Estuaries and Coasts (2018) 41:1994–2008
https://doi.org/10.1007/s12237-018-0416-5

Sedimentary Environment Influences Ecosystem Response to Nutrient Enrichment

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Received: 7 August 2017 / Revised: 9 May 2018 / Accepted: 11 May 2018 / Published online: 1 June 2018

Abstract

As coastal catchment land use intensifies, estuaries receive increased nutrient and sediment loads, resulting in habitats that are dominated by muddy organic-rich sediments. Increased mud (i.e. silt-clay (particles < 63 μm)) content has been associated with negative effects on soft sediment biodiversity and ecosystem functioning, but the simultaneous impact of nutrient enrichment on ecosystem response is unclear. Nutrient recycling and denitrification in estuarine soft sediments represent important ecosystem functions regenerating nutrients for primary producers and regulating the ability to remove excess terrestrially derived nitrogen. To test the effect of sedimentary environment on ecosystem resilience to nutrient perturbation, we experimentally enriched sediments with slow release fertiliser across an intertidal sedimentary gradient (0–24% mud content). The enrichment successfully elevated pore water ammonium concentrations (median 36 × control) to levels representative of enriched estuaries. Findings show that the sedimentary environment can influence ecosystem function response to nutrient stress. In particular, denitrification enzyme activity was suppressed by nutrient enrichment, but the effect was greater as sediment mud content increased. Furthermore, compared with sandy sediments, sediments with high mud content may restrict nutrient processing (release, uptake or transformation of organic nutrients by the benthos) facilitating ecosystem shifts toward eutrophication. These results show the value of investigating the impacts of stressors in different environmental settings and demonstrate that land use practices that increase the proportion of muddy habitats in estuaries may reduce denitrification which in turn may reduce ecosystem resilience to eutrophication.

Keywords Estuary · Denitrification enzyme activity · Benthic fluxes · Eutrophication · Sedimentation · Nitrogen

Introduction

Nutrient enrichment and sedimentation are among the primary stressors for coastal ecosystems globally (Levin et al. 2001). Estuarine soft sediment ecosystems are often described as nitrogen sinks due to their high rates of nitrogen processing and ability to naturally reduce bio-available nitrogen via denitrification (Seitzinger 1988). Denitrification may play a fundamental role in ecosystem resilience to the oversupply of nitrogen, but its ability to do this may be influenced by the sedimentary environment and potential changes to it. Nitrogen enrichment and sedimentation often occur in unison during periods of elevated rain runoff, and while it is clear that the 'muddying' (i.e. increases in fine particles < 63 μm) of estuaries can negatively affect macrofaunal diversity and ecosystem functions (Pratt et al. 2013; Thrush et al. 2003a, b; Anderson 2008), it is not known what this means when compounded with other stressors, particularly increased nutrients. The influence of sedimentary environment on the effects of nutrient stress in coastal ecosystems has rarely been investigated in the field (see O'Brien et al. 2009 for an exception) but such research is needed to better inform management with respect to setting catchment nutrient limits, for example (Hewitt et al. 2016; Chapman 2016).

Communicated by Marco Bartoli

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Ecosystem functions and benthic macrofaunal communities can be affected by increases in mud content (Rodil et al. 2011; Lohrer et al. 2004; Thrush et al. 2003a; Robertson et al. 2015) or nutrient enrichment in soft sediment habitats (Morris and Keough 2003; Fitch and Crowe 2012; Posey et al. 2006). The physical and biogeochemical properties of estuary sediments change with increasing mud content (Lohrer et al. 2004; Cummings et al. 2009) stemming from greater cohesiveness and less permeability. These sediment characteristics influence rates of pore water diffusion and solute exchange (Blackburn and Henriksen 1983; Huettel et al. 2003), ammonium (NH_4^+) adsorption (Mackin and Aller 1984), light penetration (Paterson et al. 1998; Yallop et al. 1994), the surface area available for microbial processes (Huettel et al. 2014) and can alter the activities and functional roles of resident macrofauna (Needham et al. 2011; Jones et al. 2011a). These factors all influence processes of organic matter breakdown, community metabolism, primary production and nitrogen cycling, including denitrification (Blackburn et al. 1993; Gilbert et al. 2003; Gongol and Savage 2016). Denitrification is a key process of nitrogen removal in coastal sediments and is therefore important for ecosystem resilience to nutrient enrichment. In New Zealand estuaries, and in other systems with generally low water column nitrate concentrations, the majority of denitrification is coupled to nitrification in the sediments (Gongol and Savage 2016; Seitzinger et al. 2006).

Field experiments across existing environmental gradients can be used to forecast ecosystem response or change in different environmental scenarios or stages of degradation by substituting space for time (Pickett 1989; e.g. Pratt et al. 2013; Norkko et al. 2015; Villnäs et al. 2013; Thrush et al. 2003b). This approach has successfully been used to demonstrate effects of sedimentation on ecosystem functioning (e.g. Pratt et al. 2013), but there is an underutilised opportunity to investigate effects of stressors in different environments using natural gradients (Snelgrove et al. 2014). This study investigated whether the sedimentary environment (in this case, the proportion of mud, i.e. silt/clay (particles < 63 μm)) in an estuary sandflat would influence its response to increased nutrient supply (one of the symptoms of eutrophication). This was achieved using a nutrient enrichment experiment across a natural gradient of sediment grain size on an intertidal sandflat, measuring proxies of ecosystem function (denitrification activity, primary production and community metabolism) after a 6-week period of enrichment.

In situ manipulation of pore water nutrients using slow release fertiliser is an established method in experimental soft sediment ecology (reviewed in Douglas et al. 2016), and previous work has verified its use for evaluating ecosystem response to nutrient stress (Douglas et al. 2017; Thrush et al. 2017; Gladstone-Gallagher et al. 2018). During the development of this technique, Douglas et al. (2016) found that mud content was a major controlling factor of the pore water enrichment effect, and it follows that this study investigates the influence of sedimentary environment on ecosystem function response to nutrient enrichment. Based on these previous studies, we used a level of enrichment (150 g N m^{-2}) that we knew would avoid excessive negative impacts to the macrofaunal community (see also Gladstone-Gallagher et al. 2018) and avoid unrealistic responses in ecosystem function. Due to the differences in biogeochemical properties across the sedimentary gradient, changes were expected in microphytobenthic biomass, macrofaunal community structure and ecosystem function, and consequently differences in response to nutrient enrichment. Muddy sediments typically have reduced macrofaunal biodiversity and levels of ecosystem functioning (Pratt et al. 2013; Thrush et al. 2004), and it was anticipated that this would mean less resilience (measured as maintenance of ecosystem function) to nutrient enrichment.

Methods

Experimental Design/Setup

An in situ nutrient enrichment experiment was set up in the Tuapiro estuary, Tauranga Harbour, north-eastern New Zealand (37° 29.445′S, 175° 57.007′E) in late October 2014 (austral spring). The study site encompassed a sedimentary gradient (0–24% mud content) within a 300 × 100-m area of mid intertidal flat. Where mud content values were 0%, silt and clay particles were below the detection limit of the method used. At the site-scale, tidal inundation time, salinity and temperature differences were minimal; however, localised differences in hydrodynamics and organism-sediment relationships (Anderson 2008) were probably responsible for the variation in mud content between plots. Duplicates of procedural control and enrichment plots (1 × 1 m) were set up at 12 locations (48 plots in total) to maximise the range of sediment grain size (specifically, the mud content). Sediment enrichment was achieved using slow release (70 days) nitrogen only fertiliser (Nutricote® N 70 d, 40-0-0 N:P:K, application rate 150 g N m^{-2}) buried in the sediments in 20 evenly spaced core holes (3 cm diameter, 0–15 cm depth). This N loading represented the median application rate from a literature review of nutrient enrichment studies and was chosen because it was known to elevate pore water NH_4^+ (one of the main symptoms of eutrophication) to levels representative of eutrophic estuaries, without having adverse effects on the macrofaunal community (Douglas et al. 2016; Douglas et al. 2017). Equal volumes of fertiliser (or pea gravel for procedural disturbance controls) were placed in each hole (filling the lower third, 10–15 cm), and the upper part of the intact core plug was replaced immediately to minimise sediment profile disturbance (see Douglas et al. 2016 for more detail). With this technique, less than 1.5% of the plot area was disturbed, pore

water enrichment levels varied no more than 1.3–2-fold within the plot and no enrichment was detectable more than 0.5 m outside the plot (Douglas et al. 2016).

Plots were sampled over two consecutive days in late November 2014 (early summer) after 6 weeks of enrichment. This period was based on a previous study that showed elevated pore water NH_4^+ concentrations for at least 7 weeks using this technique (Douglas et al. 2016). Benthic chambers were used to measure fluxes of solutes across the sediment-water interface and estimate community metabolism, primary productivity and nutrient regeneration rates, all commonly used proxies of ecosystem function in soft sediment habitats (Pratt et al. 2013; Rodil et al. 2011; Norkko et al. 2015; Sundback et al. 2000). Denitrification enzyme activity (DEA) assays (Groffman et al. 2006; Seitzinger et al. 1993; Douglas et al. 2017) were used to provide an index of the NO_3^- removal capacity of the resident denitrifier population and the denitrification history of sediments.

In Situ Chamber Incubations/Flux Measurements

On each sampling day (26th and 27th November 2014), incubations were conducted on one control and one enrichment plot in each of the 12 sites across the study area. The benthic chamber method used provides comparative rates of nutrient and water fluxes between plots by quantifying changes in water chemistry during incubation (Lohrer et al. 2010). Four HOBO data loggers (5-min sampling interval) were distributed across the site to monitor light intensity and temperature during incubations. Paired light and dark chambers were used to incubate sediment (0.016 m^2) and overlying water (~ 0.85 L) in the centre of each plot for approximately 3.5–4 h over midday high tides (the approximate mid-point of incubations). The chambers were made of clear Perspex and blackened to prevent light reaching the sediment or left clear allowing photosynthesis by microphytobenthos. Light intensity at the sediment surface inside light chambers was likely to be slightly reduced compared with natural levels but this was consistent across chambers. Chambers were relatively small and not fitted with stirring devices; however, some mixing did occur during sampling when water extracted through one port was replaced by ambient seawater from another. Sixty-millilitre water samples were collected from each chamber (after extracting and discarding approximately 20 mL present in the sampling tube), at the beginning and end of the incubation period. Ambient seawater was incubated in three paired light and dark bottles (1.5 L vol) in different locations across the study area at the same time as the chamber incubations, to account for water column processes. Dissolved oxygen (DO) concentrations were measured in each sample immediately after collection using an optical DO probe (PreSens Fibox PSt3). Duplicate 15-mL water samples were then collected (more than one sample was collected in case analysis re-runs were necessary), after filtering through a 1.1-μm Whatman GF/C filter for nutrient analysis. Samples were frozen at − 20 °C until analysis.

Sediment Sampling

Sediment sampling was conducted after the second set of incubations were completed (27th November 2014), as soon as possible following tidal emersion (within 1 h). Randomly placed cores were taken from each plot (excluding the incubated areas) for analysis of sediment pore water (5 × 0–2 cm depth, 2.6 cm dia. cores, pooled), sediment properties and microphytobenthic biomass (5 × 0–2 cm depth, 2.6 cm dia. cores, pooled and homogenised) and DEA assays (5 × 0–5 cm depth, 5.3 cm dia. cores, pooled and homogenised). Samples were stored in the dark, and transported to the laboratory on ice. Samples for sediment properties were frozen at − 20 °C and analysed within 6 weeks. Unfiltered seawater was collected from the site, stored on ice and then refrigerated at 4 °C for DEA assays (see below).

A transparent core (5 cm dia.) was taken randomly from the centre of each plot and used to measure the depth of the colour change as a proxy for apparent redox potential discontinuity (aRPD) (Gerwing et al. 2013; Danovaro 2009). Visual measurements have been shown to provide a good measure of aRPD as measured using electrodes or dissolved oxygen concentrations (Rosenberg et al. 2001; Gerwing et al. 2015; Gerwing et al. 2013); however, some variability in measurements may have occurred in the different sediment types. One 5-cm deep core (2.6 cm dia.) hole was made in the centre of each plot, allowed to infill, and the porewater pH measured using a waterproof pHTestr® 10 (Eutech Instruments, Oakton).

Core samples for analysis of the benthic macrofaunal community were taken 4 days after the chamber incubations and sediment sampling (1st December 2014). One core (13 cm dia., 15 cm depth) was taken from the position of the dark incubation chamber (this marked area was left undisturbed by the previous sediment sampling) in each plot. Immediately after collection, macrofaunal core samples were sieved over a 500-μm mesh and preserved in 70% isopropyl alcohol. In the laboratory, samples were stained (Rose Bengal), then all organisms were sorted, counted and identified to the lowest possible taxonomic level (usually species).

Laboratory Analyses

Within 24 h of collection, pore water was extracted from sediment (by centrifugation at 3300 rpm for 10 min), filtered (1.1 μm Whatman GF/C), then stored at − 20 °C until analysed. Nitrogen solute concentrations from benthic flux (NH_4^+) and pore water (NH_4^+, NO_2^- and NO_3^-) samples were analysed using a LACHAT Quickchem 8500 series 2 Flow Injection Analyser (detection limit 0.004 mg L^{-1}) and standard methods for seawater nutrient analysis. Benthic nitrogen flux

measurements were limited to NH_4^+ because others have consistently shown that fluxes of NO_3^- and nitrite (NO_2^-) are minimal (account for less than 1% of benthic inorganic nitrogen fluxes) in northern New Zealand estuaries (Lohrer et al. 2010; Pratt et al. 2013). Sediments were analysed for organic content (%) by weight loss-on-ignition after drying to a constant weight at 60 °C then removing the organic matter by combusting at 550 °C for 4 h. For determination of sediment grain size (% mud and median grain size (GSM)), organic matter was first removed from samples by digesting in 10% hydrogen peroxide, then measured using a Malvern Mastersizer 2000 (accuracy 0.6%). Chlorophyll *a* (Chl *a*) and degraded (phaeophytin) biomass of microphytobenthos was measured after extraction from sediments with 90% buffered acetone, using a Turner 10-AU fluorometer, before and after acidification (Arar and Collins 1997).

DEA assays were conducted the day after sampling, using the acetylene inhibition technique (Tiedje et al. 1989; Groffman et al. 2006; Groffman et al. 1999; Douglas et al. 2017), first allowing sediment samples and water to acclimate to room temperature (20 °C). DEA is a measure of the activity of the resident denitrifier population under optimal conditions without allowing for new enzyme growth (sometimes referred to as denitrification potential). It provides a snapshot of the history of the sediments, i.e. conditions for denitrification that have led to the development of the resident denitrifier population. This method was used because it allows large sample sizes at low cost and is proven as a robust way of assessing spatial differences in relative denitrification activity (Groffman et al. 2006; Groffman et al. 1999).

Assays were composed of 60-mL homogenised sediment sample, 60-mL unfiltered site water amended with chloramphenicol (to prevent new enzyme synthesis, 0.06 g L^{-1}) and unlimited NO_3^- (10 mg L^{-1} N as KNO_3) and carbon (30 mg L^{-1} C as glucose), in 440-mL glass preserving jars with modified lids fitted with rubber septa. Jars were sealed, evacuated (by vacuum pump, 4 min) and flushed (pure N_2 for 10 min) to induce anoxia; then, acetylene was added to each jar (10% of the headspace) to prevent sediment microbes from converting N_2O to N_2. Jars were kept at constant temperature (20 °C), with constant mixing (25 rpm) for 2 h. Headspace gas samples were extracted from each jar 10, 30, 60 and 120 min after the addition of acetylene and analysed for N_2O concentration using Varian CP 3800 gas chromatograph equipped with a HayeSep D column and an electron capture detector. Rates of N_2O production were calculated as the increase in concentration per area of sandflat (μmol N m^{-2} h^{-1}) (calculated using the dry weight of sediment per assay jar and the sediment density).

Data Analysis

To simultaneously account for environmental variation across the sedimentary gradient and assess the effects of nutrient addition on response variables, PERMANOVAs were conducted where the treatment (nutrient enrichment) was considered a fixed factor and mud content as a continuous co-variable. This approach also enabled assessment of the interactive effects of sediment mud content and enrichment. Response variables included sediment properties, macrofaunal community structure, proxies of ecosystem functions and denitrification activity. Results were considered significant if $p \leq 0.05$ and marginally significant if $0.05 > p \leq 0.1$.

Paired light and dark chamber measurements of oxygen and NH_4^+ fluxes were used to derive the following measures of ecosystem function. Sediment oxygen consumption (SOC) which was measured as the uptake of oxygen from the water column to the sediment in dark chambers (i.e. without the effect of photosynthesis by benthic microalgae) and can be considered as a measure of community metabolism. Gross primary productivity (GPP) was measured by subtracting the flux of oxygen in the dark chamber from the flux of oxygen in the light chamber, and when normalised by the biomass of chl *a* in the sediments provides a measure of photosynthetic efficiency ($GPP_{Chl\ a}$). The flux of NH_4^+ in dark chambers (without uptake by microalgae) can be considered as a measure of sediment nutrient regeneration. Chamber fluxes were corrected for water column processes but these made a small contribution to the total flux accounting for < 5 and < 1% for oxygen and NH_4^+, respectively.

In order to understand what aspects of macrofauna diversity were affected by nutrient addition, univariate measures (number of species (S), number of individuals (N) and numbers of adult (≥ 10 mm) and juvenile (< 10 mm) *Austrovenus stutchburyi* and *Macamona liliana*), as well as a multivariate measure of macrofaunal community were assessed. The multivariate measure was generated by combining the counts of all species into a resemblance matrix (Bray-Curtis) with treatment as a factor, after first performing a square root transformation, in order to determine effects on the macrofaunal community as a whole. *A. stutchburyi* and *M. liliana* are key bioturbating species in soft sediment ecosystems in northern New Zealand estuaries and known to be important for ecosystem functioning (Thrush et al. 2006; Sandwell et al. 2009; Pratt et al. 2013; Thrush et al. 2014; Karlson et al. 2016) so were considered separately. A principle coordinate ordination (PCO) plot using a Bray-Curtis resemblance matrix of the benthic macrofaunal community was used to visualise potential differences between treatments. Vector overlays of environmental variables were used to show strength of these factors as predictors of the macrofaunal community (Pearson's correlation).

Multiple regression (using distance-based linear models, DistLM) was used to investigate which variables explained the observed variation in ecosystem functions with and without nutrient enrichment. DistLMs were performed on univariate Euclidean distance matrices of each ecosystem function (community metabolism (SOC), primary productivity (GPP,

$GPP_{Chl\ a}$), nutrient regeneration (dark NH_4^+ flux) and DEA). A backwards elimination procedure was used with the corrected Akaike information criterion (AIC_c) and 9999 permutations to obtain the most parsimonious model. Mud was always forced to be included first in models (even if the marginal test was not significant), and where there was high collinearity among variables ($r > 0.7$), the variable explaining the least amount of variance was excluded first (Dormann et al. 2013). Predictor variables were grouped into sediment (mud), other environmental and macrofaunal community categories. All analyses were conducted using Primer v7 with PERMANOVA+ add on (Clarke and Gorley 2015).

Results

Environmental Variables

The study site encompassed a gradient of sediment mud content (0–24%, Table 1) which correlated with changes in other environmental variables (Appendix Table 4). In particular, mud content and organic content were strongly and positively correlated in both treatments ($r > 0.9$). Temperature and salinity of the water column were 19.4 °C and 31.8, respectively. Bottom water NH_4^+ concentration ranged from 0.55 to 1.9 μmol L^{-1}, water column NO_3^- and NO_2^- concentrations were not measured because they are often below detection limits and known to make up a very small proportion (~ 1%) of water column total inorganic nitrogen (Pratt et al. 2013; Lohrer et al. 2010). Microphytobenthic biomass increased with increasing mud content; however, the aRPD, pore water pH and pore water nutrient concentration were similar apart from slightly higher pore water concentrations of NO_3^- and NO_2^- in muddy sediments (Tables 1 and 2). Enrichment significantly increased pore water NH_4^+ concentration compared with controls (the enrichment median (532.4 μM) was 36 × the control median (14.6 μM)), to levels comparable to enriched estuaries globally (see Douglas et al. 2016), and this effect was independent of sediment mud content (Tables 1 and 2). Other than a small increase in pore water pH, the enrichment did not change other sediment properties or microphytobenthic biomass. Mean light intensity was lower on sampling day 1 (8826 ± 366 lux) than day 2 (22,016 ± 1258 lux) due to variable cloud cover but water temperatures were similar (20.4 ± 0.2 vs 19.6 ± 0.1 °C). This variability did not bias flux measurements because on each sampling day, incubations were conducted in one (of two) enrichment and control plots located at each of the 12 sites.

Macrofaunal Community

Both univariate and multivariate measures show that the macrofaunal community changed across the sedimentary gradient but there was no effect of treatment and no significant

Table 1 Sediment properties and macrofauna community variables as a function of treatment. Values are medians with the range in parentheses ($n = 24$ per treatment)

Variable	Control (0 g N m^{-2})	Enrichment (150 g N m^{-2})
Sediment properties		
Organic content (%)	3.2 (1.5–5.5)	3.3 (1.6–5.8)
Mud content (% particles < 63 μm)	3.5 (0–21.6)	4.0 (0–24.1)
Grain size median (μm)	151 (112–243)	151 (108–259)
pH	7.8 (7.6–8.2)	7.9 (7.6–8.5)
aRPD (mm)	25 (15–35)	20 (12–36)
Pore water concentrations (μM)		
NO_2^-	0.23 (0.12–0.49)	0.25 (0.11–0.94)
NO_3^-	0.96 (0.45–2.28)	1.03 (0.48–3.01)
NH_4^+	14.6 (0–154.2)	532.4 (12.4–24,995)
Microphytobenthic biomass (μg g^{-1} sediment)		
Chlorophyll *a*	31.6 (15.6–48.7)	34.0 (14.8–55.0)
Phaeophytin	9.7 (4.0–17.2)	10.6 (2.9–20.6)
Macrofauna (n $core^{-1}$)		
S (taxa)	18 (14–23)	16 (10–24)
N (individuals)	124 (53–208)	102 (30–262)
A. stutchburyi (< 10 mm)	9 (1–42)	6 (0–31)
A. stutchburyi (≥ 10 mm)	6 (1–11)	7 (1–13)
M. liliana (< 10 mm)	4 (1–12)	3 (0–7)
M. liliana (≥ 10 mm)	6 (1–9)	5 (1–9)

aRPD apparent redox discontinuity potential

Table 2 PERMANOVA test results for the effects of enrichment (treatment) and mud content (a continuous co-variable), on response variables. Significant terms are indicated in bold ($p \leq 0.05$), and marginally significant terms in bold italics ($p \leq 0.1$)

	Mud		Treatment		Interaction	
	Pseudo-F	p-perm	Pseudo-F	p-perm	Pseudo-F	p-perm
Sediment properties						
pH	1.57	0.22	5.72	**0.02**	2.12	0.16
aRPD (mm)	2.36	0.13	1.84	0.19	0.30	0.59
Pore water concentration (μM)						
NH_4^+	0.34	0.57	8.30	**0.001**	0.50	0.49
NO_2^-	6.17	**0.02**	0.96	0.36	0.07	0.79
NO_3^-	32.1	**0.0001**	0.25	0.62	0.03	0.86
Microphytobenthic biomass (μg g^{-1} sediment)						
Chlorophyll *a*	12.6	**0.001**	0.49	0.49	0.04	0.84
Phaeophytin	167	**0.0001**	0.23	0.62	0.23	0.63
Macrofauna (n $core^{-1}$)						
S	0.10	0.75	3.97	**0.05**	0.71	0.40
N	9.55	**0.004**	1.76	0.19	0.52	0.47
A. stutchburyi (< 10 mm)	11.31	**0.003**	0.88	0.37	0.64	0.43
A. stutchburyi (≥ 10 mm)	3.84	***0.06***	0.02	0.89	0.04	0.84
M. liliana (< 10 mm)	0.17	0.69	7.18	**0.009**	2.20	0.14
M. liliana (≥ 10 mm)	2.21	0.15	0.81	0.37	1.56	0.21
Whole community (multivariate)	11.9	**0.001**	1.72	0.13	0.41	0.84
Community metabolism						
SOC (μmol O_2 m^{-2} h^{-1})	3.53	***0.06***	0.51	0.48	0.28	0.61
Primary productivity						
GPP (μmol O_2 m^{-2} h^{-1})	15.3	**0.0004**	0.003	0.96	3.99	**0.05**
$GPP_{Chl\ a}$ (μmol O_2 μg Chl *a* g^{-1} dw m^{-2} h^{-1})	42.3	**0.0001**	0.27	0.61	1.81	0.19
Nutrient regeneration						
Dark NH_4^+ flux (μmol NH_4^+ m^{-2} h^{-1})	4.05	**0.05**	21.4	**0.0001**	4.8	**0.05**
DEA (μmol N m^{-2} h^{-1})	32.0	**0.0001**	4.62	**0.04**	7.42	**0.01**

aRPD apparent redox discontinuity potential, *S* macrofaunal taxonomic richness, *N* macrofaunal abundance, *SOC* sediment oxygen consumption, *GPP* gross primary productivity, $GPP_{Chl\ a}$ gross primary productivity normalised to chlorophyll *a* biomass, *DEA* denitrification enzyme activity

interaction (Tables 1 and 2; Fig. 1). Increasing mud content corresponded with a greater total abundance (N) and fewer adult and greater numbers of juvenile *A. stutchburyi*. The total number of species did not differ across the mud gradient and neither did the abundance of adult or juvenile *M. liliana*. The main environmental variables correlated with macrofaunal community composition were sedimentary variables and microphytobenthic biomass (Fig. 1).

Ecosystem Functioning

All measures of ecosystem function varied with sediment mud content. There was a significant mud × treatment interaction for DEA, and on average, DEA was suppressed by enrichment (Fig. 2, Table 2). Although DEA was positively correlated with sediment mud content in both control and enrichment plots (Appendix Table 5), the response in enrichment plots was non-linear and above 10% mud content DEA declined by 170% (on average) compared to control plots (Fig. 2). Between 0 and 10% mud content, R^2 values for control (0.64) and treatment plots (0.51) were similar. However, above 10% mud content, the R^2 value for enrichment plots (0.07) was much lower than that for control plots (0.71) reflecting a marked increase in the variability of DEA. Community metabolism (SOC) decreased with increasing mud content (by up to 49%), but this was only marginally significant, and there was no treatment effect (Fig. 2, Table 2). The relationship between mud content and nutrient regeneration (dark NH_4^+ flux) changed with nutrient enrichment (Fig. 2, Table 2), as indicated by the significant interaction term. In control plots, dark NH_4^+ flux was positively related to sediment mud content, but in enrichment plots, this relationship was negative (Fig. 2, Appendix Table 5). There was a significant interaction between mud and enrichment for gross primary productivity (GPP). In control sediments, GPP decreased with

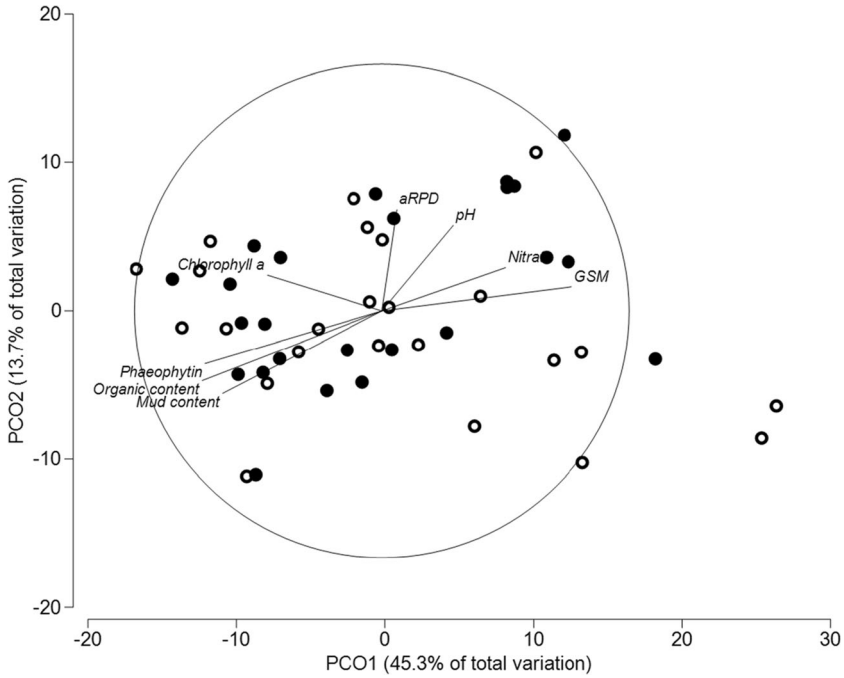

Fig. 1 Principle coordinates ordination (Bray-Curtis similarity) showing little difference in macrofaunal community between control (black circles) and enrichment (white circles) plots. Overlaid vectors show the eight most influential environmental variables. Abbreviations: grain size median (GSM), pore water nitrate concentration (Nitrate), apparent redox potential discontinuity (aRPD)

increasing mud content but this negative relationship was counteracted in the presence of nutrients (Fig. 2, Table 2, Appendix Table 5). When primary productivity was normalised by chlorophyll-*a* biomass ($GPP_{Chl\ a}$) (i.e. photosynthetic efficiency), there was no longer a significant interaction between mud and enrichment; in both treatments, $GPP_{Chl\ a}$ decreased with increasing mud content (Fig. 2, Table 2).

Multiple variables were included in explanatory DistLMs of the measured ecosystem functions (Table 3). Community metabolism (SOC) was unaffected by nutrient enrichment and the best predictors were pore water NO_3^- and macrofaunal community variables (Tables 2 and 3). Mud was the factor explaining the largest amount of variability in $GPP_{Chl\ a}$, which was also unaffected by nutrient enrichment (Table 3). Other environmental variables including aRPD and pore water NO_3^- concentration accounted for a large amount of the variation in $GPP_{Chl\ a}$ (67% explained in total), followed by large bivalves (Table 3). For ecosystem functions that showed treatment and/or interaction effects, separate models were run for each treatment. Nutrient enrichment influenced the proportion of variability in GPP, dark NH_4^+ flux and DEA accounted for by sedimentary environment (mud), other environmental variables and macrofaunal community (Table 3). Mud content was the primary factor explaining variability in GPP, dark NH_4^+ flux and DEA in control plots, but with enrichment, the amount of variability explained by mud was reduced by more than half (Table 3). The amount of variability in these ecosystem functions accounted for by other variables, especially the macrofaunal community, became greater under enriched conditions, but the total amount of variability explained was less. Under enriched conditions, factors that positively influenced GPP and DEA were those associated with oxygenation of the sediments and pore water movement (chlorophyll *a*, aRPD and abundance of macrofauna or large bivalves) (Table 3).

Discussion

A nitrogen enrichment experiment was conducted across an existing gradient of sedimentary grain size and changes in the ecosystem functions of community metabolism, primary productivity, nutrient regeneration and denitrification were measured. This experiment has provided direct evidence that the proportion of mud (fine particles < 63 μm) in sediment can influence how estuary ecosystem functions respond to nutrient enrichment. Results indicate that high sediment mud content is detrimental to denitrification activity under nutrient-enriched conditions, and furthermore, muddy sediments may restrict release of nutrients from enriched sediments, making the sediment ecosystem more likely to shift to a degraded state. The median enrichment level was 36 × control and independent of sediment mud content, yet, nutrient effects on ecosystem functions were tightly linked to the sedimentary environment. Rather than a manipulation of sediments (which would not be feasible due to slow macrofaunal recovery and re-establishment of normal sediment biogeochemistry), this experiment used an established natural gradient in sediment properties, within a site where global variables such as inundation time, salinity and temperature were similar. Although the enrichment technique did not simulate all the symptoms of eutrophication (see below), results show that sedimentary

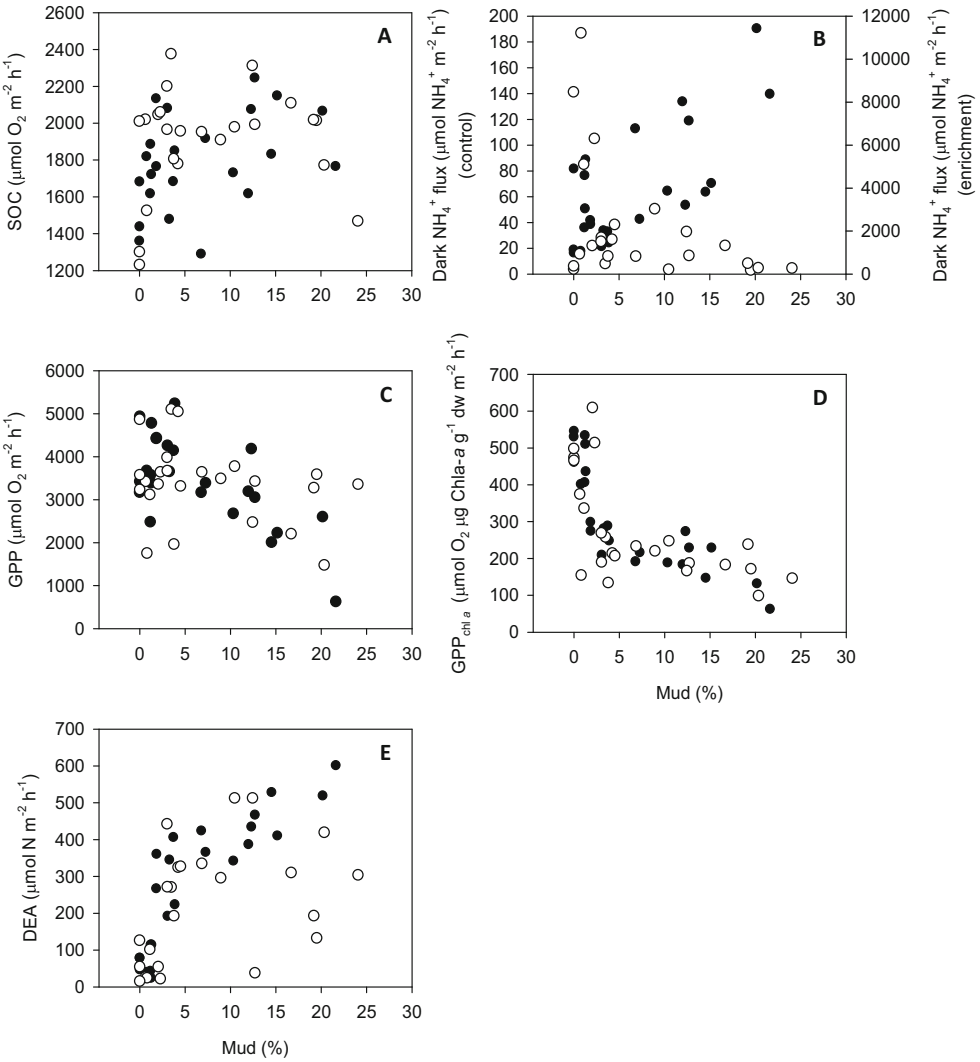

Fig. 2 Relationships between sediment mud content and **a** sediment oxygen consumption (SOC), **b** dark NH_4^+ flux, **c** gross primary production (GPP), **d** gross primary production normalised by Chl *a* biomass ($GPP_{Chl\ a}$), and denitrification enzyme activity (DEA), in control (black circles) and enrichment (white circles) treatments. Note difference in *y*-axis scales for control and enrichment plots in B

environment was a key factor influencing the response to nutrient enrichment (and therefore probably eutrophication), an important result considering increasing mud content and nutrients often occur in unison (Levin et al. 2001; Thrush et al. 2004).

Control and enrichment plot DEA were similar in sediments with low mud content, but compared to controls, DEA was reduced by enrichment in the more muddy, organic rich sediments (Fig. 2). Increases in denitrification with increasing sediment organic matter are a common phenomenon in aquatic sediments because its mineralisation provides NH_4^+ for coupled nitrification-denitrification (the main denitrification pathway in New Zealand estuaries (Gongol and Savage 2016)) (Sundback and Miles 2000; Nowicki et al. 1997; Seitzinger et al. 2006), although this can vary depending on the organic matter source and quality (Eyre et al. 2013). Results of this study imply that in enriched conditions, there is a threshold to this increase in DEA (around 10% mud content), beyond which there was a sudden reduction in activity and increase in variability (Fig. 2). This is characteristic of a stressed system approaching a tipping point (Scheffer et al. 2009; Carpenter and Brock 2006) and is likely linked to the physical properties of muddy sediments and their influence on the sediment biogeochemistry. The interface between the oxic and anoxic sediment zones can vary substantially depending on the sedimentary environment and therefore affects the coupling of nitrification and denitrification which require contrasting oxygen conditions (Joye and Anderson 2008). Because of the cohesive nature of fine sediments, greater mud content can reduce the extent and variability of sediment oxygen profiles, reduce rates of pore water transport and may contribute to a build-up of NH_4^+ (because of reduced flow of pore water out of the sediments) (Glud 2008) which can inhibit nitrification (Anthonisen et al. 1976). Competition for space, as well as diffusion limitation in muddy sediments may also limit populations of autotrophic nitrifying bacteria (which are generally less competitive than heterotrophic bacteria), limiting nitrification even when NH_4^+ is abundant (i.e. in enrichment plots) (Henriksen and Kemp 1988).

Table 3 Results of full DistLMs of ecosystem function, grouped predictor variables included in each model, and the proportion of variance each explains. Combined treatments were used for DistLMs in the absence of a significant mud × enrichment interaction or treatment effect (Table 2, SOC, $GPP_{Chl\ a}$). Where treatment effects occurred, DistLMs were run separately for control and enrichment plots (GPP, dark NH_4^+ flux, DEA). Predictor variables are grouped into sediment (mud), other environmental and macrofaunal community. Asterisks indicate significance levels of marginal tests of individual predictors included in full models $^*p < 0.1$, $^{**}p < 0.05$, $^{***}p < 0.01$. Correlation directions are indicated in parentheses

	Combined treatments		Control (0 g N m^{-2})		Enrichment (150 g N m^{-2})	
	Variables	Prop.	Variables	Prop.	Variables	Prop.
Community metabolism (SOC)						
Sediment	Mud* (+)	0.07				
Other environmental	$_{pw}NO_3^-$ ***(−)	0.21				
Macrofauna	*A. stu* (≥ 10 mm)*** (+) *M. lil* (≥ 10 mm) (+)	0.26				
	Total	0.42				
Primary productivity (GPP)						
Sediment			Mud*** (−)	0.47	Mud (−)	0.09
Other environmental			Chl *a* (+)	0.01		–
Macrofauna			*M. lil* (≥ 10 mm)*** (+)	0.32	*M. lil* (< 10 mm)** (+) *M. lil* (≥ 10 mm)*** (+)	0.34
			Total	0.71	Total	0.45
Primary productivity ($GPP_{Chl\ a}$)						
Sediment	Mud*** (−)	0.48				
Other environmental	aRPD (−) $_{pw}NO_3^-$ *** (+)	0.46				
Macrofauna	*A. stu* (≥ 10 mm)*** (−) *M. lil* (≥ 10 mm) (+)	0.2				
	Total	0.67				
Nutrient regeneration (dark NH_4^+ flux)						
Sediment			Mud** (+)	0.52	Mud** (−)	0.19
Other environmental			Chl *a** (+) aRPD (−) $_{pw}NO_3^-$ (−)	0.27	Chl *a** (+)	0.14
Macrofauna			*A. stu* (≥ 10 mm) (+)	0.003	S (−) N (−)	0.2
			Total	0.77	Total	0.4
DEA						
Sediment			Mud*** (+)	0.73	Mud** (+)	0.17
Other environmental			$_{pw}NH_4^+$** (+)	0.14	Chl *a**** (+) aRPD (+)	0.46
Macrofauna			*A. stu* (≥ 10 mm)** (+) *M. lil* (< 10 mm) (−)	0.33	N*** (+)	0.36
			Total	0.83	Total	0.64

Mud sediment mud content, *Chl a* chlorophyll *a* content, *aRDP* apparent redox discontinuity potential, $_{pw}NO_3^-$ pore water concentration of nitrate, $_{pw}NH_4^+$ pore water concentration of ammonium, *S* macrofaunal taxonomic richness, *N* macrofaunal abundance, *A. stu* (< 10 mm) juvenile *A. stutchburyi*, *A. stu* (≥ 10 mm) adult *A. stutchburyi*, *M. lil* (< 10 mm) juvenile *M. liliana*, *M. lil* (≥ 10 mm) adult *M. liliana*, *SOC* sediment oxygen consumption, *GPP* gross primary productivity, $GPP_{Chl\ a}$ gross primary productivity normalised to chlorophyll *a* biomass, *DEA* denitrification enzyme activity

In enrichment plots, higher DEA rates were associated with factors that facilitate sediment oxygenation and pore water movement: aRPD (which represents the depth of oxygen penetration), Chl *a* biomass (photosynthesis by microphytobenthos can increase oxygen penetration into the sediments) and macrofaunal abundance (N) (Table 3). Macrofauna, especially large species, play an important role in sediment biogeochemical processes and response to nutrient enrichment because their activity and burrow structures can enhance pore water movement and nutrient supply, and in particular, the oxic-anoxic interface where coupled nitrification-denitrification occurs (Woodin et al. 2016; Eyre and Ferguson 2009; Gilbert et al. 2016).

Burrow characteristics such as residence time and irrigation frequency can determine the makeup and biomass of microbial communities (Marinelli et al. 2002), and since burrow characteristics and macrofaunal behaviour vary substantially from permeable to cohesive sediments (Needham et al. 2011; Jones et al. 2011b), this can affect biogeochemical processes and associated ecosystem functions (Yazdani Foshtomi et al. 2015).

Large macrofauna were associated with higher SOC (Table 3), and this is likely to affect sediment oxygen profiles and denitrification rates, although in this study we did not detect a relationship between DEA and SOC. The presence of large macrofauna can increase sediment oxygen consumption rates directly, through respiration, and indirectly through the stimulation of biogeochemical processes and organic matter from bioturbation activities (Sandwell et al. 2009; Woodin et al. 2016; Norkko et al. 2013). Results suggest that GPP and $GPP_{Chl\ a}$ were also enhanced by large bivalves (*M. liliana*, Table 3), probably through bioadvection moving nutrient-rich pore water from within the sediment to surface layers where it can be utilised by microphytobenthos (Volkenborn et al. 2012; Woodin et al. 2016). This may influence DEA through competition with microphytobenthos for nutrients or changes in sediment oxygenation.

The nutrient enrichment level aimed to stress the ecosystem without causing strong negative effects to the macrofaunal community (e.g. high mortality or emigration) and pore water NH_4^+ concentrations were representative of enriched estuaries worldwide (see Douglas et al. 2016). In previous studies using similar enrichment levels, there were minimal or no significant effects to the macrofaunal community, with the only observed reductions occurring in the number of adult *A. stutchburyi* (Douglas et al. 2017; Gladstone-Gallagher et al. 2018). In this study, the macrofaunal community structure (measured by the multivariate metric) was not influenced by enrichment, but there was a small reduction in the abundance of juvenile *M. liliana*, and the total number of species. This may reflect species' differential sensitivity to stress; however, these effects were not exacerbated by mud content (no interaction effect).

Above 10% mud content in enrichment plots, the reduction and increased variability in DEA may reflect a shift from denitrification to dissimilatory nitrate reduction to ammonium (DNRA), or anammox (anaerobic ammonium oxidation). In enrichment plots, dark NH_4^+ flux decreased with increasing mud content which may be the result of higher levels of DNRA rather than nitrification-denitrification; however, it is more likely to be because of lower diffusion rates in sediments with higher mud content. Other studies have shown that DNRA may be favoured over respiratory denitrification in NO_3^- limited coastal sediments with high organic carbon loading (see reviews by Giblin et al. 2013; Burgin and Hamilton 2007), although sediment organic content in this study was relatively low (1.5–5.7%, Table 1). Anammox is less likely to have occurred because these bacteria are slow growing and autotrophic and unlikely to compete with heterotrophic denitrifiers for nitrite (Jetten et al. 2003).

Sediment mud content influenced ecosystem functions and response to nutrient enrichment through direct effects on biogeochemistry associated with fine sediments and indirect effects including differences in macrofaunal community in different sediment types. The main finding indicated that DEA above 10% mud content was variable and reduced which has implications for ecosystem resilience to increased N loading in estuaries which have or will experience increases in sediment mud content. However, extrapolating results requires cautions because we did not simulate all the eutrophication processes, in particular, the main way in which nutrients enter the sediment through organic matter decomposition. Also, the experiment was limited in space and time; responses were only measured at one time after enrichment, and we did not measure recovery. Future experiments should therefore consider different levels of enrichment, test other symptoms of eutrophication and make measurements at different intervals after manipulation. Investigation of other N cycling pathways as well as direct measurements of denitrification (i.e. using membrane inlet mass spectrometry) is needed to further understand the fate of nitrogen in benthic ecosystems and the effects of stressors on ecosystem functioning.

This is the first study, to our knowledge, that experimentally tests soft sediment ecosystem response to nutrient enrichment in different sedimentary environments. Isolating effects of stressors on ecosystem functioning is extremely difficult in real-world settings since rarely do stressors occur independent of others or without being directly or indirectly affected by environmental factors. Anticipating how ecosystems will respond to accelerating stressors (e.g. nitrogen oversupply) under different stress regimes (e.g. sedimentation) will be critical for the preservation of healthy estuary ecosystems and the services they provide.

Acknowledgements We thank Dudley Bell, Rebecca Gladstone-Gallagher, Bradley Monahan, Ryan Hughes, Teri O'Meara, Catherine Kelly, Simon Jopling and Jane Cope for their help with field work. Thanks to Janine Ryburn, Sarah Hailes, Lisa McCartain and Ronald Ram for assistance with laboratory analyses. We also appreciate the constructive comments of four anonymous reviewers that improved the manuscript. This project was supported by CO1x1515 4.2.1 to SFT and CAP, and a University of Waikato Doctoral Scholarship to EJD.

Author's Contributions EJD and CAP designed the study with input from SFT, AML and CS. EJD and CAP performed field research. EJD performed laboratory analyses with input from LAS. EJD and CAP analysed data, EJD wrote the manuscript with assistance from CAP and input from AML, CS, LAS and SFT.

Compliance with Ethical Standards

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

Appendix

Table 4 Pearson's correlation coefficients between environmental and community variables for control and enrichment plots ($n = 24$ per treatment). Shading indicates significant correlation at $p < 0.05$

		Control															
		OC	Mud	GSM	Chl *a*	Phaeo	pH	aRPD	$_{pw}NO_2^-$	$_{pw}NO_3^-$	$_{pw}NH_4^+$	S	N	*A. stu* (<10 mm)	*A. stu* (≥10 mm)	*M. lil* (<10 mm)	*M. lil* (≥10 mm)
Enrichment	OC		0.92	-0.91	0.63	0.93	-0.48	-0.19	-0.66	-0.67	0.37	-0.23	0.42	-0.49	0.39	-0.25	-0.36
	Mud	0.9		-0.77	0.43	0.9	-0.52	-0.32	-0.52	-0.64	0.2	-0.17	0.38	-0.47	0.35	-0.23	-0.43
	GSM	-0.89	-0.78		-0.61	-0.84	0.23	0.15	0.65	0.6	-0.4	0.19	-0.36	0.46	-0.29	0.2	0.22
	Chl *a*	0.65	0.49	-0.61		0.68	-0.03	0.47	-0.48	-0.4	0.53	-0.01	0.19	-0.05	0.42	-0.12	-0.01
	Phaeo	0.94	0.88	-0.87	0.72		-0.35	-0.12	-0.65	-0.71	0.39	-0.1	0.43	-0.42	0.44	-0.14	-0.35
	pH	-0.11	-0.02	0.05	-0.18	-0.16		0.41	0.23	0.24	0.08	0.11	-0.3	0.43	-0.24	0.24	0.39
	aRPD	-0.06	-0.13	0.09	0.53	0.05	-0.18		0.13	0.36	0.31	0.11	-0.13	0.45	0.12	0.04	0.39
	$_{pw}NO_2^-$	-0.42	-0.28	0.28	-0.43	-0.39	0.06	-0.05		0.87	-0.14	0.2	-0.13	0.65	0.02	0.49	0.24
	$_{pw}NO_3^-$	-0.73	-0.66	0.53	-0.56	-0.67	0.12	-0.01	0.74		-0.11	0.18	-0.06	0.68	-0.07	0.4	0.31
	$_{pw}NH_4^+$	-0.25	-0.16	0.14	-0.5	-0.26	0.06	-0.18	0.73	0.4		-0.38	0.11	-0.09	0.04	0.12	-0.15
	S	0.18	0.09	-0.34	0.6	0.28	-0.13	0.58	-0.08	-0.26	-0.11		0.34	0.38	0.29	0.18	0.21
	N	0.52	0.46	-0.64	0.47	0.52	0	0.31	-0.17	-0.41	-0.05	0.7		0.01	0.48	0.13	-0.06
	A. stu (<10 mm)	-0.39	-0.42	0.26	-0.2	-0.38	0.42	-0.07	0.03	0.14	-0.09	0.13	0		0.07	0.14	0.49
	A. stu (≥10 mm)	0.37	0.24	-0.35	0.39	0.34	0.07	0.06	-0.32	-0.3	-0.2	0.23	0.43	-0.15		0.12	0.05
	M. lil (<10 mm)	0.34	0.2	-0.4	0.59	0.39	-0.0	0.3	-0.3	-0.4	-0.2	0.59	0.41	0.17	0.09		-0.1
				6			1		6	8	5						
	M. lil (≥10 mm)	-0.15	-0.04	-0.05	0.13	0.02	0.07	0.2	0.14	-0.03	0.1	0.55	0.36	0.4	-0.17	0.53	

Table 5 Pearson's correlation coefficients between ecosystem functions, and environmental and community variables, for control and enrichment plots (n = 24 per treatment). Shading indicates significant correlation at $p < 0.05$

	OC	Mud	GSM	Chl *a*	Phaeo	pH	aRPD	$_{pw}NO_2^-$	$_{pw}NO_3^-$	$_{pw}NH_4^+$	S	N	*A. stu* (<10 mm)	*A. stu* (≥10 mm)	*M. lil* (<10 mm)	*M. lil* (≥10 mm)
Control																
GPP	-0.51	-0.68	0.41	0.1	-0.51	0.49	0.54	0.25	0.45	0.05	0.12	-0.15	0.56	-0.13	0.08	0.57
$GPP_{Chl\ a}$	-0.91	-0.8	0.85	-0.79	-0.91	0.38	-0.03	0.66	0.66	-0.46	0.13	-0.39	0.4	-0.47	0.26	0.28
SOC	0.33	0.39	-0.21	0.36	0.44	-0.12	0.04	-0.26	-0.31	-0.14	0.42	0.44	-0.04	0.4	0.02	0.2
Dark NH_4^+ flux	0.62	0.72	-0.45	0.19	0.52	-0.47	-0.34	-0.12	-0.21	0.16	-0.17	0.26	-0.14	0.05	-0.12	-0.43
DEA	0.95	0.86	-0.92	0.59	0.89	-0.43	-0.19	-0.62	-0.62	0.37	-0.15	0.4	-0.42	0.44	-0.31	-0.3
Enrichment																
GPP	-0.25	-0.31	0.08	0.12	-0.16	-0.05	0.43	0.16	0.15	0.07	0.36	0.12	0.31	0.03	0.47	0.55
$GPP_{Chl\ a}$	-0.75	-0.60	0.63	-0.81	-0.70	-0.01	-0.21	0.51	0.67	0.52	-0.37	-0.44	0.20	-0.35	-0.36	0.19
SOC	0.29	0.16	-0.18	0.28	0.32	-0.34	0.03	-0.45	-0.56	-0.17	0.20	0.27	-0.15	0.51	0.38	0.19
Dark NH_4^+ flux	-0.48	-0.42	0.56	-0.28	-0.53	0.26	0.04	0.00	0.13	-0.02	-0.06	-0.36	0.26	-0.04	-0.17	-0.15
DEA	0.59	0.41	-0.63	0.61	0.60	-0.12	0.08	-0.48	-0.50	-0.38	0.40	0.60	-0.05	0.51	0.43	0.20

Abbreviations (Appendices 4-5): Sediment organic content (OC), sediment mud content (Mud), Grain size median (GSM), Chlorophyll *a* content (Chl *a*), phaeophytin content (Phaeo), aRPD apparent Redox Potential Discontinuity, nitrate and ammonium $_{pw}NO_2^-$, $_{pw}NO_3^-$ and $_{pw}NH_4^+$ pore water concentrations of nitrite, macrofaunal taxonomic richness (S), macrofaunal abundance, juvenile *A. stutchburyi* (*A. stu* (<10 mm)), adult *A. stutchburyi* (*A. stu* (≥10 mm)), juvenile *M. liliana* (*M. lil* (<10 mm)), adult *M. liliana* (*M. lil* (≥10 mm)), gross primary productivity (GPP), gross primary productivity normalised to chlorophyll *a* biomass ($GPP_{Chl\ a}$), sediment oxygen consumption (SOC), nutrient regeneration (Dark NH_4^+ flux), and denitrification enzyme activity (DEA).

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