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Changes in the location of biodiversity–ecosystem function hot spots across the seafloor landscape with increasing sediment nutrient loading

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Declining biodiversity and loss of ecosystem function threatens the ability of habitats to contribute ecosystem services. However, the form of the relationship between biodiversity and ecosystem function (BEF) and how relationships change with environmental change is poorly understood. This limits our ability to predict the consequences of biodiversity loss on ecosystem function, particularly in real-world marine ecosystems that are species rich, and where multiple ecosystem functions are represented by multiple indicators. We investigated spatial variation in BEF relationships across a 300 000 m² intertidal sandflat by nesting experimental manipulations of sediment pore water nitrogen concentration into sites with contrasting macro-benthic community composition. Our results highlight the significance of many different elements of biodiversity associated with environmental characteristics, community structure, functional diversity, ecological traits or particular species (ecosystem engineers) to important functions of coastal marine sediments (benthic oxygen consumption, ammonium pore water concentrations and flux across the sediment–water interface). Using the BEF relationships developed from our experiment, we demonstrate patchiness across a landscape in functional performance and the potential for changes in the location of functional hot and cold spots with increasing nutrient loading that have important implications for mapping and predicating change in functionality and the concomitant delivery of ecosystem services.

1. Introduction

The current decline of biodiversity represents both the loss of species and changes to ecosystem function [1,2]. Changes in the functional performance of particular ecosystems will alter the way many ecosystem services are delivered and thus the benefits humanity derives from nature. Species attributes, functional traits and community characteristics (e.g. species richness) are often used to assess relationships between biodiversity and ecosystem function(s) (BEF) [3]. The form of these relationships (e.g. positive, negative, linear or nonlinear) can change with shifts in environmental characteristics, organism size [4] and density, and the spatial arrangement of individuals and communities [5]. However, it is not clear from a practical, mechanistic or theoretical perspective what the implications of these changes may be at the landscape scale or which biodiversity attributes relate best to ecosystem function [6].

To date progress in defining BEF relationships and identifying the attributes of biodiversity that link most strongly to ecosystem function has mainly been made through highly controlled, small-scale, manipulative laboratory

experiments that employ a practical but small set of species [7,8]. This has resulted in a call for more relevance to real-world situations and realistic field experiments embracing natural spatial patterns and variation in community composition [9–11]. Nevertheless, evidence is accumulating of important and positive BEF relationships, particularly as the space and time scale of experiments are extended and increasing numbers of species are incorporated into the research [12–14]. This suggests that spatial changes in biodiversity or environmental characteristics within ecological landscapes will affect functional performance. To date this has not been explicitly investigated, although, on larger continental scales, studies of terrestrial and freshwater ecosystems have highlighted strong spatial variation in BEF relationships [15,16]. A global BEF statistical model can always be built from such large-scale data sets, but its explanatory power may be low and it may be limited in predicting local relationships or changes with specific environmental conditions. Practical difficulties in mapping biodiversity and assessing ecosystem function impose critical limitations to translating many BEF relationships into real-world situations [17]. This limits the capacity of BEF studies to demonstrate why biodiversity matters.

Changes in space or time scales, or scales of biological organization, profoundly affect our understanding of ecosystem dynamics and complexity, and our ability to forecast the ecological consequences of environmental change. If BEF relationships change their functional form (e.g. from linear to exponential or parabolic) across landscapes, or with stressors, it will be difficult to simply extrapolate ecosystem functionality and potential for ecosystem service delivery. How changes in community composition across landscapes affect function and how we best use different BEF indicators to characterize (and act as surrogates for) BEF relationships are important [18]. Potentially, BEF relationships can change from place to place, creating localized hot or cold spots for specific functions, or they can change more gradually along gradients [19]. Here we combine information on the spatial structure of ecological landscapes with manipulative experiments. This allowed us to demonstrate how describing and using spatial variability in community composition, in combination with BEF relationships derived from experimental treatments, helps us to inform projections of ecosystem functional performance under conditions of environmental change.

Coastal and estuarine ecosystems, dominated by sedimentary habitats, form the interface between land and ocean, and as such they are subjected to a wide range of human impacts including climate change. Importantly, these soft-sediment ecosystems can be species rich and are known to deliver a wide range of ecosystem services [8]. The diversity of life on the seafloor and the very sharp gradients in chemical composition make these wet and salty marine sediments different from terrestrial soils [20]. Importantly, there is growing evidence of feedback between biodiversity loss and the impacts of stressors, with loss of ecosystem function escalating the impact of stressors on the ecosystem. The potential for these feedback processes is illustrated by the relationship between eutrophication and ecosystem functioning in the coastal zone [21,22]. Organisms living in sediments have long been known to drive many critical ecosystem functions, in particular the breakdown and transformation of organic material and the associated release of nutrients, facilitating their recycling, dispersion and transfer through food webs. Nitrogen is usually a limiting nutrient in the coastal zone, but also an important

driver of eutrophication. Coastal sediments are important sites of denitrification, the process that transforms the dissolved forms of nitrogen that fuel plant growth into nitrogen gas. Calculations indicate that at least 80% of terrestrial dissolved inorganic nitrogen can be denitrified in the coastal ocean margin [23]. There is a complicated set of biogeochemical interactions associated with the degradation and transformation of organic matter, and microbial communities are directly involved in these chemical transformations. However, the larger macrofauna that live within the sediment modify their environment by building tubes, burrowing and generating pore water pressure gradients that profoundly influence the microbial environment and the nature and rate of microbial processes [24,25]. The transformation of nitrogen in the coastal zone is a critical ecosystem function because when nitrogen loads are high and exceed the assimilative capacity of the ecosystem, the consequence is eutrophication [26]. As sediment nutrient loading increases and species or functional groups decrease their contribution to ecosystem functioning, the snowballing effects of decreasing assimilative capacity and increasing stress are likely to result in nonlinear change or tipping points in ecosystem's ability to cope with nutrient load.

The nature of BEF relationships is generally poorly resolved in real-world ecosystems, and there is no clear choice of how best to represent diversity in this context (e.g. key species, species richness or multivariate functional diversity measures). In coastal marine sediments, nitrogen processing is a globally important biogeochemical process that is affected by sediment-dwelling macrofauna. Biodiversity and environmental characteristics vary within and across seafloor habitats, potentially influencing how we view the functionality of coastal ecosystems in space and time. Therefore, we test (i) the role of different attributes of biodiversity in affecting the ecosystem function associated with nitrogen flux in marine sediments, (ii) how related ecosystem function indicators are influenced by different aspects of biodiversity, (iii) how the functional form of BEF relationships varies with sediment nutrient loading, and (iv) how this role varies across a natural ecological landscape and with increasing nutrient load. We demonstrate that BEF relationships are driven by multiple components of biodiversity and the functional form of these relationships changes with increasing sediment nitrogen load. This results in changes in the location of functional hot spots across the seafloor landscape with increasing sediment nutrient load.

2. Material and methods

(a) Nesting experimental sites into the diversity landscape

The experiment was conducted on Tapora Bank, Kaipara Harbour (36°39′ S, 174°29′ E), New Zealand. This is an extensive intertidal flat of permeable sandy sediments, exposed to wind-wave disturbance. Our site (300 000 m^2) encompassed appreciable variability in benthic community composition, sediment mud content and seagrass (*Zostera muelleri*) cover [27]. We surveyed this site and mapped spatial variation at multiple scales in April 2012 [28]. A total of 400 macrofaunal cores (13 cm diam, 20 cm deep) were sampled on a grid was designed to allow sampling at multiple spatial scales using a repeated sequence of sampling intervals (0.3, 1, 5, 10, 20 and 50 m) to encompass patterns on scales from centimetre to kilometre, advancing from the transect sampling

employed by Hewitt et al. [29]. This grid covered the intertidal area from the high- to low-water mark to capture tidal variation.

From this survey dataset, we identified 28 experimental locations that had different combinations of high/low abundance/richness of species with functional traits likely to affect nutrient processing in sediments (e.g. passively or actively facilitating the vertical movement of particles or pore water; see electronic supplementary material, A.A for species names). Each experimental location was centred on a grid point used in the initial survey and based on the map of functional diversity (see electronic supplementary material, A.A, figure S1), we estimated that we had a 3 m working radius around this point. At each location, three experimental plots, each 1 m^2, were created. In two of the plots, we added nitrogen (N) to the sediment; one plot received the addition of 150 g N m^{-2}, another 600 g N m^{-2} and the third acted as a disturbance control. Our high-N addition treatment elevated pore water N to concentrations commonly observed in studies of marine eutrophication [30]. To achieve these nitrogen additions, a slow release urea fertilizer (Nutricote 40-0-0, N-P-K) was injected at 20 uniformly spaced points, to a depth of approximately 15 cm, within the treatment plots using a handheld corer. Fifteen centimetres is below the depth commonly bioturbated in these sediments (S.F.T., J.E.H., C.K., A.M.L., C.A.P. & E.D. 2012, personal observations). The addition of urea-based fertilizer is commonly used in marine ecological, biogeochemical and contaminant remediation research, and we selected our concentrations based on a review of previous studies [30]. Control plots were injected with pea gravel of similar grain size to the fertilizer pellets to control for the disturbance associated with fertilizer addition. The experiment was established on 20 January 2014.

(b) Sampling the experiment

Seven weeks after the start of the experiment, we sampled the plots to measure four factors associated with nutrient flux: pore water ammonium concentration (NH$_4^+$–N), standing stock of microphytobenthos, ammonium (NH$_4^+$–N) release and dissolved oxygen (DO) consumption. We selected this sampling interval to allow time for macrobenthic species to respond to treatments and because pore water N concentrations were still tracking initial treatments [30]. In each plot, four replicate pore water samples were collected using a 2.6 cm diameter corer and the sediments sectioned into surface (0–2 cm) and deep (5–7 cm) for analysis of ammonium concentration. Benthic chlorophyll a concentration was used as a surrogate for microphytobenthos standing stock and was sampled from a standard volume of sediment in each plot (5 × 2.6 cm diam × 2 cm deep cores), as was sediment grain size. We sampled macrobenthic community composition in each plot (2 × 13 cm diam, 15 cm deep cores) and used a 0.25 m^2 quadrat that was photographed to measure surface % cover of seagrass (Z. muelleri), bare sand and shell fragments. Cover was estimated based on occurrence at 75 random points within each photo using CPCE [31]. Macrofaunal cores were sieved (500 μm mesh) and preserved in 50–70% isopropyl alcohol and rose bengal.

Solute concentrations were measured over time from in situ benthic incubation chambers [32–34] in order to evaluate ammonium release and DO consumption rates (efflux from and influx into sediments, respectively). Flux chambers measure the net effect of interacting physical, biogeochemical, plant and animal processes that influence solute exchange across the sediment–water interface and are commonly used in marine benthic BEF studies. Flux chambers were only deployed in control and high-nitrogen (600 g m^{-2}) treatment plots, and all of the incubations were conducted in the dark to control for the influence of light on photosynthetic oxygen production and ammonium uptake by microphytobenthos and seagrass. Fluxes of DO and ammonium are both strongly related to macrofaunal activity and
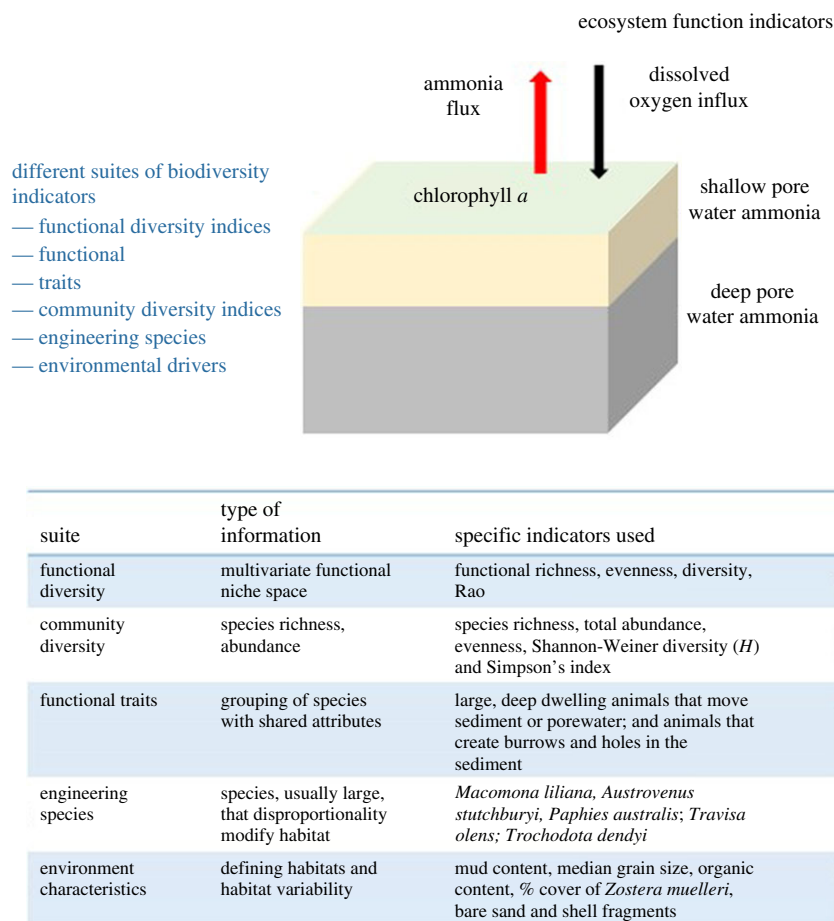
organic matter remineralization rate that is directly linked to nutrient processing capacity of the sediments. To briefly summarize the flux chamber method, 50 × 50 × 10 cm height chamber bases were pressed approximately 5 cm down into the sediment during low tide in targeted experimental plots. On the incoming tide, when water depth reached approximately 30 cm depth, Perspex domes were clamped tightly to each chamber base, sealing approximately 30 l of ambient sea water over the sediments. Opaque shade clothes were used to maintain darkness inside the chambers. Incubations occurred during a midday high-tide period of approximately 4 h. Water samples (60 ml) were withdrawn from the chambers through sampling ports at the beginning and end of the incubation period to assess solute concentrations. Dissolved O$_2$ concentrations in the water samples were measured using an optical probe. Samples were then filtered through a 0.8-μm glass fibre filter and stored frozen prior to analysis of NH$_4^+$–N using standard methods for sea water (detection limit of 1 mg m^{-3}). Fluxes were calculated as $(C_{end} - C_{initial} \times V)/A \times T$, where C is nutrient or oxygen concentration (μM l^{-1}), V is the volume of sea water inside the chamber (l), A is the area of sediment enclosed by the chamber (m^2) and T is the elapsed time between initial and final samplings (h).

(c) Laboratory analyses

In the laboratory macrofauna were sorted, identified to the lowest taxonomic resolution possible (approximately 80% to species level, with the exception of some amphipod families; the majority of these were organisms that had an abundance of 1 per site so species-level identification would not change estimates for that sample) and counted. Pore water was extracted immediately on return to the laboratory by centrifuging and filtering (1.1 μm Whatman GC glass fibre filter), and then stored at −20°C until analysis [35]. These samples were analysed for NH$_4^+$ with a Lachat QuickChem 8000 Series FIA+ (Zellweger Analytics, Milwaukee, WI, USA) using standard operating procedures for flow injection analysis. Sediment samples for the analysis of grain size were frozen at −20°C until analysis. Frozen sediment samples were defrosted and homogenized, and subsamples were taken for analysis. The subsamples were pre-treated with 10% hydrogen peroxide prior to measurements with a Malvern Mastersizer-S [36]. Sediments for chlorophyll a analysis were freeze-dried and then 5 g extracted in 90% acetone and measured using a Turner Designs 10 AU fluorimeter [37].

(d) Defining a suite of biodiversity indicators

We measured a suite of variables considered likely to affect changes in pore water concentrations, nitrogen efflux or DO influx associated with biodiversity or nitrogen addition (figure 1). These included a number of indicators related to different elements of biodiversity: (i) community diversity indices (i.e. species richness, total abundance, evenness, Shannon–Wiener diversity (H') and Simpson's index); (ii) habitat characteristics (i.e. sediment mud content, median grain size, % cover of Zostera); (iii) abundances of ecological engineering species selected based on authors previous research [5,25,38–41] (Bivalves—Macomona liliana, Austrovenus stutchburyi, Paphies australis; Polychaetes—Travisa olens; Holothuria—Trochodota dendyi); (iv) abundances of two macrobenthic community functional traits related to nutrient processing (i.e. large, deep dwelling organisms that move sediment or pore water, and organisms that create burrows and holes in the sediment); and (v) functional diversity indices and measures of trait diversity (i.e. trait evenness, trait Shannon–Wiener index and trait Simpson's index). The functional diversity and trait diversity measures were based on known biological traits of the taxa. We used traits related to general categories of living position, sediment topographic feature created, direction of sediment particle movement, degree of motility, feeding behaviour and body size because we expected

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suite	type of information	specific indicators used
functional diversity	multivariate functional niche space	functional richness, evenness, diversity, Rao
community diversity	species richness, abundance	species richness, total abundance, evenness, Shannon-Weiner diversity (H) and Simpson's index
functional traits	grouping of species with shared attributes	large, deep dwelling animals that move sediment or porewater; and animals that create burrows and holes in the sediment
engineering species	species, usually large, that disproportionality modify habitat	*Macomona liliana, Austrovenus stutchburyi, Paphies australis*; *Travisa olens; Trochodota dendyi*
environment characteristics	defining habitats and habitat variability	mud content, median grain size, organic content, % cover of *Zostera muelleri*, bare sand and shell fragments

Figure 1. Marine sediment with a surface layer of microphytobenthos, an aerobic surface sediment layer atop deeper anaerobic sediments. The range of ecosystem function indicators we use are shown (black text) along with the suite of biodiversity indicators (grey text). (Online version in colour.)

them to affect nitrogen processing in the sediments by pumping pore water, moving sediment particles and organic material and changing sediment topography [17,21,24,25]. Fuzzy coding was used to assign species to traits [42], with allocation across each general category summing to 1 (see electronic supplementary material, B for the coding of each taxa).

The dbFD package in R [43] was used to calculate the three indices [44] of functional richness (F_{Ric}), functional evenness (F_{Eve}) and functional divergence (F_{Div}), as well as functional dispersion (F_{Dis}) [45] and Rao's quadratic entropy (Q) [46]. As all the traits were numeric (probabilities with values ranging from 0 to 1), they were standardized to mean of 0 and unit variance and FD was run based on Euclidean distances. Calculation of all indices except F_{Ric} was weighted by abundance. The number of principal coordinates analysis (PCA) axes to keep as 'traits' for calculating F_{Ric} and F_{Div} was set at the maximum number of axes that allowed the number of species to be greater than the number of traits, with the presence of negative eigenvalues set to be corrected by using the square root of the distances. However, dimensionality reduction occurred with only three of the axes retained. F_{Ric} output values were not standardized by the 'global' F_{Ric} to run between 0 and 1, nor were the Rao's Q output values scaled by its maximal value over all frequency distributions.

(e) Statistical modelling

Generalized linear models (GLMs) with nitrogen treatment as a fixed factor and appropriate error structures and link functions (see below) were used to determine which of the indicators of ecosystem function (pore water nitrogen concentrations in the surface and deep of the sediment, efflux of ammonium from the sediment, DO influx and chlorophyll *a* concentrations) displayed

significant treatment differences. Where a significant effect was detected, Bonferroni comparisons were used to determine which treatments differed. Only for indicators of ecosystem function that revealed treatment effects did we go on to develop predictive models.

Models were developed to explore three questions: (i) which aspects of biodiversity and habitat characteristics were most useful in predicting the ecosystem functions, (ii) whether relationships between the predictors and the ecosystem functions were generally linear or not, and (iii) whether best predictors changed with our experimental addition of N. To answer the first question, and to limit problems associated with correlated predictors, we used a hierarchical model development (see electronic supplementary material, A.B). In order to maximize our ability to detect changes in predictors with experimental additions of N (question 3), models were run for each treatment type (control, medium and high) separately (see electronic supplementary material, A.C).

BEF statistical models were developed using GLMs (SAS INSIGHT v. 9.3; SAS Institute Inc) with nonlinearities incorporated by using log transformations, polynomials and multiplicative terms (see electronic supplementary material, A.B). While the use of other error structures were investigated, only normal errors with identity links were required (defined by residual by predicted plots and residual normal plots). Parsimonious models were produced by backwards selection based on largest *p*-values, with terms only removed if doing so did not result in a significant increase in deviance [47]. Best models were selected based on the residual by predicted plots, residual normal plots and partial leverage plots, stability of the parameter estimates and the Akaike information criterion (AIC) [48–50]. In total 10 best BEF statistical models were derived: three treatment types for shallow and deep pore water (6 models in total); and two

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Table 1. Experimental manipulation of sediment nitrogen loading has significant effects on pore water and ammonium efflux. Note flux measurements were made only in the high (H) and control (C) experimental plots.

source	DF	MS	F	p	multiple comparison
chlorophyll a (μg g^{-1})					
model	2	44.991525	1.29	0.2819	
error	81	34.977434			
total	83				
pore water surface N (μM)					
model	2	83 119 734.9	23.28	<0.0001	H > M = C
error	80	3 570 495.5			
total	82				
pore water deep N (μM)					
model	2	302 406 220	32.47	<0.0001	H > M > C
error	81	9 314 281			
total	83				
NH$_4$ efflux (μM m^{-2} h^{-1})					
model	1	116 268 240.6	33.73	<0.0001	H > C
error	51	3 447 318.9			
total	52				
DO influx (μM m^{-2} h^{-1})					
model	1	13 778 879.6	3.39	0.0713	C > H
error	51	4 061 589			
total	52				

treatment types for nitrogen efflux and DO influx (4 models in total).

Best results were obtained using untransformed data but frequently with second degree polynomials, thus a number of functional forms were observed: linear, convex, concave and unimodal in either a positive or negative direction. For each model, we determined the relative importance of each predictor variable. There are a number of ways of doing this [51]; here we use the importance of each predictor variable to the response's mean (i.e. 'level' importance [52]). As polynomial terms in the final models precluded assessing this importance by simply comparing standardized coefficients, for each variable the predicted effect on the response variable was calculated over the range of the variable in the model data, and divided by the sum of the effects of all variables. For models with no polynomial terms, this is equivalent to using the standardized coefficients.

(f) Analysis of the sensitivity of statistical models to selected traits

Sensitivity analysis is often performed by dropping variables from the analyses, and we chose not to follow this approach because we had strong mechanistic reasons to link our selected traits to nitrogen processing and sediment oxygen consumption in marine sediments. Therefore, if we were to drop traits, we would expect our models to perform more poorly. Instead we chose to assess sensitivity by including extra traits that could potentially act as surrogates for different indirect effects of animals on sediment nitrogen processing and oxygen consumption. Comparisons were made between results we obtained using the traits we considered directly linked to the ecosystem function (see 'Defining a suite of biodiversity indicators' above, and electronic supplementary material, B) and when two other trait types that could be indirectly linked to ecosystem function (body shape

and hardness) were also included (electronic supplementary material, C). Changes in the traits included could have affected the values for the multivariate indices (F_{Ric}, F_{Div}, F_{Eve}, Rao's Q and F_{Dis}) and the univariate trait indices (trait evenness, Shannon–Wiener and Simpson's indices). For the three sets of traits dimensionality reduction occurred and only the first three PCA axes were used to calculate F_{Ric} and F_{Div}. As a result the quality of the reduced-space representation of F_{Ric} varied from 0.44 to 0.46.

(g) Mapping the experimentally determined ecosystem function back onto the landscape

Ecosystem function was predicted across the larger landscape, based on the initial intensive survey [28]. To assess changes in functionality across the sandflat associated with increasing sediment nitrogen concentration, we used the BEF statistical models derived from the control and high nutrient addition experimental plots, applied to the survey data. The resulting model predictions were standardized to range from 0 to 10 to show the relative change in function. We used averaging and linear interpolation over a 20 m grid cell size to map the spatial variation in individual ecosystem function variables across the 300 000 m^2 site. Paired t-tests were used to determine the significance of differences between the predictions of the two BEF statistical models.

3. Results

Treatment effects for surface and deep pore water N concentration and ammonium efflux were detected 7 weeks after the experimental manipulation of sediment nitrogen loading (table 1). There was a weaker indication that DO influx into

Table 2. The relative importance (%) of different suites of biodiversity indicators associated with the predictive models of the four ecosystem functions from each of the three experimental N additions (control, medium, high). Relative importance values are derived from electronic supplementary material, A. Biodiversity indicators: environment = habitat characteristics (mud content, % cover of *Zostera*); community = species richness, total abundance, evenness, Shannon−Wiener diversity (H') and Simpson's index; single trait = large, deep dwelling organisms that move sediment or pore water, and organisms that create burrows and holes in the sediment; functional diversity = F_{Ric}, F_{Div}, F_{Eve}, Rao's Q, trait evenness, trait Shannon−Wiener index and trait Simpson's index; ecological engineers = *Macomona liliana*, *Austrovenus stutchburyi*, *Paphies australis*; *Travisa olens*.

biodiversity indicator	pore water shallow			pore water deep			ammonium efflux		DO influx	
	control	medium	high	control	medium	high	control	high	control	high
environment	—	—	—	—	—	12	9	—	—	10
community	38	5	—	32	—	42	—	67	67	39
single trait	—	—	—	—	—	5	—	7	18	—
functional diversity	25	76	56	42	56	36	24	—	—	39
ecological engineers	38	18	43	26	44	6	66	26	15	13

the sediment was decreased by elevating sediment nitrogen concentration ($p = 0.0713$). However, we could not detect a treatment effect on surface sediment chlorophyll *a* concentration and consequently did not investigate this further.

(a) Changes in the functional form of biodiversity and ecosystem function relationships and the relative importance of different biodiversity indicators

Both the structure and functional form of BEF relationships derived separately for controls, and the 150 and 600 g N m^{-2} treatments were influenced by changes in biodiversity and environmental characteristics across the experimental site (see electronic supplementary material, A.D and A.E, for the statistical results of the 10 models). The results of these models were summarized to determine both the relative importance of different biodiversity indicators and the differences in the functional form of the BEF relationships.

Nineteen biodiversity indicators and environmental factors in total were selected across the ten models as being important for predicting changes in our function variables (shallow and deep pore water ammonium, ammonium efflux and DO influx). The variables selected as predictors often varied between treatments. Community (richness and abundance) and functional diversity measures (diversity, F_{Div}; richness, F_{Ric}) contributed to driving the function variables, but macrofaunal ecosystem engineer species particularly bivalves (*Austrovenus*, *Macomona*, *Paphies*) and large worms (*Travisia*) were also important. The most common drivers were F_{Ric}, and the densities of large *Austrovenus* and *Macomona* (occurring in 7, 7 and 6, respectively, of the 10 models; electronic supplementary material, A.D).

To assess the overall importance of different types of factors (i.e. environmental characteristics, community indices, functional traits, functional diversity indices or the presence of ecological engineers), we aggregated and summed the relative contribution of the individual variables to each BEF model (table 2). The presence of engineering species, particularly large bivalves, was consistently an important predictor. Functional diversity and community indices were also generally important, although the role of community indices was more variable. Single traits and environmental characteristics

were less important, with environmental factors contributing little to the function models when included. Adding nitrogen to the sediment always changed the relative contribution of different biodiversity indices (table 2; electronic supplementary material, A.D). Only for deep pore water ammonium did the dominant biodiversity measure (F_{Ric}) remain the same with nitrogen addition.

Analyses of the sensitivity of our analysis to the traits selected showed that our results, as presented, changed little with the addition of extra traits with generally less than 5% changes in model R^2 and the relative importance of different factors contribution to the models (see electronic supplemental material, C). This reflected the generally high correlations observed between the indices calculated on the three sets of traits.

(b) Nonlinearity in biodiversity and ecosystem function relationships

All models, except for DO influx, included nonlinear relationships (figure 2). Different biodiversity indicators drove the nonlinearity in ecosystem functions as the nitrogen load in the sediment increased. The most consistent indicator leading to nonlinear responses was F_{Ric}, along with the abundance of ecological engineering species *Austrovenus* and *Macomona* (accounting for 5, 2 and 3 nonlinear relationships, respectively, out of 11 identified). No consistent functional form of BEF relationships across experimental treatments was apparent for the different ecosystem functions (figure 2; electronic supplementary material, A).

(c) Mapping the experimentally determined ecosystem function back onto the landscape

These changes in drivers, and their relative contributions, result in the BEF models predicting significant changes in ecosystem function variables across our 300 000 m^2 study site as nitrogen loading increases from present conditions to 600 g N m^{-2} (table 3 and figure 3). Regions of high surface pore water ammonium concentrations under current conditions switched their distribution from high shore to low shore and the distribution of high-function regions became
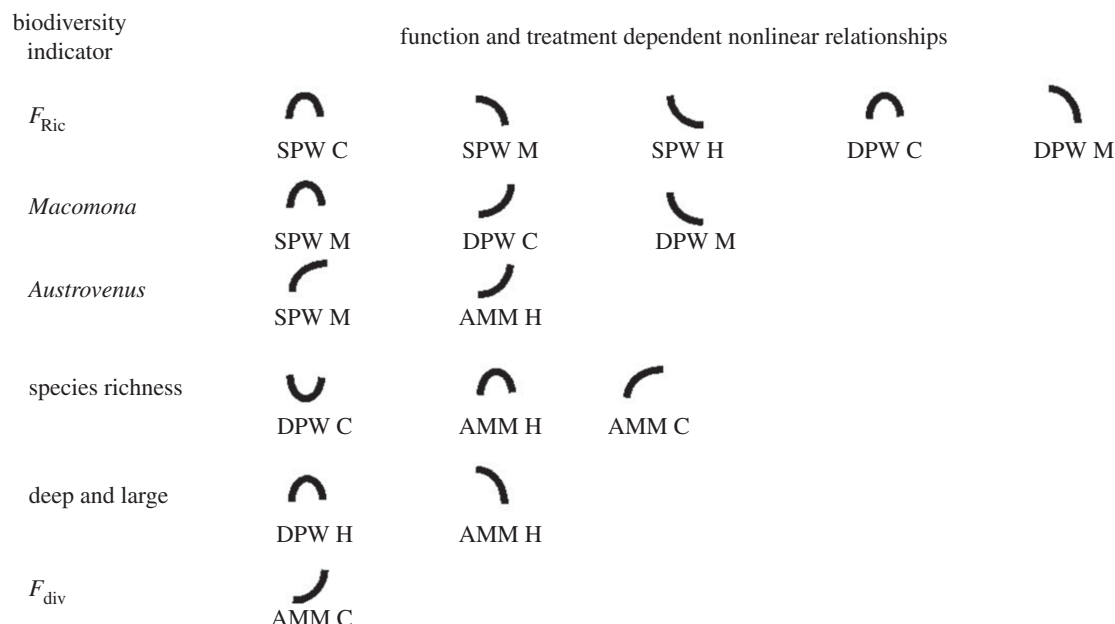
Figure 2. The form of nonlinear relationships associated with different biodiversity indicators on ecosystem function variables. SPW, surface pore water ammonium; DPW, deep pore water ammonium; AMM, ammonium efflux. Experimental treatments: C, control; M, medium-nitrogen addition; H, high-nitrogen addition.
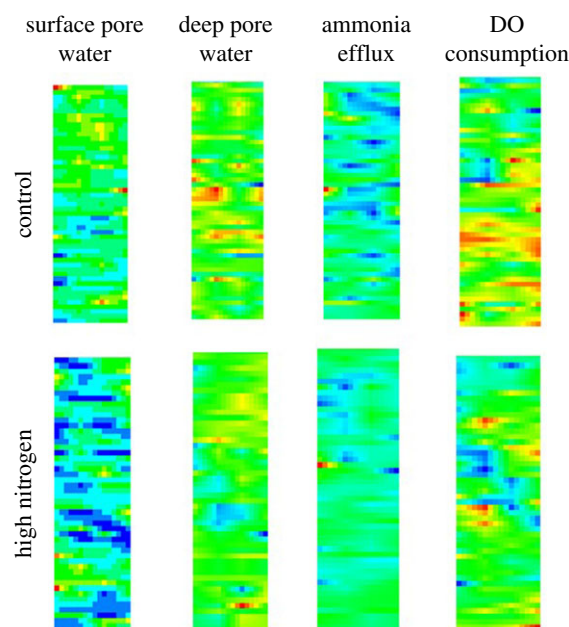
Table 3. Significance of differences in BEF statistical models derived from high and control nitrogen additions projected across the 300 000 m^2 study site. Paired t-test, $n = 319$.

variable	t	$p > |t|$
pore water shallow (μM)	16.81	<0.0001
pore water deep (μM)	−4.10	<0.0001
ammonium efflux (μM m^{-2} h^{-1})	−11.22	<0.0001
DO influx (μM m^{-2} h^{-1})	−3.57	0.0004

Figure 3. Maps of relative functional performance under control and high nitrogen treatments, projected across the 300 000 m^2 study site. Projections are normalized to allow the spatial variation in function to be seen despite the increase in nitrogen concentration. Hot colours indicate areas of high functionality and cold colours low functionality, except for sediment DO consumption where high consumption (high functionality) is blue.

increasingly patchy with increasing nitrogen load. Specific locations of highest predicted surface pore water concentration also changed. Deep pore water ammonium concentrations showed a similar pattern to surface concentrations. However, spatial changes in functionality across the site were different for ammonium efflux. For example, increasing the sediment's nitrogen content resulted in a much more homogeneous pattern of elevated efflux, while for DO influx, the pattern was intermediate between pore water ammonium concentration and ammonium efflux. Thus the factors driving sediment nutrient processing are changing in response to sediment nutrient concentration.

4. Discussion

Our experiment was, to our knowledge, the first attempt to nest a BEF experiment into a natural landscape of variation in community composition and subject the BEF relationships to environmental change. Multiple elements of biodiversity were important in driving change in ecosystem function indicators associated with sediment nitrogen dynamics. Given that both individual ecosystem engineer species and functional diversity indices were important, multiple metrics are probably needed to fully capture BEF relationships. A key finding of our experiment was that many of our BEF

relationships were nonlinear and the functional form of individual response variables changed with both increases in sediment nitrogen concentration and particular ecosystem function indicators (figure 2). These ecosystem-function-specific nonlinear BEF relationships emphasize the potential for threshold responses and that thresholds for different functional indicators may be tripped under different levels of environmental change. The functionality of the sandflat was not uniform, and the relative functionality changed across the landscape with increasing nutrient loading. This has important implications for detecting change in ecosystem

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function and in mapping ecosystem functions in relation to determining the delivery of ecosystem services.

Spatially dependent shifts in functional performance (figure 3) emphasize the importance of context, specifically in our study of sediment nutrient loading and variation in macrobenthic community structure. This is the real-world consequence of shifting biodiversity in habitats that are well recognized for their contribution to nutrient processing [23]. Importantly spatial shifts in functional performance has strong implications for scaling-up BEF relationships, mapping nutrient processing or relating detailed process-based measurements to ecosystem service delivery. Averaging is often used in models to overcome this 'noisy' pattern, but this can lead to problems with aggregation errors [53]. Averaging over variability can also lead to a lack of sensitivity in identifying how environmental change, stress or disturbance will impact BEF relationships. For example, our experiment detects changes in the factors that influence nutrient processing with increased nitrogen loading and in the functional form of that response. Shifts in the functional form of BEF relationships, from linear to nonlinear, positive to negative, offer potential insights into the functional resilience of ecosystems as they work to process increasing contaminant loads or recover from elevated disturbance levels. The potential to identify thresholds in how these systems respond to change by shifting their functional performance is a powerful attribute of our experimental design.

The relative importance of ecological engineers, single trait-based functional groups or broader measures of biodiversity in BEF relations is contentious [6]. We demonstrated that large engineering species that affect particle transport and pore water pressure gradients were particularly important in affecting ecosystem function indicators associated with sediment nitrogen processing. This is mechanistically consistent with how macrofauna can modify the environment for microbial communities that transform organic matter and nutrients in sediments, as well as pump pore water deep in the sediment and transport dissolved nutrients [25]. However, the individual functional trait group that was mechanistically associated with species important in deep burrowing and particle transport in the sediment only contributed to two BEF relationships. In both situations this involved high nitrogen additions (deep pore water ammonium concentration and ammonium efflux). We had expected that the specificity of this functional group and the inclusion of multiple species within it would have made it a more consistent and important explanatory factor based on previous studies of functional traits in marine sediments. Many BEF experiments document the importance of particular species on function (identity effects), although the functional consequence of the loss of these species can be dependent on the community that remains [22]. We also found functional and community diversity measures to be important [54], with functional richness the most consistent biodiversity predictor in the BEF models. Functional richness was based on multiple traits and represents the volume of trait space within the community. Often functional diversity or biological traits are used as surrogates for function, because measuring function is difficult over large scales. Our results emphasize the need to assess the nature of relationships between function and specific biodiversity indicators as they can change as the ecological landscape changes or environmental drivers change. This will be assisted by developing better mechanistic understanding of ecological interaction networks [41].

The relationships between terrestrial plant diversity and primary production have been a cornerstone in BEF research [3,13,55]. In our system we could not detect an effect on the most productive plants (i.e. the microphytobenthos biomass), although these plants do respond to the release of nitrogen from the sediments and are a significant contributor to primary production in many coastal ecosystems [38,56,57]. We have seen similar results in other harbours affected by wind waves [41]. In these permeable sandy sediments, bedload transport of surface sand grains and associated microphytes can dilute the localized effects of experimental treatments. Seagrass was also patchily distributed around our study site, and this more structural species only made a small relative contribution to deep pore water ammonium concentration. A pan-Europe study of plant diversity–primary productivity relationships demonstrated that, although overall a log-linear BEF relation was apparent at individual locations, the specifics of the parameter estimates differed, emphasizing the important limitations of global models in predicting local patterns [58,59]. The changes we observe across the sandflat landscape have important implications for both scaling up BEF relationships in marine sediments and understanding how the performance of ecosystem functions will change associated with elevated levels of environmental change.

Biodiversity ecosystem function relationships are important to understand because they underpin many ecosystem services [1]. Understanding how real-world ecosystems actually change is not only critical to our fundamental understanding but also in communicating changes in ecosystem benefits, especially under substantial predicted future environmental change [60]. BEF field experiments often warn of complicated, context-dependent results and the difficulty of making predictions without detailed system knowledge [21,22]. Our results based on nesting a BEF experiment into a natural landscape of variation in community composition revealed that multiple elements of biodiversity can be involved in defining BEF relationships and the importance of these biodiversity elements can change, even for closely related aspects of ecosystem function (e.g. pore water nutrient concentrations and fluxes across the sediment–water interface). Many of our BEF relationships were nonlinear, and the functional form of these relationships changed with sediment nutrient load. The functionality of the sandflat was spatially structured but changed across the landscape with increasing nutrient loading. Defining BEF relationships across ecological landscapes and under different levels of nutrient loading is a practical way of improving and empirically generalizing our understanding of the importance of different factors in driving ecosystem function. At the landscape scale, shifts in the location of functional hot spots or the relative spatial uniformity in functional performance may provide useful insight into the resilience of coastal and estuarine resilience in the face of increasing nutrient loading and eutrophication.

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rspb.royalsocietypublishing.org *Proc. R. Soc. B* **284**: 20162861

References

1. Duncan C, Thompson JR, Pettorelli N. 2015 The quest for a mechanistic understanding of biodiversity–ecosystem services relationships. *Proc. R. Soc. B* **282**, 20151348. (doi:10.1098/rspb.2015.1348)

2. Naeem S, Duffy JE, Zavaleta E. 2012 The functions of biological diversity in an age of extinction. *Science* **336**, 1401–1406. (doi:10.1126/science.1215855)

3. Hooper DU *et al.* 2005 Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecol. Monogr.* **75**, 3–35. (doi:10.1890/04-0922)

4. Norkko A, Villnäs A, Norkko J, Valanko S, Pilditch C. 2013 Size matters: implications of the loss of large individuals for ecosystem function. *Sci. Rep.* **3**, 2646. (doi:10.1038/srep02646)

5. Lohrer AM, Thrush SF, Hewitt JE, Kraan C. 2015 The up-scaling of ecosystem functions in a heterogeneous world. *Sci. Rep.* **5**, 10349. (doi:10.1038/srep10349)

6. Butterfield BJ, Suding KN. 2013 Single-trait functional indices outperform multi-trait indices in linking environmental gradients and ecosystem services in a complex landscape. *J. Ecol.* **101**, 9–17. (doi:10.1111/1365-2745.12013)

7. Stachowicz JJ, Bruno JF, Duffy JE. 2007 Understanding the effects of marine biodiversity on communities and ecosystems. *Annu. Rev. Ecol. Syst.* **38**, 739–766. (doi:10.1146/annurev.ecolsys.38.091206.095659)

8. Snelgrove PVR, Thrush SF, Wall DH, Norkko N. 2014 Real world biodiversity-ecosystem functioning: a seafloor perspective. *Trends Ecol. Evol.* **29**, 398–405. (doi:10.1016/j.tree.2014.05.002)

9. Srivastava DS, Vellend M. 2005 Biodiversity-ecosystem function research: is it relevant to conservation? *Annu. Rev. Ecol. Syst.* **36**, 267–294. (doi:10.1146/annurev.ecolsys.36.102003.152636)

10. Diaz S, Symstad AJ, Chapin FS, Wardle DA, Huenneke LF. 2003 Functional diversity revealed by removal experiments. *Trends Ecol. Evol.* **18**, 140–146. (doi:10.1016/S0169-5347(03)00007-7)

11. Midgley GF. 2012 Biodiversity and ecosystem function. *Science* **335**, 174–175. (doi:10.1126/science.1217245)

12. Bracken MES, Friberg SE, Gonzalez-Dorantes CA, Williams SL. 2008 Functional consequences of realistic biodiversity changes in a marine ecosystem. *Proc. Natl Acad. Sci. USA* **105**, 924–928. (doi:10.1073/pnas.0704103105)

13. Isbell F *et al.* 2011 High plant diversity is needed to maintain ecosystem services. *Nature* **477**, 199–202. (doi:10.1038/nature10282)

14. Gamfeldt L, Lefcheck JS, Byrnes JEK, Cardinale BJ, Duffy JE, Griffin JN. 2015 Marine biodiversity and ecosystem functioning: what's known and what's next? *Oikos* **124**, 252–265. (doi:10.1111/oik.01549)

15. Hector A *et al.* 2007 Biodiversity and ecosystem functioning: reconciling the results of experimental and observational studies. *Funct. Ecol.* **21**, 998–1002. (doi:10.1111/j.1365-2435.2007.01308.x)

16. Woodward G *et al.* 2012 Continental-scale effects of nutrient pollution on stream ecosystem functioning. *Science* **336**, 1438–1440. (doi:10.1126/science.1219534).

17. Thrush SF, Lohrer AM. 2012 Why bother going outside: the role of observational studies in understanding biodiversity-ecosystem function relationships. In *Marine biodiversity futures and ecosystem functioning Frameworks, methodologies and integration* (eds DM Paterson, M Solan, R Aspenal), p. 256. Oxford, UK: Oxford University Press.

18. Johnson PTJ, Ostfeld RS, Keesing F. 2015 Frontiers in research on biodiversity and disease. *Ecol. Lett.* **18**, 1119–1133. (doi:10.1111/ele.12479)

19. Larsen TH, Williams NM, Kremen C. 2005 Extinction order and altered community structure rapidly disrupt ecosystem functioning. *Ecol. Lett.* **8**, 538–547. (doi:10.1111/j.1461-0248.2005.00749.x)

20. Hedges JI, Oades JM. 1997 Comparative organic geochemistries of soils and marine sediments. *Org. Geochem.* **27**, 319–361. (doi:10.1016/S0146-6380(97)00056-9)

21. Villnäs A, Norkko J, Lukkari K, Hewitt J, Norkko A. 2012 Consequences of increasing hypoxic disturbance on benthic communities and ecosystem functioning. *PLoS ONE* **7**, e44920. (doi:10.1371/journal.pone.0044920)

22. O'Connor NE, Bracken MES, Crowe TP, Donohue I. 2015 Nutrient enrichment alters the consequences of species loss. *J. Ecol.* **103**, 862–870. (doi:10.1111/1365-2745.12415)

23. Erisman JW, Galloway JN, Seitzinger S, Bleeker A, Dise NB, Petrescu AM, Leach AM, de Vries W. 2013 Consequences of human modification of the global nitrogen cycle. *Phil. Trans. R. Soc. B* **368**, 20130116. (doi:10.1098/rstb.2013.0116)

24. Volkenborn N *et al.* 2012 Intermittent bioirrigation and oxygen dynamics in permeable sediments: an experimental and modeling study of three tellinid bivalves. *J. Mar. Res.* **76**, 794–823. (doi:10.1357/002224012806770955)

25. Woodin SA, Volkenborn N, Pilditch CA, Lohrer AM, Wethey DS, Hewitt J, Thrush SF. 2016 Same pattern, different mechanism: locking onto the role of key species in seafloor ecosystem process. *Sci. Rep.* **6**, 26678. (doi:10.1038/srep26678)

26. Diaz RJ, Rosenberg R. 2008 Spreading dead zones and consequences for marine ecosystems. *Science* **321**, 926–929. (doi:10.1126/science.1156401)

27. Kraan C, Dormann CF, Greenfield BL, Thrush SF. 2015 Cross-scale variation in biodiversity–environment links illustrated by coastal sandflat communities. *PLoS ONE* **10**, e0142411. (doi:10.1371/journal.pone.0142411)

28. Greenfield BL, Kraan C, Pilditch CA, Thrush SF. 2016 Mapping functional groups can provide insight into ecosystem functioning and potential resilience of intertidal sandflats. *Mar. Ecol. Prog. Ser.* **548**, 1–10. (doi:10.3354/meps11692)

29. Hewitt JE, Legendre P, McArdle BH, Thrush SF, Bellehumeur C, Lawrie SM. 1997 Identifying relationships between adult and juvenile bivalves at different spatial scales. *J. Exp. Mar. Biol. Ecol.* **216**, 77–98. (doi:10.1016/S0022-0981(97)00091-9)

30. Douglas EJ, Pilditch CA, Hines LV, Kraan C, Thrush SF. 2016 *In situ* soft sediment nutrient enrichment: a unified approach to eutrophication field experiments. *Mar. Poll. Bull.* **111**, 287–294. (doi:10.1016/j.marpolbul.2016.06.096)

31. Kohler KE, Gill SM. 2006 Coral Point Count with Excel extensions (CPCe): a Visual Basic program for the determination of coral and substrate coverage using random point count methodology. *Comput. Geosci.* **32**, 1259–1269. (doi:10.1016/j.cageo.2005.11.009)

32. Jones HFE, Pilditch CA, Bruesewitz DA, Lohrer AM. 2011 Sedimentary environment influences the effect of an infaunal suspension feeding bivalve on estuarine ecosystem function. *PLoS ONE* **6**, e27065. (doi:10.1371/journal.pone.0027065)

33. Needham HR, Pilditch CA, Lohrer AM, Thrush SF. 2011 Context-specific bioturbation mediates changes to ecosystem functioning. *Ecosystems* **14**, 1096–1109. (doi:10.1007/s10021-011-9468-0)

34. Sandwell DR, Pilditch CA, Lohrer AM. 2009 Density dependent effects of an infaunal suspension-feeding bivalve (*Austrovenus stutchburyi*) on sandflat nutrient fluxes and microphytobenthic productivity. *J. Exp. Mar. Biol. Ecol.* **373**, 16–25. (doi:10.1016/j.jembe.2009.02.015)

35. Lohrer AM, Halliday NJ, Thrush SF, Hewitt JE, Rodil IF. 2010 Ecosystem functioning in a disturbance-recovery context: contribution of macrofauna to primary production and nutrient release on intertidal sandflats. *J. Exp. Mar. Biol. Ecol.* **390**, 6–13. (doi:10.1016/j.jembe.2010.04.035)

36. Singer JK, Anderson JB, Ledbetter MT, McCave IN, Jones KPN, Wright R. 1988 An assessment of analytical techniques for the size analysis of fine-grained sediments. *J. Sediment. Petrol.* **58**, 534–543.

10

rspb.royalsocietypublishing.org   Proc. R. Soc. B 284: 20162861

37. Arar E, Collins G. 1997 *In vitro determination of chlorophyll a and pheophytin a in marine and freshwater algae by fluorescence*. Cincinnati, OH: National Exposure Research Laboratory, US Environmental Protection Agency.

38. Thrush SF, Hewitt JE, Gibbs M, Lundquist C, Norkko A. 2006 Functional role of large organisms in intertidal communities: community effects and ecosystem function. *Ecosystems* **9**, 1029–1040. (doi:10.1007/s10021-005-0068-8)

39. Lohrer AM, Townsend M, Rodil IF, Hewitt JE, Thrush SF. 2012 Detecting shifts in ecosystem functioning: the decoupling of fundamental relationships with increased pollutant stress on sandflats. *Mar. Poll. Bull.* **64**, 2761–2769. (doi:10.1016/j.marpolbul.2012.09.012)

40. Woodin SA, Wethey DS, Hewitt JE, Thrush SF. 2012 Small scale terrestrial clay deposits on intertidal sandflats: behavioral changes and productivity reduction. *J. Exp. Mar. Biol. Ecol.* **413**, 184–191. (doi:10.1016/j.jembe.2011.12.010)

41. Thrush SF *et al.* 2014 Experimenting with ecosystem interaction networks in search of threshold potentials in real-world marine ecosystems. *Ecology* **95**, 1451–1457. (doi:10.1890/13-1879.1)

42. Hewitt JE, Thrush SF, Dayton PD. 2008 Habitat variation, species diversity and ecological functioning in a marine system. *J. Exp. Mar. Biol. Ecol.* **366**, 116–122. (doi:10.1016/j.jembe.2008.07.016)

43. Laliberté E, Legendre P, Shipley B. 2014 FD-package measuring functional diversity from multiple traits, and other tools for functional ecology. See http://idg.pl/mirrors/CRAN/web/packages/FD/FD.pdf.

44. Villeger S, Mason NWH, Mouillot D. 2008 New multidimensional functional diversity indices for a multifaceted framework in functional ecology. *Ecology* **89**, 2290–2301. (doi:10.1890/07-1206.1)

45. Laliberte E, Legendre P. 2010 A distance-based framework for measuring functional diversity from multiple traits. *Ecology* **91**, 299–395. (doi:10.1890/09-0199.1)

46. Botta-Dukát, Z. 2005 Rao's quadratic entropy as a measure of functional diversity based on multiple traits. *J. Veg. Sci.* **16**, 533–540. (doi:10.1111/j.1654-1103.2005.tb02393.x)

47. Crawley MJ. 1993 *GLIM for ecologists*. Oxford, UK: Blackwell Scientific Publications.

48. Burnham KP, Anderson DR. 1998 *Model selection and multimodel inference*. New York, NY: Springer.

49. Chatterjee S, Hadi AS. 1988 *Sensitivity analysis: linear regression*. New York, NY: John Wiley & Sons.

50. Faraway J. 2004 *Linear models with R*. Boca Raton, FL: Chapman & Hall.

51. Grömping U. 2006 Relative importance for linear regression in R: the package relaimpo. *J. Stat. Softw.* **17**, 1–27. (doi:10.18637/jss.v017.i01)

52. Achen CH. 1982 *Interpreting and using regression*. Thousand Oaks, CA: Sage.

53. Rastetter EB, King AW, Cosby BJ, Hornberger GM, O'Neill RV, Hobbie JE. 1992 Aggregating fine-scale ecological knowledge to model coarser-scale attributes of ecosystems. *Ecol. Appl.* **2**, 55–70. (doi:10.2307/1941889)

54. Gagic V *et al.* 2015 Functional identity and diversity of animals predict ecosystem functioning better than species-based indices. *Proc. R. Soc. B* **282**, 20142620. (doi:10.1098/rspb.2014.2620)

55. Tilman D, Reich PB, Knops JMH, Wedin DA, Mielke T, Lehman C. 2001 Diversity and productivity in a long-term grassland experiment. *Science* **294**, 843–845. (doi:10.1126/science.1060391)

56. Lohrer AM, Thrush SF, Gibbs MM. 2004 Bioturbators enhance ecosystem function through complex biogeochemical interactions. *Nature* **431**, 1092–1095. (doi:10.1038/nature03042)

57. Cahoon LB. 1999 The role of benthic microalgae in neritic ecosystems. *Oceanogr. Mar. Biol.* **37**, 47–86.

58. Hector A *et al.* 1999 Plant diversity and productivity experiments in European grasslands. *Science* **286**, 1123–1127. (doi:10.1126/science.286.5442.1123)

59. Hector A *et al.* 2010 General stabilizing effects of plant diversity on grassland productivity through population asynchrony and overyielding. *Ecology* **91**, 2213–2220. (doi:10.1890/09-1162.1)

60. Oliver TH *et al.* 2015 A synthesis is emerging between biodiversity-ecosystem function and ecological resilience research: reply to Mori. *Trends Ecol. Evol.* **31**, 89–92. (doi:10.1016/j.tree.2015.12.008)