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Macrofaunal Functional Diversity Provides Resilience to Nutrient Enrichment in Coastal Sediments

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Abstract

The degradation of ecosystems is often associated with losses of large organisms and the concomitant losses of the ecological functions they mediate. Conversely, the resilience of ecosystems to stress is strongly influenced by faunal communities and their impacts on processes. Denitrification in coastal sediments is a process that may provide ecosystem resilience to eutrophication by removing excess bioavailable nitrogen. Here, we conducted a large-scale field experiment to test the effect of macrofaunal community composition on denitrification in response to two levels of nutrient enrichment at 28 sites across a biologically heterogeneous sandflat. After 7 weeks of enrichment, we measured denitrification enzyme activity (DEA) along with benthic macrofaunal community composition and environmental variables. We normalised treatment site specific DEA values by those in ambient sediments (DEA_{CN}) to reveal the underlying response across the heterogeneous landscape. Nutrient enrichment caused reductions in DEA_{CN} as well as functional changes in the community; these were both more pronounced under the highest level of nutrient loading (on average DEA_{CN} was reduced by 34%). The degree of suppression of DEA_{CN} following moderate nitrogen loading was mitigated by a key bioturbating species, but following high nitrogen loading (which reduced the key species density) the abundance and diversity of other nutrient processing species were the most important factors alleviating negative effects. This study provides a prime example of the context-dependent role of biodiversity in maintaining ecosystem functioning, underlining that different elements of biodiversity can become important as stress levels increase. Our results emphasise that management and conservation strategies require a real-world understanding of the community attributes that facilitate nutrient processing and maintain resilience in coastal ecosystems.

Key words: denitrification; benthic community; nutrient processing; eutrophication; enzyme assay; intertidal; functional traits; sandflat.

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Introduction

Enrichment of the ocean through anthropogenic alteration of the nitrogen cycle is leading to degradation of marine ecosystems and the services they provide (Nixon 1998). This occurs because nitrogen is essential for primary production and its oversupply in (generally) nitrogen-limited systems can cause blooms of algae, increases in organic matter, alteration of nutrient ratios and changes to habitats, communities and food webs (Vitousek and others 1997). Most of the terrestrial nitrogen received by the marine environment is removed through denitrification in coastal sediments (estimated up to 80%), a natural ecosystem process that removes bioavailable nitrogen (Galloway and others 2003). Denitrification (D_N) can therefore provide resilience to eutrophication, which is recognised as a global threat to the functioning of coastal ecosystems and the goods and services they provide (Vitousek and others 1997; Laursen and others 2002).

Benthic macrofauna, such as bivalves and polychaetes, play a critical role in coastal marine nitrogen cycling. Particle and water transport related to feeding and movement activity (that is, bioturbation) promotes transport of nutrients and oxygen throughout the sediment profile enhancing rates of nitrogen transformation (Kristensen and others 1985, 1991; Pelegri and others 1994; Gilbert and others 1998; Webb and Eyre 2004; Laverock and others 2011). In sediments with an oxic layer and low water column nutrient concentrations, nitrification and D_N are often coupled (Sloth and others 1995; Seitzinger and others 2006). The distinct oxygen conditions these processes require (that is, presence of oxygen for nitrification and anoxia for D_N) mean that the interface between the oxic and anoxic sediments is an important site for coupled D_N. The activities of benthic macrofauna cause this interface to be dynamic in space and time (Volkenborn and others 2010, 2012), enhancing coupled D_N (Stief 2013). Moreover, bioturbation can also enhance un-coupled D_N by increasing the supply of nitrate to sediments from the water column (Kristensen and others 1991; Nogaro and Burgin 2014). However, if macrofauna are negatively affected by nutrients and/or other stressors, their positive influence on D_N will be diminished.

Degradation of biodiversity through loss of species can reduce an ecosystem's ability to withstand stress or adapt to changing conditions (Villnäs and others 2013). Species loss can be deleterious to key ecosystem processes contributing to feedback loops that invoke changes in community and overall ecosystem function (Thrush and others 2006, 2014). Given the complex interaction between bioturbating macrofauna and nitrogen cycling and that species with traits relevant to nutrient processing will vary in their sensitivity to stress (that is, response diversity) (Elmqvist and others 2003; Hewitt and others 2010; Mori and others 2013; de Juan and others 2014), nonlinear responses to losses in biodiversity and ultimately resilience are likely (Naeem and others 1994; Chapin and others 2000). Identification of the elements of macrofaunal diversity that contribute to D_N is necessary to understand the potential ecosystem response to nutrient oversupply and to adequately conserve the necessary aspects of biodiversity. These elements include both local- (alpha), and landscape-scale (gamma) diversity that contribute to the overall heterogeneity of communities (beta diversity), which can provide a measure of ecosystem stability (Doak and others 1998; Thrush and others 2008). As diversity and density of marcofauna decrease, D_N rates are also likely to decrease, which may in turn further intensify eutrophication impacts, creating a strong feedback (Loreau and others 2001; Folke and others 2004; Hewitt and others 2010; Hewitt and Thrush 2010).

Nitrogen loading to coastal ecosystems is increasing globally (Galloway and others 2008), and there is a pressing need to understand how it alters D_N and interactions with macrofaunal diversity in real-world settings. Although field studies have quantified D_N in a range of coastal habitats (for example, Piehler and Smyth 2011; Eyre and others 2013; Foster and Fulweiler 2014) they do not make linkages to macrofauna diversity or the diversity response to nutrient stress, and the consequences for D_N are absent. Similarly, despite a considerable amount of research examining aquatic sediment nitrogen cycling (reviewed by Huettel and others 2014), and much highlighting the importance of macrofauna [reviewed by Stief (2013)], studies have so far not been able to address potential feedbacks between biodiversity and stressors. To date, nutrient enrichment field experiments have tested the responses of macrofaunal communities (Morris and Keough 2003; Posey and others 2006; Fitch and Crowe 2012), whereas others have measured effects on ecosystem functions including D_N (Koop-Jakobsen and Giblin 2010; Oakes and others 2011; Vieillard and Fulweiler 2012), but no study has combined the two and assessed the role of macrofauna in D_N response to nutrient enrichment.

We simulated eutrophication in situ using sediment nutrient enrichment in experimental plots

across a sandflat with a heterogeneous landscape of macrofaunal community abundance and diversity. The study focused on two species of shellfish (*Austrovenus stutchburyi* and *Macomona liliana*) recognised as key species for nutrient processing (Thrush and others 2006; Sandwell and others 2009; Jones and others 2011; Pratt and others 2013; Thrush and others 2014), as well as 46 other species with traits important for nutrient processing (Greenfield and others 2016). We used denitrification enzyme activity (DEA) assays to provide an index of nutrient processing and nitrogen removal; a proven method for comparisons of denitrification activity in aquatic systems that permits large sample sizes (Barnes and Owens 1998; Livingstone and others 2000; Bernot and others 2003; Wall and others 2005; Teixeira and others 2010; Bruesewitz and others 2011; Jones and others 2011). We expected treatments that caused substantive increases in pore water ammonium (NH_4^+) concentrations would be detrimental to the diversity of nutrient processing macrofauna (Pearson and Rosenberg 1978; Gray and others 2002), leading to reductions in DEA. Alternatively, increased pore water NH_4^+ concentrations could enhance DEA via coupled D_N provided surface sediment remained oxygenated by macrofauna and/or in permeable sediments by advective pore water flushing due to physical processes (Huettel and others 2014).

Methods

Experimental Design

Twenty-eight sites across a 300,000 m^2 intertidal sandflat in the Kaipara Harbour were selected based on a macrofauna community survey at the study site (Kraan and others 2015) and an analysis of species functional traits that characterise life history, morphology and behaviours that influence sediment biogeochemistry and stability (Greenfield and others 2016). From Greenfield and others, we identified a functional group of 46 species that possessed traits that influence sediment biogeochemistry (for example, deposit feeding, free mobility and burrow building) and therefore are important for nutrient processing. The selected sites encompassed a spectrum of abundance and species richness of this functional group as well as sediment properties (Table 1) to maximise the variation in nutrient processing capacity. The experiment ran for 7 weeks and at each site, 1 procedural control and 2 nutrient enrichment treatment plots (1 × 1 m) were established in a 5 × 5 m area by adding slow release fertiliser (or pea gravel for controls) buried in the sediments. Fertiliser [Nutricote® N (70 days, 40-0-0 N:P:K)] was applied to each plot in a series of 20 evenly spaced 3-cm-diameter 15-cm-deep holes made in the sediment using a hand-held corer. Each hole received an equal volume of fertiliser (or pea gravel) and the intact sediment core plugs were replaced immediately to minimise disturbance to the sediment (see Douglas and others 2016 for details). We considered the control plots to be representative of ambient sediments because less than 2% of the plot area was impacted and previously; with a similar level of disturbance, we found no procedural effects on intertidal macrofaunal community composition, benthic respiration, nutrient fluxes and primary production when sampled after 4–7 days (Gladstone-Gallagher and others 2014, 2016). Moreover, photographs of plots taken four and 7 weeks after disturbance indicated no trace of coring, even in plots containing seagrass. Application rates (medium 150 g N m^{-2}, high 600 g N m^{-2}) were based on a literature review of previous enrichment experiments and resulted in significantly elevated pore water NH_4^+ concentrations for at least 7 weeks in surface (0–2 cm) and deeper (5–7 cm) sediments (Table 1; Douglas and others 2016).

Sample Collection and Analyses

All sampling was conducted on March 17, 2014, 7 weeks after fertiliser enrichment. For DEA analyses, five sediment cores (5 cm depth, 5.3 cm dia.) were collected from each plot, pooled, transported on ice to the laboratory, kept at 4°C and analysed within 48 h of collection. Prior to conducting assays, samples were brought to room temperature (20°C). DEA assays were used as an index of D_N to give a relative measure of sediment nutrient processing and nitrogen removal capacity. DEA assays were conducted using the chloramphenicol-amended acetylene inhibition technique (Tiedje and others 1989; Groffman and others 1999; Bruesewitz and others 2006; Groffman and others 2006). This method does not measure actual denitrification rates since acetylene inhibits nitrification; however, it measures the activity of the resident denitrifier population under optimal conditions (total anoxia, constant mixing, unlimited nitrate and organic carbon) but without allowing new enzyme growth.

Assays were conducted in glass jars (440 mL volume) with lids fitted with *n*-butyl rubber septa. Homogenised wet sediment samples (60 mL) were placed into jars with 54 mL unfiltered seawater

Table 1. Sediment Properties and Macrofaunal Variables in Different Treatments

Variable	Control (0 g N m^{-2})	Medium (150 g N m^{-2})	High (600 g N m^{-2})
Sediment properties			
Seagrass (% cover)	16 (0–84)	20 (0–97)	21 (0–75)
OC (%)	0.9 (0.6–2.0)	0.9 (0.6–2.0)	1.0 (0.6–1.8)
Mud (% < 63 μm)	1.78 (0–15)	0.62 (0–14)	0.42 (0–12)
GSM (μm)	215 (177–241)	220 (182–242)	219 (190–250)
Microphytobenthic biomass (μg g^{-1} sediment)			
Chl *a*	9.3 (3–23)	10.0 (5–32)	9.5 (5–28)
Phaeophytin	4.4 (1.5–18)	6.4 (1.6–22)	4.0 (1.1–19)
Pore water NH_4^+ (μM)			
Surface sediments (0–2 cm)	24 (0–198)	253 (0–2210)	1849 (111–10,239)
Deeper sediments (5–7 cm)	74 (15–484)	1209 (99–10,275)	5846 (565–18,842)
Macrofauna (n $core^{-1}$)			
S (taxa)	10 (6–16)	10 (4–15)	8 (3–16)
N (individuals)	60 (15–376)	39 (12–519)	32 (7–301)
A. stutchburyi (<10 mm)	6 (0–91)	2 (0–99)	2 (0–64)
A. stutchburyi (≥10 mm)	1 (0–22)	1 (0–14)	1 (0–21)
M. liliana (<10 mm)	5 (1–25)	4 (0–14)	2 (0–9)
M. liliana (≥10 mm)	2 (0–4)	1 (0–3)	1 (0–6)

Values are medians with minimum and maximum in parentheses (n = 28).
OC = sediment organic content; Mud = sediment mud content; GSM = grain size median; Chl a = chlorophyll a content; S = number functional group species; N = number of functional group individuals.

from the site. Chloramphenicol was added to prevent new enzyme synthesis at a final concentration of 0.06 g L^{-1}. Assays were amended with unlimited carbon (30 mg L^{-1} C as glucose) and nitrate (10 mg L^{-1} N as KNO_3). Anaerobic conditions were obtained by sealing the jars, evacuating with a vacuum pump for 4 min, then purging with pure N_2 gas for 10 min. Pure acetylene was added as 10% of the headspace volume to prevent the conversion of N_2O to N_2. Assay jars were placed on shakers at 125 rpm and incubated at 20°C for 2 h. Headspace gas samples (6 mL) were collected at 10, 30, 60 and 120 min after the addition of acetylene. Gas samples were analysed using a Varian CP 3800 gas chromatograph equipped with a HayeSep D column and an electron capture detector.

Sediment dry weight (DW) in each assay jar was determined (after 48 h at 60°C) and N_2O production rates (μg g DW^{-1} h^{-1}) calculated from the linear increase in concentration over time ($r^2 > 0.8$). DEA was expressed per unit area of sandflat (μmol N m^{-2} h^{-1}) by multiplying the production rate by the sediment density (g DW cm^{-3}, determined by drying a known volume of the assay sediment) and sample depth (5 cm). Our analysis had a minimum DEA detection limit of 1 μmol N m^{-2} h^{-1} and in preliminary testing replicate subsamples ($n = 5$) from homogenised sediment had a coefficient of variation (mean/SD) of 7%, whereas the coefficient of variation between five replicate 1 m^2 plots in a 25 m^2 area at five sites was between 10 and 15%.

Environmental variables were characterised as follows. Seagrass (*Zostera muelleri* Irmisch ex. Asch.) coverage (%) was estimated using photographs (taken before sampling) of the central 0.25 m^2 of each plot and a random point count method (see Kohler and Gill 2006). Sediment cores from each plot were collected for analysis of pore water NH_4^+ ($n = 4$, 2.6 cm dia., 0–2 and 5–7 cm depths, separated and depth sections pooled), sediment organic content, mud content (% < 63 μm), grain size median, chlorophyll *a*, phaeophytin ($n = 5$, 2.6 cm dia., pooled, 0–2 cm depth) and macrofauna community composition ($n = 2$, 13 cm dia. pooled, 15 cm depth). Laboratory protocols are described in detail elsewhere (Douglas and others 2016), but briefly pore water was extracted by centrifugation, filtered (1.1 μm Whatman GC glass fibre filter), frozen (−20°C) and then analysed for NH_4^+ concentration using a Lachat QuickChem 8000 Series FIA+ (Lohrer and others 2010), sediment grain size was analysed with a Malvern Mastersizer 2000 after removal of organic matter (Singer and others 1988), sediment organic content was determined by loss on ignition (550°C, 4 h) (Parker 1983) and microphytobenthic biomass was determined by extraction of pigments from freeze dried sediment (90% acetone) and measuring fluorescence using a Turner Designs 10-AU flourometer (Arar and Col-

lins 1997). Macrofaunal cores were sieved (500 µm mesh), preserved (50% iso-propyl alcohol) and stained (Rose Bengal), and then, all organisms were counted and identified (usually to species level).

Statistical Analysis

A permutational multivariate analysis of variance (PERMANOVA, using a Euclidean distance matrix) was used to test for significant treatment effects on environmental variables (seagrass cover, sediment properties and microphytobenthic biomass). Due to the experimental design (that is, the spatial scale and selection of sites to maximise macrofauna diversity), there was, as expected, high intersite variability in DEA, macrofauna and environmental variables (Figure 1; Table 1). To compensate this natural heterogeneity and reveal potential treatment effects, we normalised site specific treatment response parameters by the corresponding control plot values so effect size was relative to the site specific background level. Normalisation assumes control plot values are representative of a site, a justifiable assumption given the small interplot distances (2 m) and strong positive correlations between control and treatment plot sediment properties and primary producer biomass/coverage (Pearson's $r > 0.75$, $P < 0.001$; raw data in Online Appendix 5). Treatment response variables (DEA and macrofauna community measures) were also correlated (Online Appendix 1). Control normalised (CN) DEA and community values were tested for differences from control values (that is, $DEA_{CN} \neq 1$; one-sample *t*-tests) and between fertiliser addition treatments (medium vs. high; two-sample *t*-tests) using Statistica 11 (StatSoft Inc 2012).

Distance-based Linear Models (DistLM) were used to identify significant individual predictors (marginal tests) and then the best combination of predictor variables (backwards elimination procedure) of DEA_{CN} at different levels of nutrient enrichment. Predictor variables included environmental variables and univariate measures of macrofaunal community composition. We used the corrected Akaike information criterion (AIC_c) which is the most appropriate selection criterion when the number of variables is large compared to the sample size (Burnham and Anderson 2002). Predictor variables were normalised (between −2 and 2) to enable comparison among variables with different units without altering the distribution. Where there was co-linearity among variables ($r > 0.7$), the variable explaining the lesser amount of variability was excluded from full models (Dormann and others 2013). Variance partitioning analysis (Borcard and others 1992; Anderson and Cribble 1998) was used to determine how much of the model variance was attributed to grouped predictor variables, sediment pore water NH_4^+ concentration [surface (0–2 cm) and deep (5–7 cm)], environmental variables [seagrass cover, sediment organic content (OC), median grain size (GSM), sediment mud content (% < 63 µm; mud), chlorophyll *a* (chl *a*), phaeophytin, distance from shore] and macrofaunal community variables (see below). All multivariate analyses were conducted using PRIMER 7.0 with PERMANOVA+ add-on (Clarke and Gorley 2015) with untransformed data.

For measures of macrofaunal community composition, we just considered the 46 species identified by Greenfield and others (2016) with traits important for nutrient processing. On average this functional group comprised 52% of the taxa and 63% of the abundance, and preliminary analyses indicated that this group had greater effects on DEA than the macrofaunal community considered as a whole. We included in analyses the number of species (*S*) and individuals (*N*) belonging to this functional group, and the abundances of juvenile (<10 mm) and adult (≥10 mm) *A. stutchburyi* and *M. liliana*. *Austrovenus stutchburyi* and *M. liliana* were included as separate predictors as both species have been shown to strongly influence ecosystem functioning (that is, are key species) on New Zealand sandflats (Thrush and others 2006; Sandwell and others 2009; Jones and others 2011; Pratt and others 2013; Thrush and others 2014) and we separated adults and juveniles because impacts on ecosystem differ with size (Hewitt and others 1997; Norkko and others 2013).

Results

Nutrient Enrichment Effect on DEA

Nutrient treatment (150 and 600 g N m^{-2}) significantly increased pore water NH_4^+ concentrations throughout the sediment profile (Douglas and others 2016), but had no significant effects on sediment properties, seagrass cover or microphytobenthic biomass (Table 1; all PERMANOVA pseudo-$F = 0.77$, $P > 0.5$, not shown). There was substantial variability in DEA values in all treatments across the study site, with control plot values ranging from 7.6 to 183.2 µmol N m^{-2} h^{-1} (Figure 1A). The site specific DEA response to enrichment (DEA_{CN}) ranged from 0.12 to 2.0 in medium

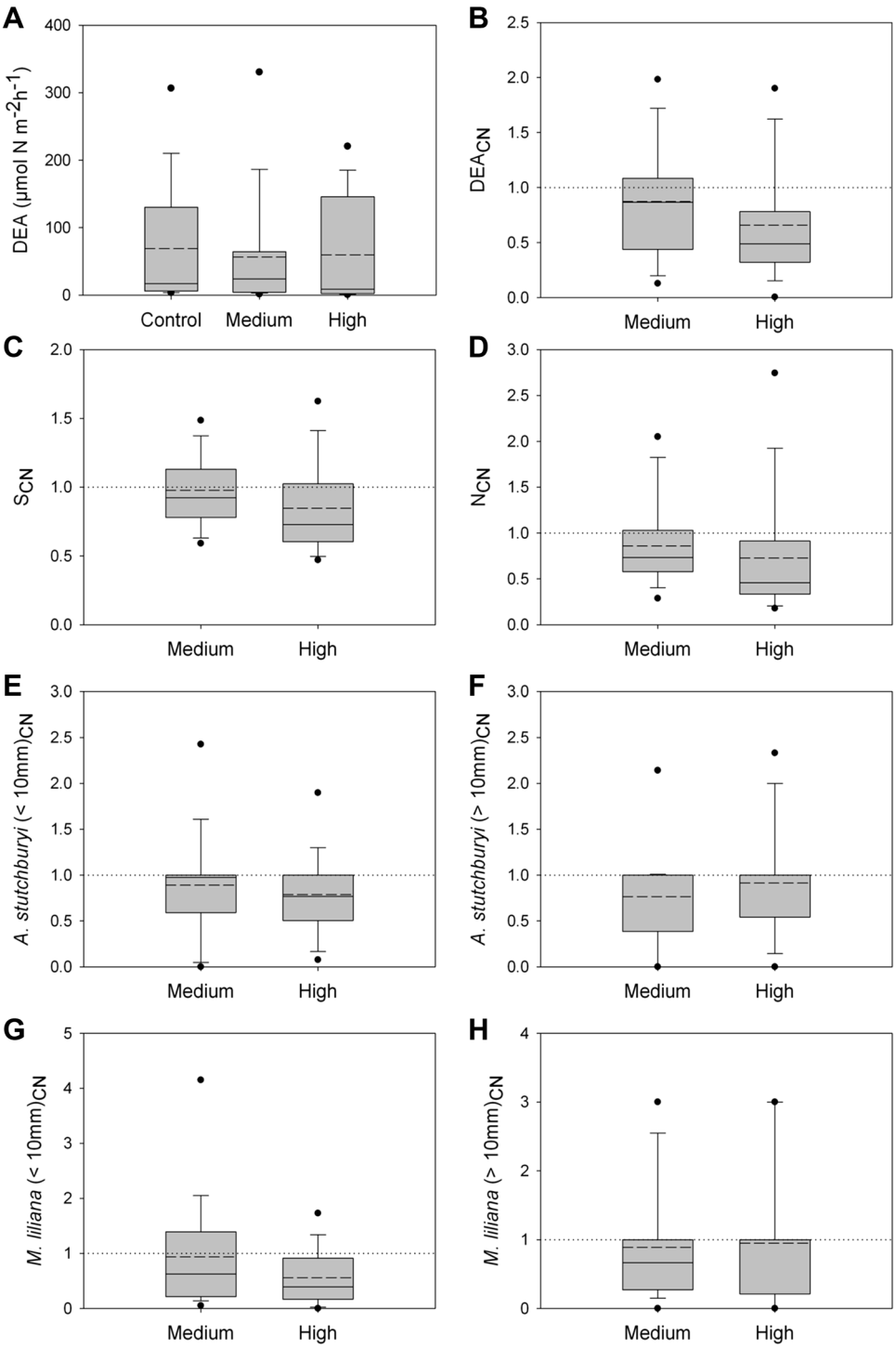

Figure 1. Effect of nutrient enrichment treatment on **A** DEA, and control normalised, **B** DEA (DEA_{CN}), **C** macrofaunal functional group diversity (S_{CN}), **D** macrofaunal functional group abundance (N_{CN}), **E** juvenile (<10 mm) and **F** adult (≥10 mm) *A. stutchburyi*$_{CN}$ abundance, and **G** juvenile (<10 mm) and **H** adult (≥10 mm) *M. liliana*$_{CN}$ abundance. *Boxes* are 25th and 75th percentiles, whiskers show 10th and 90th percentiles, *black dots* show 5th and 95th percentiles. *Solid line* is median, *dashed line* is mean, and in the normalised plots, the *dotted line* is provided for reference to the control value.

treatment plots (that is, 12–200% of control values), and 0.001 to 1.9 in high treatment plots (that is, 0.1 and 190% of control values). In the medium treatment 18 of 28 sites, and in the high treatment 21 of 28 sites, DEA values were less than in controls (that is, $DEA_{CN} < 1$), indicating that DEA was, on average, suppressed by enrichment (Figure 1B). In approximately 25% of treatment plots, enrichment enhanced DEA by greater than 20%. Reductions in DEA_{CN} were only significant in the high treatment; however, reductions were greater in the high compared with the medium treatment (although only marginally significant; Figure 1B; Table 2).

Table 2. Treatment Effects on Control Normalised (CN) DEA and Macrofaunal Community Measures

Treatment		Difference from control		Difference between treatment means	
Variable	Mean	*t*	*P*	*t*	*P*
DEA_{CN}					
Medium	0.87	−0.13	0.20	***1.86***	***0.07***
High	**0.66**	**−3.41**	**0.002**		
S_{CN}					
Medium	0.98	−0.45	0.66	**2.85**	**0.008**
High	**0.85**	**−2.50**	**0.02**		
N_{CN}					
Medium	0.86	−1.60	0.12	1.44	0.16
High	**0.73**	**−2.05**	**0.05**		
A. stutchburyi (<10 mm)$_{CN}$					
Medium	0.89	−1.01	0.32	1.26	0.22
High	**0.79**	**−2.54**	**0.02**		
A. stutchburyi (≥10 mm)$_{CN}$					
Medium	**0.77**	**−2.13**	**0.04**	−0.92	0.37
High	0.91	−0.77	0.45		
M. liliana (<10 mm)$_{CN}$					
Medium	0.94	−0.30	0.76	***1.94***	***0.06***
High	**0.56**	**−4.54**	**0.0001**		
M. liliana (≥10 mm)$_{CN}$					
Medium	0.89	−0.72	0.48	−0.26	0.80
High	0.95	−0.30	0.77		

Test results for differences between treatments and controls (one-sample t test), and between medium and high treatment (two-sample t-test).
Control normalised DEA_{CN} = Denitrification Enzyme Activity; S_{CN} = number functional group species; N_{CN} = number of functional group individuals.
Significant differences ($P \leq 0.05$) are indicated in bold and marginal significant differences ($P \leq 0.1$) in bold italics.

Predictors of DEA

DEA was significantly correlated with a number of environmental variables (Online Appendices 2, 3, 4). In general, sites with higher control plot DEA were those with more sediment OC and mud, smaller median grain size, more seagrass coverage and more phaeophytin biomass. Control plot DEA was significantly correlated with DEA in both treatment plots (Online Appendix 1); that is, sites with naturally high DEA were also high following enrichment. Normalisation of medium and high treatment DEA by control values effectively removes spatial environmental influences, and consequently, these variables (and control plot DEA) did not explain a substantial proportion of DEA_{CN} (Table 3, Online Appendices 3, 4). The predictors included in the full models of DEA_{CN} differed depending on the level of enrichment (Table 3; Figure 2). In the medium treatment, surface sediment pore water NH_4^+ concentration had a positive effect on DEA_{CN}, but community variables explained more of the response. Key bioturbators showed a strong influence on medium treatment DEA_{CN}; together, juvenile and adult *M. liliana* and adult *A. stutchburyi* made up 32% of the total 54% explained variance. The effects of these two species on DEA_{CN} were different, *M. liliana* positive and *A. stutchburyi* negative (Table 3). Unlike the medium treatment, pore water NH_4^+ concentration was not an important predictor of DEA_{CN} in the high treatment; only community variables were included in the full model explaining 39% of the variance, and key species did not have a significant role (Table 3; Figure 2B). Most (37%) of the explained variance was attributed to the abundance of nutrient processing species (*N*) which was positively correlated with DEA_{CN}. The amount of unexplained variance in DEA_{CN} increased with the level of nutrient enrichment from 46 to 61%.

Nutrient Enrichment Effect on the Macrofaunal Community

Analysis of control normalised measures of the nutrient processing functional group composition revealed significant treatment effects (Table 2; Figure 1C–H). The number of species (S_{CN}) was

Table 3. DistLM Results for Treatment Plot Control Normalised DEA (DEA_{CN})

Treatment	Group	Variable	Pseudo-*F*	Prop.	Full model (%)
Medium	Pore water	NH_4^+ (0–2 cm)	7.16	0.21** (+)	19
	Community	*M. liliana* (<10 mm)	5.19	0.16** (+)	32
		M. liliana (≥10 mm)	2.56	0.09 n.s. (+)	
		A. stutchburyi (≥10 mm)	3.09	0.11* (–)	
				Total	54
High	Environment	Mud	3.68	0.12** (+)	–
		Phaeophytin	2.81	0.10* (+)	–
	Community	*S*	5.98	0.19*** (+)	–
		N	10.98	0.30** (+)	37
		M. liliana (≥10 mm)	0.50	0.02 n.s. (–)	9
				Total	39

Prop. is the proportion of variability in DEA_{CN} explained by each variable when considered individually. Full model shows the variables included in the best DistLM of DEA_{CN} and the variance attributed to each.
NH_4^+ (0–2 cm) = surface sediment pore water ammonium concentration (μM); Mud = sediment mud content (%); phaeophytin (μg g^{-1} sediment); S = number functional group species; N = number of functional group individuals
*Significance levels are *P ≤ 0.1, **P ≤ 0.05, ***P ≤ 0.01, and correlation directions are in parentheses.*

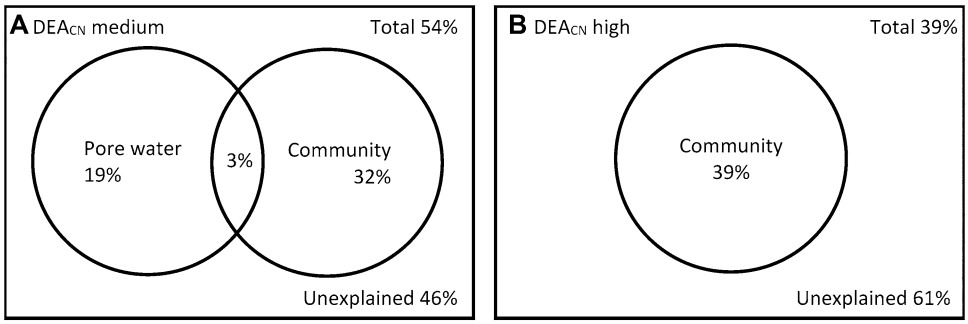

Figure 2. Diagrams presenting partitioning of variance in DEA_{CN} in **A** medium and **B** high treatment attributed to unique and shared effects of measures of community and pore water ammonium concentration (realised treatment effect). Results from variance partitioning analysis of full DistLM as described in Table 3.

lower in the high than control and medium treatments, and there were reductions in the total abundance (N_{CN}), but this was only significant in the high treatment. The abundance of key bioturbating species were also negatively impacted with nutrient enrichment. Adult and juvenile *A. stutchburyi* densities were reduced in the medium and high treatments, respectively. For *M. liliana*, only juveniles (which were numerically dominant) were affected, only in the high treatment (Table 2).

Discussion

We examined the role of macrofauna diversity in moderating nutrient oversupply using an indirect measure of nutrient processing capacity (DEA) across 28 sites with substantial natural variability in the community composition of nutrient processors. DEA was spatially highly variable which was expected given the heterogeneity of the sandflat and sites with naturally high DEA were also high following nutrient enrichment. By normalising treatment plot DEA by control values, we revealed the response to nutrient addition and demonstrate in a real-world setting that benthic macrofaunal diversity is important to the preservation of denitrification (D_N) following nutrient stress. This is significant because D_N is a process that can mitigate eutrophication, and nutrient enrichment commonly has negative effects on benthic macrofauna (Pearson and Rosenberg 1978).

Fertiliser addition on average suppressed DEA (that is, $DEA_{CN} < 1$) especially in the high treatment, and we assume this suppression was due to inhibition of nitrification (although we did not measure this process directly). Most of the D_N in this system is likely to be coupled to nitrification because control plot DEA strongly correlates with

sediment organic content (suggesting organic matter mineralisation is the primary source of *N*; Online Appendix 2) (Sloth and others 1995; Seitzinger and others 2006), and New Zealand estuaries typically have low pore water and water column nitrate concentrations (Lohrer and others 2004; Thrush and others 2006; Lohrer and others 2010). Nitrification inhibition would occur if the enriched sediments became periodically anoxic or the oxic layer depth decreased (preventing or reducing nitrification of NH_4^+ even when present in great quantity) (Joye and Hollibaugh 1995; Magalhães and others 2005; Foster and Fulweiler 2014). Shifts towards anaerobic conditions may have been caused by the NH_4^+-induced reduction in the abundance of bioturbating species (Table 2; Figure 1C–H) which would reduce oxygenation of the sediments (Diaz and Rosenberg 1995, 2008; Glud 2008) and further exacerbated by dead macrofauna stimulating microbial metabolism during decay (Kelly and Nixon 1984; Blackburn and others 1993). But note there was no detectable enrichment of sediment organic content in treatment plots that could be related to macrofauna mortality (Table 1).

Although enrichment suppressed DEA_{CN} at most sites, the response represented a continuum from inhibition to enhancement. DistLM showed that 39–54% of response to enrichment could be explained, most of it by macrofaunal diversity. It is difficult to speculate on the source(s) of the unexplained variation in DEA_{CN}, but on a dynamic intertidal sandflat spatial and temporal variations in sediment biogeochemistry caused by hydrodynamic forcing (Green and Coco 2014; Huettel and others 2014), foraging and excretion by large predators (for example, Thrush and others 1994; Hines and others 1997; Jauffrais and others 2015), detrital inputs (for example, Eyre and Ferguson 2002; Eyre and others 2013) and microbial diversity (for example, Yazdani Foshtomi and others 2015) could all contribute, as could any initial small-scale variation between plots within a site. Nevertheless, the fact that a substantial proportion of the DEA response could be explained by macrofauna diversity despite the complexity of the field setting emphasises its importance in regulating the effects of enrichment.

When NH_4^+ was supplied in the medium treatment, the density of *M. liliana* was critical in mediating the response of DEA. Both the concentration of surface sediment pore water NH_4^+ and abundances of *M. liliana* were significantly positively correlated with DEA_{CN}. This agrees with our expectation that factors that promote the coupling of nitrification and D_N (that is, bioturbation-induced increases in sediment oxygenation and solute transport) would lessen the negative effect of enrichment on DEA (that is, DEA_{CN} declines from 1 would be less). *Macomona liliana* is a surface deposit feeding bivalve known to influence sedimentary oxygen and nitrogen fluxes (Thrush and others 2006; Volkenborn and others 2012; Pratt and others 2015). The feeding and burrowing behaviour of this species injects pulses of oxygen-rich water into sediments as well as creating hydrostatic pressure gradients in the sediment profile. This increases the oxic–anoxic interface (both spatially and temporally), accelerates solute exchange and forces nutrient-rich anoxic water shallower in the sediment profile (and into the oxic nitrification zone) (Volkenborn and others 2012). Others have shown that under well-flushed conditions (that is, via bioturbation and/or in permeable sediments advective pore water flushing) nitrification is positively correlated with NH_4^+ concentrations (Caffrey and others 2003; Huettel and others 2014); in this case, bioturbation by *M. liliana* appears to be the flushing mechanism.

Adult *M. liliana* (≥10 mm) live deep in the anoxic zone of the sediments (about 10 cm depth) (Hewitt and others 1997) and therefore are likely to have a strong positive influence on coupled D_N. In our study, adult *M. liliana* did not show significant individual effects on DEA_{CN}; this is unsurprising given that they were in low densities, and sampling two 0.13 m^2 area cores per plot unlikely gives an accurate representation of the resident individuals. Despite this, adult *M. liliana* still featured in models explaining variance in DEA_{CN} in both treatments, suggesting an influence on the activity of the resident denitrifier population. Our grouping of juvenile *M. liliana* included all those less than 10 mm, encompassing young juveniles (≤ 5 mm) that occupy surface sediments (<2 cm depth, within typical oxic zones) and larger juveniles (5–10 mm) that occupy sediments between 2 and 10 cm depth (Hewitt and others 1997), below the typical oxic depth of these types of sediments. Juveniles (<10 mm) showed a strong positive effect on medium treatment DEA_{CN} and despite being shallower dwelling than adults, their activities are likely to increase oxic zones and the transport of nutrient-rich pore water (relative to un-bioturbated sediments) also facilitating coupled D_N.

Negative ecosystem effects increased with increased nutrient enrichment (that is, from medium to high); in particular loss of key species and decreases in DEA performance. The high nutrient treatment reduced the abundance of juvenile *M.*

liliana and subsequently the positive influence on DEA_{CN} seen in the medium treatment was gone. With reduced abundance of this key species under high nutrient stress, the fundamental role in explaining DEA_{CN} (and supporting coupled D_N) was taken up by the remaining community of nutrient processing macrofauna. Both the diversity (S) and abundance (N) of the functional group were significantly positively correlated with DEA_{CN}, indicating that both are important for maintaining coupled D_N (and therefore nitrogen removal) under high nutrient stress (albeit at reduced efficiency). It is possible that pore water NH_4^+ concentrations, particularly in high treatments, reached a threshold where nitrification was either saturated or suppressed (Anthonisen and others 1976; Henriksen and Kemp 1988). Maintenance of nutrient processing from bioturbation is important for resistance to negative feedbacks that cause nitrification inhibition. Our study has shown that different elements of biodiversity, especially functional group species abundance and diversity, and key species size and abundance, are important for ecosystem functioning under increasing nutrient stress. Nutrient stress caused reduced diversity of nutrient processors which may lead to reductions in ecosystem resilience to nutrient enrichment. Such effects may be further exacerbated by multiple stressor effects associated with habitat loss, pollution and fisheries exploitation (Rothschild and others 1994; Thrush and Dayton 2002; Lohrer and others 2004; Solan and others 2004).

Land-use intensification and terrestrial nutrient loading to the marine environment will continue to increase therefore maintenance of soft sediment nutrient processing will be paramount for coastal ecosystem resilience to eutrophication. This in situ study has demonstrated that under nutrient stressed conditions, key species, and then functional group abundance and diversity govern an essential nitrogen removal process that may ultimately mitigate shifts towards eutrophication. Furthermore, our results provide an example of how community response diversity contributes to ecosystem resilience to nutrient enrichment stress (Elmqvist and others 2003; Mori and others 2013). Increasing stress to soft sediment ecosystems can cause loss of bioturbators, decoupling of processes and changes in ecosystem functioning (Lohrer and others 2011; Pratt and others 2013). This is a concern for sediment nitrogen removal given the demonstrated dependence of soft sediment ecosystem processes on macrobenthic communities. Although both the medium and high levels of nutrient stress led to reductions in nutrient processing, the effects were greater with the higher level of stress, due to the reduced abundance of a key species and decoupling of processes that occurred in this treatment type. This supports the notion that losses of large or functional species that play pivotal roles in ecosystem processes leads to loss of ecosystem resilience (Thrush and others 2006; Norkko and others 2013), with implications for future management of coastal ecosystems. If stress thresholds are crossed, causing reductions in key nutrient processing species and functional diversity, there may be long-term effects on ecosystem resilience to eutrophication. This could contribute to tipping points and major regime shifts in coastal ecosystems (Thrush and others 2014).

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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