)

Table 3.4. Attributes of seagrass health for time zero, control and treatment plots at each site (values are mean +/- standard deviation). The abbreviations featured in the column represent the following: Seagrass coverage (% cover), below-ground biomass (BGB), above-ground biomass (AGB), leaf count (L#), leaf width (LW), leaf length (LL), carbon content (C%), and nitrogen content (N%).

Site	**Cover (%)**	**BGB (0.01m^2)**	**AGB (0.01m^2)**	**L # (count)**	**LW (mm)**	**LL (mm)**	**C (%)**	**N (%)**
BL								
Time zero	57.17 ± 11.00							
Control	71.83 ± 11.00	102.48 ± 36.79	36.81 ± 21.24	164.50 ± 53.25	1.24 ± 0.18	50.12 ± 12.51	26.55 ± 17.06	1.60 ± 0.97
Treatment	16.00 ± 13.40	88.81 ± 16.25	10.89 ± 12.37	66.33 ± 48.31	0.89 ± 0.19	32.57 ± 13.23	7.72 ± 15.50	0.61 ± 1.20
Mean	43.92	95.64	23.85	115.42	1.07	41.34	17.13	1.10
OTU								
Time zero	57.20 ± 6.24							
Control	70.83 ± 6.82	283.32 ± 102.13	136.04 ± 32.63	137.67 ± 24.36	2.56 ± 0.19	55.34 ± 12.24	33.59 ± 7.26	1.85 ± 0.59
Treatment	61.33 ± 22.76	284.04 ± 77.76	142.38 ± 116.34	122.83 ± 68.96	2.62 ± 0.09	47.62 ± 12.13	35.60 ± 5.62	2.17 ± 0.31
Mean	66.08	283.68	139.21	130.25	2.59	51.48	34.60	2.01
TUA								
Time zero	87.50 ± 6.16							
Control	100 ± 0.00	217.74 ± 47.38	198.76 ± 69.25	303 ± 66.64	1.85 ± 0.17	65.56 ± 15.34	34.34 ± 5.01	1.89 ± 0.36
Treatment	99.17 ± 2.04	245.83 ± 26.52	235.80 ± 96.86	290.67 ± 50.78	1.78 ± 0.17	65.15 ± 22.53	31.97 ± 6.38	2.16 ± 0.44
Mean	99.55	243.62	236.79	307.45	1.80	66.86	32.88	2.04
ATH								
Time zero	64.78 ± 5.73							
Control	70.33 ± 11.20	144.36 ± 13.19	141.16 ± 51.88	118.33 ± 24.31	2.72 ± 0.09	61.46 ± 9.87	38.61 ± 1.73	1.87 ± 0.08
Treatment	47.83 ± 10.34	147.30 ± 43.82	128.50 ± 63.77	90.17 ± 41.19	2.72 ± 0.10	50.49 ± 11.61	38.10 ± 2.39	2.25 ± 0.16
Mean	59.08	145.83	134.83	104.25	2.72	55.98	38.35	2.06
MAT								
Time zero	27.63 ± 4.43							
Control	65.17 ± 10.36	75.37 ± 33.99	46.28 ± 11.59	62.67 ± 15.71	2.08 ± 0.10	42.49 ± 5.74	34.23 ± 7.09	1.64 ± 0.13
Treatment	33.40 ± 10.28	67.96 ± 24.56	39.95 ± 27.42	49.50 ± 39.07	1.97 ± 0.23	38.78 ± 7.51	33.03 ± 6.90	1.91 ± 0.49
Mean	48.67	71.67	43.11	56.08	2.03	40.63	33.63	1.77
TP								
Time zero	60.33 ± 6.50							
Control	68.83 ± 9.33	140.88 ± 43.89	127.51 ± 33.41	119.33 ± 34.77	2.62 ± 0.05	61.13 ± 7.64	37.46 ± 0.89	1.71 ± 0.12
Treatment	59.17 ± 12.83	113.65 ± 30.28	108.66 ± 44.68	105 ± 35.45	2.66 ± 0.09	62.34 ± 9.94	37.55 ± 1.90	2.06 ± 0.14
Mean	64	127.26	118.08	112.17	2.64	61.74	37.50	1.89

Table 3.5. Summary of two-way PERMANOVA (Euclidean) comparing seagrass health between sites, treatment plots, and site by treatment plot interaction. P-values < 0.05 are shown in bold

Source	**Df**	**MS**	**Pseudo-F**	**P**
Site	5	53.83	29.23	**0.0001**
Treatment	1	31.97	17.36	**0.0001**
Site *X* treatment	5	15.11	8.20	**0.0001**
Residual	55	1.84		

Table 3.6. Summary of PERMANOVA post-hoc pair-wise comparisons testing for differences in attributes of seagrass health in control plots between sites. P-values < 0.05 and 0.10 are shown in bold with ** and *, respectively. See **Table 3.4** for a description of abbreviations used.

Site comparison	Overall	% Cover	BGB	AGB	L#	LW	LL	C%	N%
BL vs TUA	**0.013****	0.224	**0.008****	**0.014****	**0.012****	**0.002****	0.107	0.419	0.482
BL vs OTU	**0.002****	0.867	**0.003****	**0.002****	0.303	**0.002****	0.484	0.380	0.602
BL vs MAT	**0.027****	0.300	0.241	0.353	**0.003****	**0.002****	0.210	0.390	0.919
BL vs ATH	**0.002****	0.823	**0.033****	**0.002****	**0.077***	**0.003****	0.114	**0.079***	0.460
BL vs TP	**0.002****	0.605	0.135	**0.003****	0.115	**0.002****	**0.089***	0.244	0.748
TUA vs OTU	**0.017****	0.150	0.239	0.221	**0.002****	**0.003****	0.235	0.819	0.897
TUA vs MAT	**0.002****	0.110	**0.007****	**0.015****	**0.002****	**0.013****	**0.012****	0.968	0.104
TUA vs ATH	**0.016****	0.209	**0.029****	0.284	**0.002****	**0.002****	0.587	**0.030****	0.890
TUA vs TP	**0.015****	0.171	**0.050****	0.177	**0.002****	**0.002****	0.528	**0.091***	0.242
OTU vs MAT	**0.003****	0.295	**0.002****	**0.002****	**0.003****	**0.002****	**0.043****	0.864	0.482
OTU vs ATH	**0.007****	0.956	**0.004****	0.835	0.216	**0.081****	0.357	**0.062***	0.892
OTU vs TP	**0.018****	0.692	**0.016****	0.651	0.319	0.488	0.348	0.234	0.575
MAT vs ATH	**0.003****	0.445	**0.003****	**0.003****	**0.003****	**0.002****	**0.006****	**0.060***	**0.002****
MAT vs TP	**0.002****	0.534	**0.008****	**0.004****	**0.006****	**0.002****	**0.003****	0.250	0.368
ATH vs TP	0.870	0.827	0.876	0.600	0.982	**0.003****	0.951	0.175	**0.029****

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The presence of a significant interaction indicated that treatment effects were site-specific and required pair-wise tests to elucidate effects at each site separately (**Table 3.5**). A post-hoc pair-wise comparison of all plots revealed that seagrass health varied between control and treatment at BL, ATH, and MAT, as well as in a variety of attributes (**Table 3.7**). Seagrass cover within treatment plots at these sites was lower than that observed at time zero (**Table 3.4**). All three sites illustrated a significant difference in seagrass cover between control and treatment plots, which was shown to be a negative association with enrichment. BL also exhibited treatment effects in above-ground biomass, leaf count, leaf width, leaf length, C% and N%, with all of these attributes being negatively affected (**Table 3.4**, **Table 3.7**). Below-ground biomass decreased somewhat at BL (**Table 3.4**), though not significantly. Treatment plots at ATH exhibited a decrease in N% (**Table 3.4**, **Table 3.7**), while MAT showed no treatment effect in any attribute other than cover. These negative trends can be illustrated via MDS ordination (**Figure 3.1**), where treatment plots from BL cluster away from the seagrass attribute overlay. Control plots from BL in this ordination cluster towards the positive end of this overlay, indicating relatively higher attribute levels of seagrass health. The separation of control and treatment plots can also be seen at ATH and MAT (**Figure 3.1**), though this separation is not as distinct as at BL. The physical degradation of seagrass cover over time in treatment plots can be seen visually via photographs (**Figure 3.2**), while control plots show no discernible changes by comparison.

Table 3.7. P-values of PERMANOVA post-hoc pair-wise comparisons testing for treatment effects in seagrass attributes at different sites. P values < 0.05 are shown in bold and with **. See **Table 3.4** for a description of abbreviations used.

Site	Overall	% Cover	AGB	L#	LW	LL	C%	N%
BL	**0.003****	**0.006****	**0.002****	**0.004****	**0.002****	**0.003****	**0.003****	**0.002****
OTU	0.243	0.101	0.957	0.188	0.530	0.135	0.585	0.239
TUA	0.225	0.999	0.572	0.225	0.567	0.156	0.397	0.443
ATH	**0.038****	**0.009****	0.672	0.191	0.937	0.115	0.684	**0.002****
MAT	**0.008****	**0.003****	0.624	0.613	0.446	0.440	0.773	0.237
TP	0.162	0.164	0.424	0.484	0.309	0.815	0.943	0.114

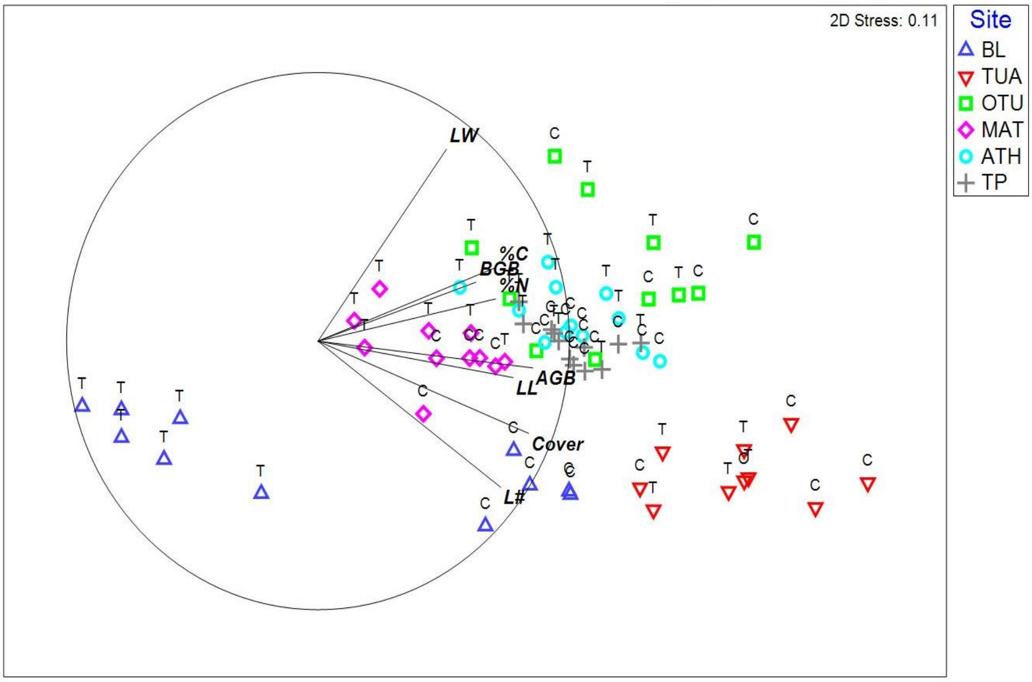

Figure 3.1 Non-metric MDS ordination (Euclidean) illustrating differences in seagrass health between plots (C = control, T = treatment) as well as sites (see legend). The ordination has been overlaid with attributes of seagrass health in order to elucidate variation in plot distribution. Each point represents one seagrass core sample. See **Table 3.4** for a description of abbreviations used in the overlay

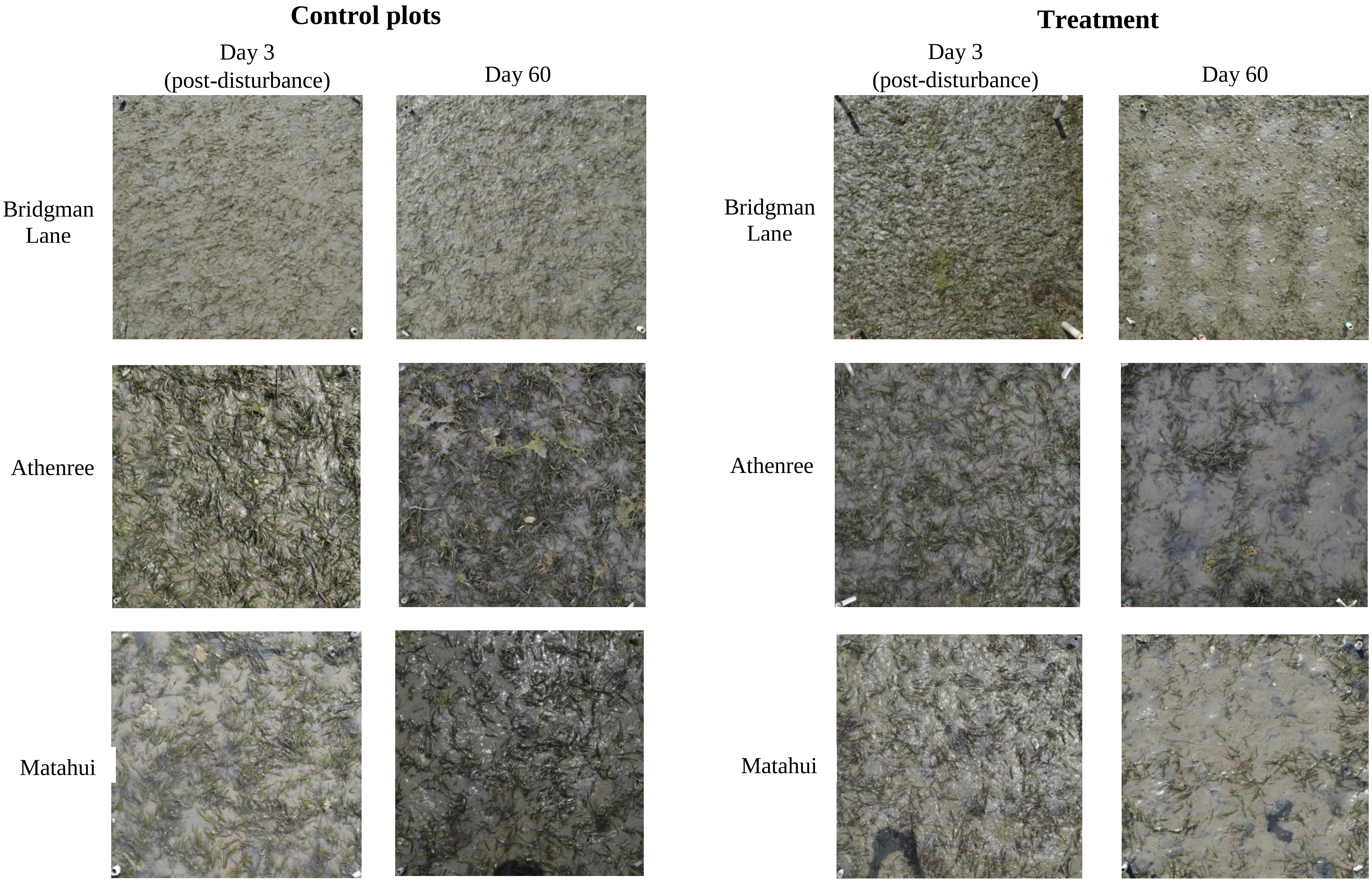

Figure 3.2. Photographs comparing the seagrass cover of control and treatment plots at Bridgman Lane, Matahui, and Athenree at 3 day recovery and 60 day observations. Sites were chosen based on PERMANOVA pair-wise comparison (**Table 3.7**).

No single characteristic of the sediment can be used to group all three sites that showed a response to enrichment (**Table 3.1**). While BL had low mud content, ATH and MAT had much higher by comparison. Both BL and MAT had comparable median grain sizes, while ATH had one of the highest. The NH_4^+ concentration of control plots at MAT was approximately triple that of ATH, whilst BL had the lowest of any site by a large margin.

In order to discern if any of the control plots had changed over the course of the experiment, observations of seagrass cover were made in plots that had no gravel or fertiliser added before the experiment was conducted. These were referred to as "time zero" plots, and were considered to represent the standard of seagrass health at each site prior to the experiment. When comparing the cover of time zero plots and control plots from the conclusion of the experiment (**Table 3.8**), a difference between the two observations can be seen. A difference between sites is also observed, as well as an observation by site interaction, indicating differences in cover between sites – though these differences are site-specific.

Table 3.8. Summary of two-way PERMANOVA (Euclidean) comparing seagrass cover between observations (time zero vs. control), sites, and observation by site interaction. P-values < 0.05 are shown in bold.

Source	**Df**	**MS**	**Pseudo-F**	**P**
Observation	1	4278.1	65.63	**0.0001**
Site	5	2793.2	42.85	**0.0001**
Observation *X* site	5	392.03	6.01	**0.0003**
Residual	60	65.192		

In order to elucidate which sites experienced a difference in cover between time zero and control plots, a post-hoc pairwise test was conducted (**Table 3.9**). This test showed that every site, bar ATH, exhibited a difference in cover between these observations, with varying levels of significance. OTU, TUA, and MAT showed a significant difference in cover, while BL and TP showed a marginally significant difference. When looking at the values for cover (**Table 3.4**), this difference is shown to be a positive one, with each site exhibiting an increase in cover between time zero and control plot observations. MAT shows the largest increase between observations, followed by OTU and TUA. BL exhibits a notable increase in cover - its large standard deviation possibly explaining why this site exhibited a marginally significant difference, rather than a significant one. Finally, TP shows the smallest increase amongst sites that were found to show differences in cover.

Table 3.9. P-values of PERMANOVA post-hoc pair-wise comparisons testing for differences in seagrass cover between time zero and control observations at different sites. P-values < 0.05 and 0.10 are shown in bold with ** and *, respectively.

Site	Seagrass cover (P)
BL	**0.0519***
OTU	**0.0086****
TUA	**0.0014****
ATH	0.3298
MAT	**0.0021****
TP	**0.0978***

3.3 Macrofauna community assemblages

Two measures of macrofauna community assemblage, abundance and richness, varied between sites (**Table 3.10**). BL had the lowest average abundance per plot, yet had a species richness per plot similar to that of MAT. OTU had an average abundance per plot approximately half of that seen at TP, though OTU had a higher average richness per plot. TUA had the highest average abundance as well as the highest species richness per plot. ATH exhibited the second highest values in both measures. The response of these measures to treatment at each site was not uniform, with both positive and negative associations with treatment plots amongst the sites (**Table 3.10**). Both species abundance and richness decreased at TUA and TP, while MAT, BL, and OTU all showed increases in both measurements. ATH exhibited a decrease in abundance, while species richness remained relatively the same between control and treatment plots. A two-way PERMANOVA analysis conducted on macrofauna community assemblages revealed a significant difference in composition between sites (**Table 3.11**), though no treatment effect on overall composition was detected.

Table 3.10. The abundance (average macrofauna count per plot) and richness (average number of taxon per plot) of macrofaunal communities in control and treatment plots at each site (values are mean +/- standard deviation).

Site	**B.L.**	**OTU**	**TUA**	**ATH**	**MAT**	**TP**
Abundance						
Control	32.50 ± 10.15	69.67 ± 23.53	169.40 ± 37.66	125.33 ± 71.12	49.75 ± 16.52	149.50 ± 85.071
Treatment	38.00 ± 22.90	81.20 ± 15.99	114.17 ± 32.90	103.00 ± 71.12	57.33 ± 36.24	116.00 ± 42.45
Mean	35	74.91	142.55	114.17	54.30	132.75
Richness						
Control	14.33 ± 3.67	16.50 ± 3.78	23.00 ± 1.23	18.50 ± 2.66	14.25 ± 1.26	17.17 ± 2.99
Treatment	18.40 ± 7.13	17.80 ± 1.30	19.17 ± 1.60	19.33 ± 4.18	14.33 ± 3.20	13.50 ± 1.38
Mean	16.18	17.09	20.91	18.92	14.30	15.33

Table 3.11. Summary of two-way PERMANOVA (Bray-Curtis) comparing macrofauna assemblage between sites, treatment plots, and site by treatment plot interaction. P-values < 0.05 are shown in bold.

Source	**df**	**MS**	**Pseudo-F**	**P**
Site	5	14320	12.9	**0.001**
Treatment	1	1438.8	1.21	0.242
Site *X* treatment	5	2337.3	1.97	**0.001**
Residual	59	1184.6		

The presence of a significant interaction indicated that treatment effects were site-specific and required pair-wise tests to elucidate effects at each site separately (**Table 3.11**). Treatment effects on community assemblages at TUA and TP were found to be significant (**Table 3.12.**). Examining plot configurations via MDS ordination (**Figure 3.3**) revealed a response in community assemblage to enrichment, with groups forming relative to control or treatment plots at these sites

Table 3.12. P-values of PERMANOVA post-hoc pair-wise comparisons testing for treatment effects in macrofauna assemblages at different sites. P-values < 0.05 are shown in bold and with **.

Site	**Macrofauna assemblage (P)**
BL	0.166
OTU	0.146
TUA	**0.012****
ATH	0.432
MAT	0.140
TP	**0.041****

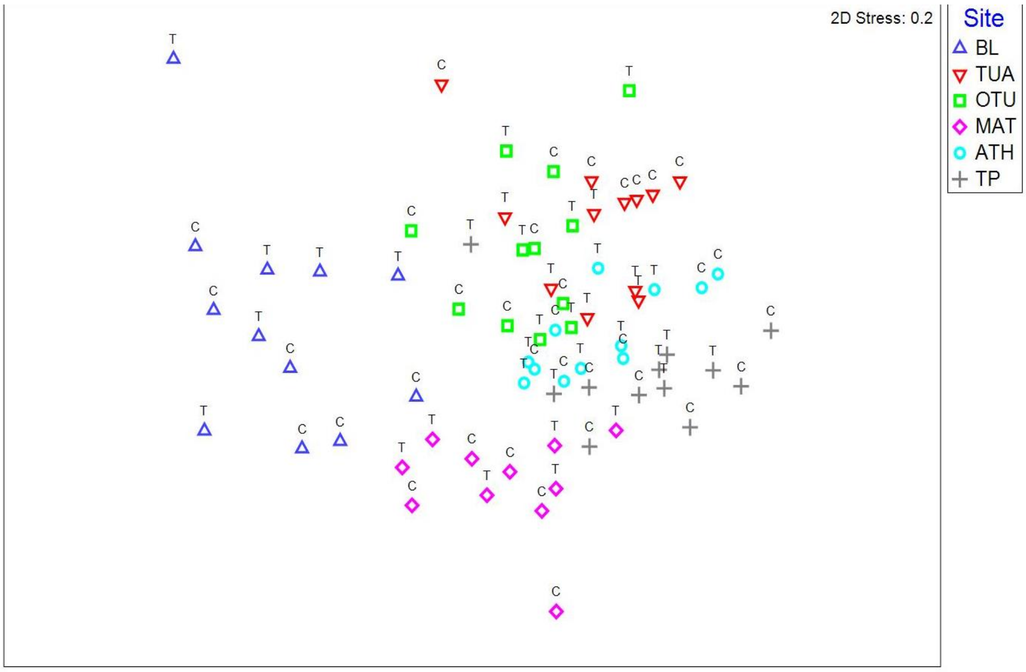

Figure 3.3. Non-metric MDS ordination (Bray-Curtis) illustrating differences in macrofauna assemblages between plots (C = control, T = treatment) as well as sites (see legend). Each point represents one macrofauna core sample.

The number of species that contributed > 50% to the cumulative dissimilarity in community assemblages between control and treatment plots differed between TUA and TP (**Table 3.13**). Five species, contributing approximately 10% each, comprised > 50% of the cumulative dissimilarity at TUA, while TP needed only two species to comprise > 50%. This reflected the high species richness of TUA when compared to TP (**Table 3.10**). Two species contributed to the 50% limit of dissimilarity between control and treatment plots at both sites - the polychaete worm, *Heteromastus filiformis*, and the bivalve, *Linucula hartvigiana* (**Table 3.13**). The contribution of *H. filiformis* was weakest at TUA, and approximately threefold by comparison at TP. The weak contribution of *H. filiformis* at TUA may be due to the contribution of *Prinospio aucklandica*, another polychaete worm, at this site (**Table 3.13**). The relationship between *H. filiformis* abundance and enrichment was positive at TUA, but negative at TP (**Table 3.10**). The decrease in *H. filiformis* abundance at TP is a notable one (**Figure 3.4.a**), and is likely the driver of the decline in overall abundance at this site (**Table 3.13**) due to the high contribution of dissimilarity associated with it. The abundance of *L. hartvigiana* experienced a decline in response to enrichment at both of these sites, though the decrease was much more significant at TP (**Figure 3.4.b**). The mean abundance of *H. filiformis* and *L. hartvigiana* at these two sites is notably higher than at all others (**Appendix 1**).

Table 3.13. Results of SIMPER analysis, using Bray-Curtis dissimilarity, illustrating taxon that contributed > 50% to the cumulative dissimilarity between control and treatment plots at TUA and TP. Increases/decreases in average abundance of treatment plots relative to control for each taxon are represented by +/-, respectively.

Taxon	**TUA**		**T.P.**	
	Contribution %	Dissimilarity %	Contribution %	Dissimilarity %
Control vs treatment		**56.18**		**50.27**
Prinospio aucklandica	13.63 -			
Heteromastus filiformis	11.98 +		34.25 -	
Phoxocephalidae	11.11 -			
Linucula hartvigiana	10.11 -		16.02 -	
Austrovenus stutchburyi	9.34 -			

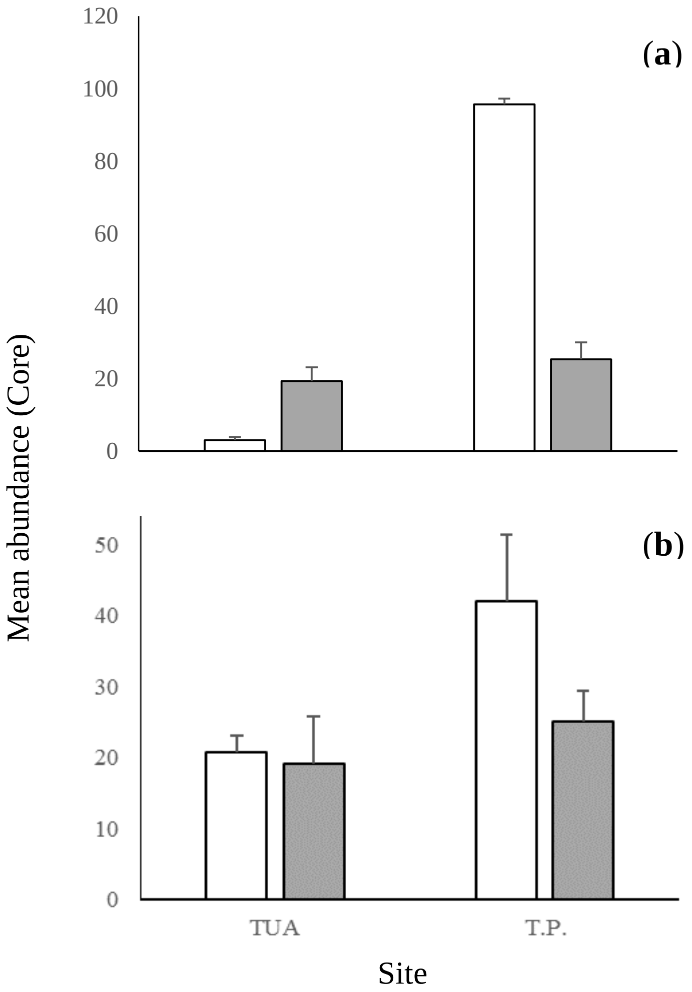

Figure 3.4. Mean abundance (+/- SE) of *Heteromastus filiformis* (a) and *Linucula hartvigiana* (b) in control (empty bars) and treatment (filled bars) plots at TUA and TP.

Both TUA and TP can be grouped by two sediment characteristics – high chl *a* and high phaeo content (**Table 3.1**). TUA and TP had much higher chl *a* and phaeo than all other sites, with TUA having the highest in both characteristics, followed by TP. While both sites showed an increase in these characteristics, an increase in these sediment characteristics was seen at all sites (**Table 3.1**). TUA exhibited a higher (though more variable) NH_4^+ concentration in its control plots than that found at TP (**Table 3.1**), while the concentration of treatment plots at TP was significantly larger and had a huge variation. The OM content of sediment at

TUA was double that found at TP. As one of the sandy sites, TUA had a mud content and grain size much lower than that at TP - the site that was observed as the muddiest. This difference in grain size and mud content can be visualised via MDS ordination (**Figure 3.5**). Grain size and mud content were placed in the overlay of this ordination as they had been shown to contribute > 10% to the variation in community assemblages (**Table 3.14**). This MDS ordination shows TUA and TP forming groups in conjunction with the grain size and mud content overlay, reflective of their respective values in these characteristics. The control and treatment plots from TP assemble distinctively in conjunction with mud content, suggesting that this characteristic may play a role in overall community assemblage at this site.

Neither TUA nor TP exhibited any significant difference between control and treatment plots in attributes of seagrass health (**Table 3.7**), though the two sites had differences between them in attributes overall. TUA had the highest seagrass cover of any site, while TP had a lower cover – though comparable to other sites classed as muddy (**Table 3.4**). Cover was shown to contribute to the variation in community assemblage (**Table 3.14**), and as such was used in an MDS ordination (**Figure 3.5**) to examine whether groups formed in conjunction with this attribute. In this ordination, plots from TUA assembled in conjunction with cover, suggesting that the community composition of this site was related to this attribute. TUA had double the above/below ground biomass, three times the leaf count, and a longer leaf length than that of TP (**Table 3.4**). The leaf width and C% of TP were higher than at TUA. The only attribute that two sites had that were of comparable levels was N% (**Table 3.4**).

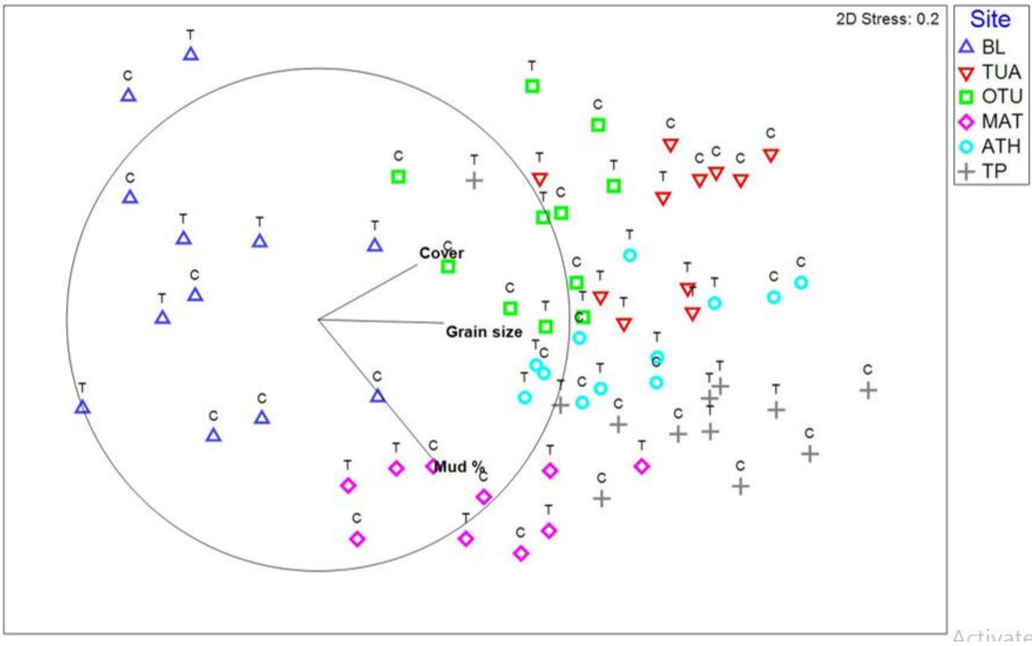

Figure 3.5. Non-metric MDS ordination (Bray-Curtis) illustrating differences in macrofauna assemblages between plots (C = control, T = treatment) as well as sites (see legend). The ordination has been overlaid with sediment characteristics and attributes of seagrass health that contributed > 10% to variation in community assemblages in order to elucidate variation in plot distribution. Each point represents one macrofauna core sample**.**

Table 3.14. Results of a DistLM analysis illustrating variables that explain > 5% of the variation in macrofauna assemblages. Variables with a contribution > 10% are represented in **Figure 3.5**.

Variable	**% of variation explained**
Cover	15.18
Grain size	15.01
Mud content	10.69
BG biomass	7.96
NH_4^+	5.62

Chapter Four:
Discussion

This study examined the effects of nutrient enrichment on seagrass metrics and macrofaunal community assemblages at sites across a gradient of sediment mud content. Different metrics were measured as attributes of seagrass meadows, the amalgamation of which was referred to as seagrass health. The overall community composition of macrofaunal assemblages was measured, with species richness and abundance examined as aspects. No other study to date has examined all of these variables in tandem *in situ*, and therefore this study provides a novel opportunity to examine the possible relationships between them.

The NH_4^+ concentration of sediment pore water was elevated in order to simulate nutrient loading as an aspect of long-term eutrophication. This was conducted using a method previously shown to elevate nutrients locally without affecting the nutrient levels of nearby control plots. The pore water NH_4^+ concentration was successfully elevated within treatment plots at each site, and was lost from the sediment at varying levels based on mud content and grain size. Seagrass cover increased over the course of the experiment within control plots at all sites, though natural growth was expected as the experiment ran during the seagrass growing season. Three of the six sites exhibited a response in overall seagrass health within treatment plots, one sandy site (BL) and two muddy sites (ATH, MAT). All three of these sites showed a decrease in cover compared to control plots, while ATH additionally showed an increase in N%, and BL exhibited a significant decrease in every attribute, bar BGB.

The average abundance and richness of macrofaunal community assemblages increased at certain sites and decreased at others. However, changes in these measures were not unique to sediment classes and therefore cannot be attributed to them. Two sites showed a significant difference in community assemblages with treatment, one sandy (TUA) and one muddy (TP). Both sites showed a decrease in overall abundance and richness, and only two species were shown to significantly contribute to the dissimilarity at both sites. These species were the polychaete worm, *Heteromastus filiformis* and the bivalve, *Linucula hartvigiana*. A decrease in abundance of *H. filiformis* was strong enough to be considered the driver of the decrease of overall community abundance at TP. TUA exhibited a small increase in *H. filiformis* abundance, which contributed approximately one third the amount of variation in community assemblage as at TP. The abundance of the bivalve *L. hartvigiana* exhibited a decrease in at both sites – though only significantly at TP.

The variables shown to contribute > 10% of the variation in community assemblages (seagrass cover, grain size, and mud content) differed significantly between TUA and TP. Despite this, both sites experienced a significant response in macrofaunal assemblage to treatment, and neither of these two sites showed any treatment effects in any attributes of seagrass health. The sediment pore water of treatment plots at TUA suggests that the plots experienced leaching, though the N% content of leaves at this site did not differ between control and treatment plots, indicating that the tissues had not been saturated. The cover and AGB of TUA were far higher than at any other site, and may suggest that the seagrasses here distributed nutrients lost from the sediment enough to avoid degradation or stress. Denitrification may also result in the elimination of up to 25 % of mineralised NH_4^+ from the sediment (Gilles & Christiane, 1988), and as such eutrophication induced hypoxic conditions (Jorgensen & Richardson, 1996) present at TUA may have enhanced activity by nitrifying bacteria. This still would have exposed the macrofauna to high concentrations of NH_4^+ while moving from the sediment pore water to the overhead water column, explaining the significant decline in both abundance and richness while the seagrasses remained relatively unaffected. The other sandy site which lost its elevated NH_4^+ concentrations from treatment plots at a similar level (BL) showed a significant reduction in almost every aspect of seagrass health. However, BL had much lower cover as well as AGB before the addition of nutrients than at TUA, and showed no difference in macrofauna community assemblages. Therefore, it may be the state of seagrass health pre enrichment, rather than its response to nutrient loading, that denotes the response of macrofaunal community assemblages at sandy sites.

An elevated NH_4^+concentration was found in the majority of treatment plots at TP, though a few exhibited a loss in concentration to an extent that caused notable variation amongst plots. This may have been caused by bioturbation by the bivalve *L. hartvigiana,* which is known to mix the upper layers of sediment, and was found in higher numbers at this site than at any other. Macrofauna that mix sediment via bioturbation have been known to alter local pore water nutrient concentrations (Waldbusser & Marinelli, 2006). The overall abundance of *L. hartvigiana* was second highest at TUA, which may have contributed to the exchange of NH_4^+ between the sediment pore water and the water column at this site as well. Additionally, TP contained a low species richness in control plots when compared to TUA, though a similar overall abundance by comparison.

As such, sediment characteristics, seagrass metrics, and the composition of macrofaunal assemblages may work in tandem to denote the magnitude and type of response evocated in community assemblages. None of the sites which exhibited a response in seagrass health also showed a response in macrofaunal assemblage, and variations in either of these responses were not unique to a particular sediment class.

4.1 Sediment pore water enrichment and nutrient loss

The treatment plots of each site showed evidence of enrichment without elevating the nutrients of any control plots. The concentration of treatment plots at the conclusion of the experiment was used as a proxy for the level at which the plots had leached nutrients. Two of the sandy sites, BL and TUA, exhibited low NH_4^+ concentrations that were comparable to those seen in their control plots. This was assumed to be a result of extensive leaching at these sites, based on the inclination that sandier sediments lose nutrients from pore water at rates different to muddier ones (Corbett, 2010). The sandy site which retained a high NH_4^+ concentration, OTU, differed from BL and TUA in that it had a much coarser grain size. This may suggest that although OTU had low mud content, its grain size was too coarse for significant leaching to occur, perhaps requiring the combination of low mud content and grain size. The muddy sites all retained high pore water NH_4^+ concentrations in treatment plots, though measurements within treatment plots varied heavily. This variation may have be caused by fertiliser pellets still present within the sediments at this site skewing readings of the pore water concentrations (pers. obs., **Appendix 2**). However muddy sediments are known to retain nutrients naturally due to lower levels of interaction with the overhead water column in comparison to sandier sediments (Koch, 2001). The pore water NH_4^+ concentrations of control plots were considered a proxy for ambient concentrations at each site. Low ambient concentrations may be an indicator of sensitivity to enrichment, e.g. at BL and TP, where significant responses in attributes of seagrass health and macrofaunal community assemblages were seen, respectively.

4.2 Attributes of seagrass health

Increases in seagrass cover were shown between time zero plots and control plots at the end of the experiment. The increase in cover is likely a result of the experiment taking place over the seagrass growing season, rather than being stimulated by elevated nutrient levels. This is supported by Douglas et al. (2015, submitted), from which the method of enrichment used in this experiment originated, where nutrient concentrations were shown to coincide with ambient levels at a distance of 0.5 m from enriched plots. Seagrass cover within treatment plots was lower than in control plots at every site with the exception of TUA. As such, we can ascertain that the concentration at which treatment plots were enriched was enough to stunt seagrass growth amongst the five sites affected, though only significantly at BL, ATH, and MAT. The seagrass cover in treatment plots at these three sites was lower than at time zero observations, suggesting that growth in treatment plots was not only stunted but reduced with enrichment. This coincides with enrichment studies that have found that while enrichment can enhance seagrass growth, prolonged exposure at high enough levels impedes growth and physiological attributes (Brun et al., 2002; Montefalcone, 2009; Marba et al., 2013)

Significant decreases in every attribute, bar BGB, were seen at BL. Below ground tissues are more resistant to physical disturbance than above ground tissues (Duarte & Chiscano, 1999) and therefore less likely to be removed by wave action when degraded or stressed. As such, BGB may have simply physically persisted at this site as a result of being buried in the sediment, rather than its tolerance to elevated NH_4^+ concentrations. This may have been why BGB remained similar between control and treatment plots at this site. Even so, while not statistically significant, BL still showed an overall decreased in BGB, therefore exhibiting a negative trend in all attributes in response to enrichment. This site was classified as the sandiest, and was therefore predicted to respond notably to enrichment relative to mud content as suggested by relevant literature (van Katwijk et al., 1997; Fonesca

& Bell, 1998). However, the sheer magnitude of response in seagrass metrics seen at BL, especially in comparison to sites of similar mud content, was surprising. None of the individual attributes of seagrass health in treatment plots could be used as indicators of response at BL - as so much of the meadow died that significant differences in all attributes in comparison to control sites were to be expected. Perhaps if a similar experiment was conducted with observations of attributes at intervals of degradation, their value in determining the state of meadow health could be ascertained as they respond through time.

Sediment classes could not be used to explain which sites exhibited significant changes in seagrass metrics, with BL classed as sandy, and ATH and MAT classed as muddy. The NH_4^+ concentration of treatment plots at all three sites varied widely. Treatment plots at BL contained the lowest concentration, likely due to leaching, and exhibited changes in seagrass cover by the first observation, roughly two weeks after addition (pers. obs). Treatment plots at ATH and MAT retained high NH_4^+ concentrations, with seagrass cover at these sites deteriorating by the fourth observation – much closer to the end of the experiment. This difference was notable enough that BL was considered a site of interest well before the experiment reached its conclusion. By comparison, ATH and MAT only exhibited a notable decrease in cover by the fourth observation. Therefore sediment composition alone cannot be used to explain why these three sites in particular exhibited a significant response in attributes of seagrass health to enrichment. Perhaps the dosage at which fertiliser was added was too little to instigate a response. A stronger concentration may be needed to prompt a response in seagrass variables across a gradient of sediment. A median dose was chosen (200 N g/m^2) for this based on a literature review of nutrient loading on seagrasses in order to result in a response without mass death. However some studies have instigated responses in seagrass metrics with weaker dosages (88 N g/m^2) (Udy & Dennison, 1997), while others used stronger dosages (686 N g/m^2) without mass death as a result (Armitage et al., 2005) How these would vary across sites with differing sediment characteristics may merit investigation.

4.3 Macrofauna community assemblages

The abundance and richness of the macrofauna community assemblages in treatment plots increased at some sites and decreased at others – tending to do either in tandem at most sites. As anticipated, community assemblages were shown to vary between sites, as the composition of macrofauna communities has been shown to vary with traits of associated seagrass meadows (Mills & Berkenbusch, 2009) and aspects of the local environment (Frost et al., 1999). Sediment classes could not be used to predict whether sites would show increases or decrease of species abundance or richness, as this varied with site sediment characteristics. However, the two sites which exhibited a negative trend in both of these measures (TUA, TP) also showed a significant response in community assemblage to treatment. The control and treatment plots from TP assembled distinctively in conjunction with mud content via MDS ordination, suggesting that this characteristic may play a role in overall community assemblage at this site. However, as TUA was classed as a sandy site, and TP as the muddiest, sediment classification alone could not be used to explain why community assemblage responded at both sites.

Five species were shown to have contributed > 50% of the dissimilarity between control and treatment plots at TUA. By contrast, only two species were needed to contribute the same amount of dissimilarity at TP. As TUA had a much higher species richness in control plots when compared to TP, this is likely a reflection of the high number of species present, rather than a list of species especially sensitive to NH_4^+ enrichment. Only two species contributed to the dissimilarity in assemblages at both TUA and TP - the polychaete worm, *Heteromastus filiformis,* and the bivalve, *Linucula hartvigiana.* The contribution of *H. filiformis* to dissimilarity between control and treatment plots was weaker at TUA than at TP, which may just be a product of the high species richness at TUA. Polychaete worms, such as *H. filiformis*, have been shown as indicators of health within seagrass meadows (Cardoso et al., 2007), often decreasing in abundance as eutrophication occurs. As *H. filiformis* significantly decreased in abundance at TP and not TUA, this may be an indication that the individual traits of each of site denote the response of macrofauna to enrichment.

The abundance of *L. hartvigiana* was relatively similar between control and treatment plots at TUA – whereas it decreased with treatment by approximately 50% at TP. This difference in sensitivity may be due to the variation in sediment characteristics (Berkenbusch & Rowden, 2007), seagrass attributes (Mills & Berkenbusch, 2009), or macrofaunal community composition between these two sites (Al-Wedaei et al., 2011). The abundance of *L. hartvigiana* at both TUA and TP is much higher than at any other site – even after being significantly reduced in the treatment plots of TP. This may suggest that the presence of this bivalve at these sites, at the abundance of which they are found, could have mitigated the community response to nutrient enrichment. Through bioturbation, *L. hartvigiana* is known to mix the upper layers of sediment (Greenfield et al., 2013), likely intensifying the exchange of nutrients between the sediment pore water and the water column (Mortimer et al., 1999).

While not within a seagrass meadow, Gallagher et al. (2013) observed that macrofaunal community responses to the burial of detritus varied with sediment characteristics in a New Zealand estuary. This study suggested that the response of macrofauna to sediment mixing varies between areas with fine sediment and high mud content vs sandy areas. This mirrors the response of *L. hartvigiana* abundance to enrichment, with a much lower reduction in abundance seen at the sandy site (TUA), and a strong negative association with enrichment seen at the muddier site (TP). As the control plots mimicked the level of disturbance exerted on the plot by the addition of nutrients, it is unlikely that the physical mixing of sediment is causing this response in the treatment plots of these sites. Rather, it is more likely that the elevated NH_4^+ concentration of the sediment pore water is causing the reduction of *L. hartvigiana* at the muddy site (TP), while having an insignificant impact on this same species at the sandier site (TUA).

4.4 Relationships between responses in seagrass attributes and macrofauna community assemblages to nutrient enrichment

Examining the attributes of seagrass health at the sites that showed treatment effects on community assemblages (TUA, TP) revealed no common measures between both, bar N%. TUA exhibited the highest cover, above/below ground biomass, leaf count and leaf length of any site, and at levels much higher than those of TP. Neither TUA nor TP experienced any significant changes in seagrass health with treatment. As seagrass attributes vary amongst these sites, it is doubtful that a response in any attribute of seagrass health explains changes in the community composition of both sites. However, a study compiling observations of seagrass cover from 1959-2011 (Park, 2016) suggests that seagrasses meadows at these sites vary in their distribution over time. This study showed that from 1996-2011, cover at TUA had decreased by 28%, while increasing at TP by 74% over the same period. Changes over such large scale observations may implicate a response to factors outside the scope of this experiment – and explain why these sites in particular responded to treatment while others of similar characteristics did not.

Only one site showed no response to treatment in either attributes of seagrass health or macrofaunal community assemblage – the sandy site, OTU. The only unique sediment characteristic of this site was the high NH_4^+ concentration of its treatment plots, far higher than at any other site. Perhaps the lack of response in any seagrass or macrofaunal measurements is due to the fact that this site retained the majority of its nutrients within the sediment pore water. This site also contained the highest BGB, both before and after treatment, perhaps at a level high enough to mitigate the release of NH_4^+ into the water column (Hasegwa et al., 2008). It could be that the coupled characteristics of low mud content and low grain size are needed for substantial nutrient leaching to occur, rather than just low mud content alone. This speculation is supported by TUA, which had a higher mud content than OTU, though a smaller median grain size, and experienced heavy nutrient loss.

TUA and TP exhibited a significant macrofauna community assemblage response with treatment, while no significant response in seagrass attributes was observed. TUA maintained the highest seagrass cover with treatment, even though the above-ground tissues would have been exposed to elevated nutrients they moved from the pore water to the above water column. The N% levels of enriched leaves from TUA was comparable to those seen at TP – despite the fact that TP had retained a high level of pore water NH_4^+ concentration. It is possible that the thick seagrass cover at TUA diluted the NH_4^+ concentration across the seagrass at the treatment plots, mitigating the saturation of the above-ground tissues (Duarte, 1990; Stapel et al., 1996). This explains how even though the pore water concentration at the conclusion of the experiment insinuates that leaching occurred in treatment plots, the N% of leaves at TUA was not found to differ from those within control plots. This is further supported by the way that plots from TUA assembled in conjunction with cover via MDS ordination, suggesting that the community composition of this site was related to cover.

4.5 Summary of major findings

The concentration of sediment pore water NH_4^+ was elevated within seagrass meadows at sites across a gradient of varying sediment characteristics. This study compared the attributes of seagrass health between control and treatment plots at these sites, as well as the response of macrofaunal community assemblages within them. Of course, the present findings do not address all facets of nutrient elevation within seagrass meadows, but invite further research in this direction.

The major findings of this study were:

1. The pore water NH_4^+ concentration within treatment plots was retained at different levels between different sites. Sites with low mud content and finer grain sizes lost more NH_4^+ than sites with high mud content and coarser grain sizes.

2. Bridgman Lane (BL), the site classed as sandiest and lost the most NH_4^+ experienced a significant degradation in almost all attributes of seagrass health, with observations of cover suggesting that this happened early in the

experiment. Two sites with high mud content (ATH, MAT) exhibited responses in seagrass cover towards the conclusion of the experiment.

3. Two sites, one sandy (TUA) and one muddy (TP), showed a response in macrofaunal community assemblages with enrichment. At both of these sites mean abundance and richness decreased. The classes in which these sites were placed based on mud content and grain size could not be used to explain why both sites showed a response.

4. Two species contributed to the dissimilarity of macrofaunal community assemblages with treatment at TUA and TP, *H. filiformis* and *L. hartvigiana*. The abundances of these species responded differently to treatment between these sites. TUA experienced an increase in abundance of *H. filiformis* and small a decrease of *L. hartvigiana*, while both of these species decreased significantly in abundance at TP.

5. Neither of the two sites that exhibited a response in macrofaunal community assemblages to enrichment also showed a response in attributes of seagrass health. Seagrass meadows at TUA contained some of the highest measurements of health, whereas those at TP were similar to other sites classed as muddy.

6. It is likely that the individual traits of TUA and TP contributed to the response of their associated macrofaunal assemblages, rather than any traits these sites had in common. The combination and interaction of traits at these sites selectively may explain why community assemblages responded to treatment at both sites, despite variations sediment characteristics and attributes of seagrass health.

4.6 Limitations of the study and suggestions for future research

The results of this study suggest that while responses in attributes of seagrass health and macrofaunal community assemblages may be tied to sediment characteristics, these responses can be mitigated by other variables. Distinguishing between these variables is a delicate task, as they may act to buffer, enhance, or simply respond in tandem to nutrient enrichment. That is not to say that speculation is not without merit, but that it should be conducted with caution when expecting to see a straight-forward response in any one attribute amongst sites of varying characteristics.

While the site that experienced the most leaching, BL, exhibited signs of degradation early in the experiment, other sites that showed responses in seagrass health with treatment did so closer to the conclusion of the experiment. Many sites showed a retention of pore water concentration in treatment plots, which may leach into the water column over time. It could be that meadows at these sites would exhibit responses to nutrients over a period of time greater than that observed in this study – or perhaps at such a rate that the seagrass at these sites can cope without any significant detriment. Any future work concerning the effects of long-term nutrient saturation may want to consider observations longer than 60 days. Perhaps by conducting the experiment outside of the growth season, a different response in seagrass metrics may be seen.

The final pore water concentrations of treatment plots alluded to the level at which nutrients were leached from each site. However, obtaining these values at intervals during the experiment would have provided us with an actual rate at which this occurred, e.g. by using flux chambers. This could have been used to elucidate why sites that experienced leaching responded differently to enrichment. This study examined nutrient loading based on a median concentration from a review of studies where seagrasses underwent enrichment. Subjecting meadows to a range of concentrations based on such a review could result in a range of responses and may be worth examining.

I recommend an observation of macrofauna community assemblages at sites of intense pore water NH_4^+ concentration loss (e.g. BL, TUA) at an earlier period when the meadows show physical signs of degradation – in order to ascertain the structure of communities under duress from enrichment at sites such as these. By the conclusion of the experiment, the sediment pore water of BL was similar between control and treatment plots, and the assemblages may have reverted back to pre-addition compositions despite massive seagrass die off. By contrast, TUA experienced leaching, but a significant change in its community assemblage without any changes in attributes of seagrass health. A closer examination between sites such as these may shed more light on the role of seagrass health and sediment characteristics on the response of macrofauna to enrichment.

Chapter Five:

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Chapter Six:

Appendices

Appendix 1.

Table A.1.1. Average abundance of each taxon within control (C) and treatment (T) plots at each site.

Taxon	BL C	BL T	OTU C	OTU T	TUA C	TUA T	MAT C	MAT T	ATH C	ATH T	TP C	TP T
Anthropleura aureoradiata	0.7	1.3	1.0	3.0	0	0.7	0	0	0.7	0.3	0	0
Aonides trifida	2.0	0.7	6.7	5.0	6.7	5.3	0	0.3	4.7	1.7	1.3	1.0
Aricidea	0	0.3	1.0	3.0	4.0	1.3	0	0	5.7	2.0	3.0	1.0
Arthritica bifurca	0.7	0.3	0	0.3	2.3	1.0	6.3	2.3	8.7	0.7	2.7	11.7
Austrohelice crassa	0	0.7	0	1.7	2.0	0.3	0.0	0.7	3.0	1.0	0.7	0
Austrovenus stutchburyi	1.7	2.0	10.0	4.3	33.7	20.7	0.7	1.0	1.7	2.7	9.7	4.0
Cantharidus tenebrosus	0	0	2.7	1.0	0.3	0	0.3	0	0	0	2.0	0
Capitella	0	0	1.0	2.3	3.3	2.0	0	0.3	2.7	3.0	0.7	0
Chiton glaucus	0	0	0	0	2.0	0.3	0	0.0	0.0	0.3	0	0

Table A.1.1. (cont.) Average abundance of each taxon within control (C) and treatment (T) plots at each site.

Taxon	BL C	BL T	OTU C	OTU T	TUA C	TUA T	MAT C	MAT T	ATH C	ATH T	TP C	TP T
Circotrema zelebori	0	0	0	0	0	0	0	0	0	0	0.3	0
Colorustylis lemurum	0	0	0	1.3	0.7	0	0	0	0	0	0	0
Cominella glandiformis	2.0	1.7	0.3	0.3	1.3	0.7	0.7	0.7	0	0.3	1.0	1.7
Dexaminidae	0	0	0	0	0	0	0	0.7	0	0	0	0
Diloma subrostrata	0	1.0	0	0	0	0.7	0.7	1.0	0.3	1.3	0.3	0
Exosphaeraroma planulum	0	0	0	0	0	0	0.3	0	0	0	0	0
Halicarcinus whitei	1.0	0.3	0.3	1.3	2.3	6.7	1.0	1.7	0.7	1.0	0.3	1.0
Hemiplax hirtirpes	0	0	0.7	0.3	0	0	0.7	0	0	0	0.3	0
Heteromastus filiformis	6.0	3.7	6.3	7.0	3.0	19.3	9.7	25.3	26.3	26.3	95.7	51.7
Linucula hartvigiana	1.0	0.3	1.7	3.0	23.0	23.3	0	0	1.3	2.0	42.0	25.0
Macomona liliana	5.0	3.3	0.7	2.0	9.0	1.0	2.3	1.0	2.0	3.3	2.0	3.0
Melita awa	0	0.7	1.0	4.7	2.7	0.7	0	1.0	4.0	1.3	0	0
Nereididae	5.0	8.7	3.3	3.0	1.7	2.3	1.3	2.7	4.0	4.3	2.0	1.7

Table A.1.1. (cont.) Average abundance of each taxon within control (C) and treatment (T) plots at each site.

Taxon	BL C	BL T	OTU C	OTU T	TUA C	TUA T	MAT C	MAT T	ATH C	ATH T	TP C	TP T
Notoacmea scaphia	0.7	0	1.0	1.0	0.7	0	5.0	0.7	1.7	0.7	3.0	3.0
Nucula nitidula	0	1.3	0	0	0.3	2.0	0	0	0.7	0.7	0	0
Oligochaeta	0.7	0.3	3.3	1.3	1.0	4.3	0.3	3.3	3.3	6.3	6.0	0
Paracalliope	0.3	0	2.0	31.3	1.7	2.3	0.3	0.7	10.0	5.7	0	0
Paradoneis lyra	0.3	0.7	3.7	7.7	3.7	3.0	0.7	0.7	5.0	1.7	2.3	4.7
Paramoera	0	0	0	0	7.3	0.3	0	0	12.3	4.3	0	0
Partiriella regularis	0	0	0	0	2.0	0.7	0	0	0	0	0	0
Pelicaria vermis	0	0	0	0	0.3	0	0	0	0	0	0	0
Phoxocephalidae	0	0	13.7	14.0	28.7	18.0	29.7	16.0	12.3	7.0	6.3	11.0
Prinospio aucklandica	1.7	3.3	23.7	18.3	28.0	25.3	0.3	4.3	36.3	43.3	12.3	20.0
Scalibregmatidae	0	0.3	0.7	0	0.3	0	0	0	0	0	0	1.3
Scolecolepides benhami	7.0	4.0	0.7	0.3	0	0.7	1.3	2.3	1.3	0.3	0.3	0

Table A.1.1. (cont.) Average abundance of each taxon within control (C) and treatment (T) plots at each site.

Taxon	BL C	BL T	OTU C	OTU T	TUA C	TUA T	MAT C	MAT T	ATH C	ATH T	TP C	TP T
Sphaerosyllis semiverrucosa	0.3	0.3	0	0	0.7	0	0	0	0	0.3	0.7	3.7
Syllinae	0	0	0	0	1.7	0	0	0	0	0	0	1.0
Torridoharpinia hurleyi	0	0	0.7	0	1.7	1.3	1.0	1.7	1.0	2.3	0.3	2.0
Zeacumanthus lutulentus	1.0	7.0	0	1.0	4.3	0.7	1.0	0.7	0.7	0.7	1.7	0.3
Zeacumanthus subcarinatus	1.7	5.0	0.3	0	6.3	0.3	0	0	0	0	0	0

Appendix 2.

Figure A.2.1. Fertiliser pellets still present in cores from TP treatment plots (personal observation)