

Species densities, biological interactions and benthic ecosystem functioning: an *in situ* experiment

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ABSTRACT: Understanding how biota affect the functioning of ecosystems is imperative if we are to predict the impacts of ongoing biodiversity change on ecosystem service provision. Evidence from marine sediments—the most widespread habitat on earth—suggests that ecological function delivery is driven by the presence and densities of certain species. However, most experiments have been conducted using fixed density treatments and run for short durations (<4 wk) within homogenous laboratory microcosms. In nature, the impact of changing density in one species may depend on consequent changes in the densities of others. Moreover, evidence from vegetation assemblages suggests that the influence of complementarity among species increases in heterogeneous environments and over time. Here, we simulated a realistic pattern of biodiversity change by transplanting the macroinfaunal bivalve *Scrobicularia plana* into an intertidal mudflat at various densities. The impact on redox potential discontinuity (RPD) depth (a proxy for benthic functioning) was measured at 1, 5 and 9 wk. Increasing *S. plana* density negatively affected RPD depth (i.e. RPD depth became shallower) by causing the density of a functionally dominant species, *Corophium volutator*, to decline. Furthermore, the influence of density-dependent interspecific interactions (among macroinfauna) on RPD depth became increasingly positive as the experiment progressed. Our results reaffirm the direct functional importance of certain species in a natural ecosystem and highlight the indirect importance of other species to which their density is tightly coupled. An implication is that species loss could enhance functioning if it causes the density of a functionally dominant species to increase. Nevertheless, the apparent temporal emergence of interspecific facilitation suggests that diverse species assemblages promote high function delivery.

KEY WORDS: Complementarity · *Corophium volutator* · Facilitation · Interspecific interactions · Marine biodiversity · Redox potential · *Scrobicularia plana*

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INTRODUCTION

In an era of rapid environmental change, there is a need to determine how alterations to biodiversity affect the functioning of ecosystems (Naeem et al. 2012). It is now well established that changes to species densities can impact key ecological functions, such as nutrient cycling and productivity, with the magnitude of impact depending on the organism's bi-

ological traits (Chapin et al. 1997, Diaz & Cabido 2001). Evidence from field experiments suggests that complementarity enhances functioning when more species are present (i.e. the community performs better than expected from the performances of the individual constituent species; Loreau & Hector 2001). More recent laboratory microcosm experiments have shown that complementarity can emerge through interspecific facilitation (positive species interactions;

Cardinale et al. 2002, Tiunov & Scheu 2005) and niche partitioning (the ability of different species to utilise different sections of the niche space; Caliman et al. 2011, Cardinale 2011). However, with most experimental assemblages having been constructed randomly from a species pool (Tilman et al. 2014), questions have been raised about the applicability of these findings to natural communities experiencing non-random compositional change (e.g. Srivastava & Velend 2005). Indeed, studies have shown that the impact of biodiversity loss on the delivery of ecological functions (hereafter 'function delivery') depends on the order of extinction and differs between random and realistic scenarios (Solan et al. 2004, Larsen et al. 2005, Bracken et al. 2008, Bracken & Low 2012). In marine systems, the issue of real-world applicability is particularly pertinent as most experiments have been conducted within controlled and homogenous environments (Gamfeldt et al. 2015). Efforts to increase realism in biodiversity–ecosystem function (BEF) research are now being encouraged (e.g. Hillebrand & Matthiessen 2009, Gamfeldt et al. 2015).

Marine sediments are the most widespread habitat on Earth and play a major role in climate regulation, waste assimilation and the production of food for human consumption (Snelgrove et al. 2014). Therefore, to predict the impact of global change on the provision of these ecosystem services, we must determine how ongoing changes to biodiversity affect the underlying functioning of marine sediments. Experimental evidence supports the proposition that marine sediment biodiversity regulates ecosystem functioning (Gamfeldt et al. 2015, Strong et al. 2015). Consistent with terrestrial counterparts, studies point to the importance of certain species (or species-specific traits) in driving function delivery (Emmerson et al. 2001, Ieno et al. 2006, Norling et al. 2007, Godbold et al. 2009a,b, Braeckman et al. 2010, Godbold et al. 2011). For example, a study of Skagerrak macroinfauna found that oxygen consumption and benthic–pelagic nutrient fluxes were dominated by the deep-burrowing thalassinid shrimp *Calocaris macandreae* (Norling et al. 2007), while organic matter consumption by enchinoderm species from Gullmarfjord, Sweden, was found to be dominated by the active deposit-feeding sea urchin *Brissopsis lyrifera* (Godbold et al. 2009a). Aggregate density, or the density of functionally dominant species, also appears to be an important factor (Emmerson et al. 2001, Ieno et al. 2006, Braeckman et al. 2010), whereas complementarity among species has not emerged as an important driver of marine sediment functioning (but for evidence of complementarity in

the functioning of freshwater/brackish sediments, see Mermillod-Blondin et al. 2003, Karlson et al. 2010, Caliman et al. 2011).

The above-mentioned marine sediment BEF studies have generally used short-term experiments (<4 wk), conducted within homogenous laboratory microcosms (but see Godbold et al. 2009b, 2011), to isolate biotic influence over functioning from the confounding effects of environmental heterogeneity. The typically small number of species considered has permitted the examination of all (or many) possible assemblages, thus allowing biodiversity effects caused by complementarity to be distinguished from those caused by the presence and/or density of individual species (Ieno et al. 2006, Benedetti-Cecchi & Maggi 2012). However, complementarity among species has been shown in vegetation assemblages to increase over time and in heterogeneous environments (e.g. Stachowicz et al. 2008, Wacker et al. 2008), raising the possibility that complementarity would emerge in marine sediments if experiments were run in the field, and for longer durations. The impact of changing density in one species may also depend on consequent changes in the densities of other species (e.g. Ruesink & Srivastava 2001, O'Connor & Crowe 2005); a possibility that is excluded when using closed microcosms with fixed density treatments. Therefore, while the design of such experiments has allowed BEF mechanisms to be unambiguously identified, the degree to which findings characterise BEF relationships in natural marine sediments is unclear. To assess how real-world biodiversity change affects ecosystem functioning requires that biodiversity is manipulated within natural, open ecosystems.

Here, a field experiment was conducted over an extended period (9 wk) to determine how realistic changes in species densities impact benthic ecosystem functioning. We transplanted the macroinfaunal bivalve *Scrobicularia plana* into plots on an intertidal mudflat and measured the redox potential discontinuity (RPD) depth—an index of sediment oxygen content (Gerwing et al. 2015), which in turn drives organic matter decomposition (Hulthe et al. 1998) and nutrient regeneration (Ruardij & van Raaphorst 1995). Sediment reworking and burrow ventilation by macroinfauna act to increase RPD depth (Solan et al. 2004, Birchenough et al. 2012), which may create a positive feedback by improving conditions for other macroinfauna that also perform these activities (Bouma et al. 2009). We propose that such facilitative interactions will manifest among individuals of different species, which are likely to occupy different

niches (Silvertown 2004), whereas intraspecific interactions are more likely to cause interference and, thus, reduced activity (e.g. Duport et al. 2006). Experimental plots were sampled on 3 occasions; we predicted that RPD depth would initially respond to *S. plana* density or consequent changes to the density of another species (see 'Materials and methods: Study species and potential paths of impact on functioning'), but over time would be increasingly driven by positive density-dependent interspecific interactions (reflecting interspecific facilitation) and/or negative density-dependent intraspecific interactions (reflecting intraspecific antagonism) among the macroinfaunal assemblage.

MATERIALS AND METHODS

Study species and potential paths of impact on functioning

Scrobicularia plana is a large (up to 65 mm) and thus potentially functionally dominant bivalve (Thrush et al. 2006, Norkko et al. 2013) that lives buried to depths of 20 cm in muddy intertidal sediments throughout the coasts of the NE Atlantic (Santos et al. 2011). Local densities of *S. plana* can vary from absent to 100s of ind. m⁻² within a few years (Essink et al. 1991, Ysebaert & Herman 2002). Its patchy distribution is apparently caused by sensitivity to local environmental conditions at the time of settlement (Santos et al. 2011), whereas adults are tolerant of a wide range of physical and chemical conditions (Freeman & Rigler 1957, Beukema 1979, Boldina-Cosqueric et al. 2010). Realistic biodiversity change can therefore be simulated by transplanting *S. plana* adults into suitable habitats where its density is low.

From its burrow, *S. plana* extends its siphons to the sediment surface where it draws down water for respiration (Green 1967), feeds on settled or suspended detritus and expels pseudofaeces (Hughes 1969). The species therefore has the potential to directly increase sediment oxygen content through burrow irrigation, while consumption and deposition of organic matter could indirectly increase or decrease oxygen content, respectively, by altering sediment oxygen demand (Gray et al. 2002). Such activities may also facilitate or inhibit other species within the assemblage (Bouma et al. 2009), which could in turn impact the functioning of the ecosystem.

We hypothesised 4 paths through which *S. plana* density could affect RPD depth (Fig. 1). The first, P1,

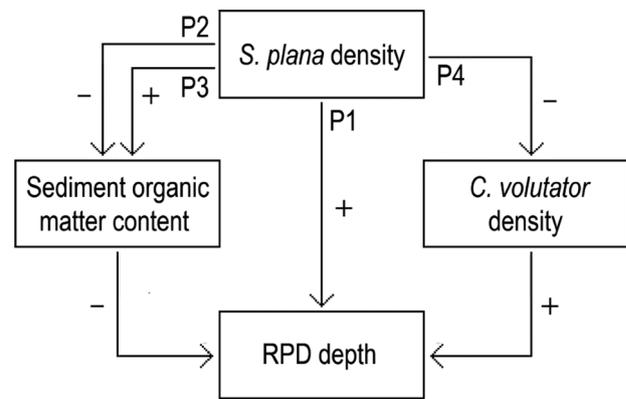


Fig. 1. Hypothesised paths for an impact of *Scrobicularia plana* density on redox potential discontinuity (RPD) depth: Path 1 (P1) is a direct positive effect; Path 2 (P2) is an indirect positive effect mediated by a decrease in sediment organic matter content, which is expected to have a negative influence over RPD depth; Path 3 (P3) is an indirect negative effect mediated by an increase in sediment organic matter content; and Path 4 (P4) is an indirect negative effect mediated by a decrease in the density of *Corophium volutator*, which is expected to have a positive influence over RPD depth

is a direct positive effect (an increase in RPD depth) due to burrow irrigation. P2 is an indirect positive effect mediated by net consumption of sediment organic matter and a subsequent decrease in sediment oxygen demand; whereas P3 is an indirect negative effect mediated by net deposition of organic matter (pseudofaeces) and a subsequent increase in sediment oxygen demand. P4 is an indirect negative effect mediated by a decrease in the density of another widely distributed burrowing macroinvertebrate, *Corophium volutator*. Previous studies have shown that *C. volutator* has a positive influence on RPD depth (e.g. Limia & Raffaelli 1997) and is negatively affected by large bivalves (e.g. Jensen 1985, Beadman et al. 2004).

Changes to density-dependent intra- and interspecific interactions within the macroinfaunal assemblage represent additional mechanisms by which the transplants were predicted to affect RPD depth, through antagonism and facilitation, respectively. As these mechanisms do not relate specifically to *S. plana*, they were considered separately from the afore-mentioned paths.

Experimental site

The experiment was conducted on an intertidal mudflat in the Mersey Estuary, Liverpool, UK

(53° 21' 22" N, 02° 55' 28" W). At the time of the experiment, the macroinfaunal assemblage was dominated by the mud shrimp *C. volutator*, the ragworm *Hediste diversicolor* and oligochaetes *Tubificoides* spp. *S. plana* occurred sporadically and at low density, as did the bivalve *Macoma balthica* and the polychaete *Eteone longa*. Few epifaunal invertebrates inhabited the site, but the shore crab *Carcinus maenas* and the brown shrimp *Crangon crangon* were present. The gastropod *Hydrobia ulvae* was abundant on the upper shore but was apparently absent mid-shore, where the experiment was conducted (D. S. Clare pers. obs.). Sediment organic matter content at the site was ~7%.

Running the experiment

S. plana adults (>20 mm) were transplanted into circular plots on the seabed (20 cm diameter; 0.03 m²) at 3 densities: 510 g m⁻² (1.0 × *D*), 1020 g m⁻² (2.0 × *D*) and 255 g m⁻² (0.5 × *D*), and were contained using cages (described in detail below). *D* refers to the density of the species at the donor site on the Dee Estuary, Wirral Peninsula, UK (53° 21' 14" N, 3° 10' 18" W), where it was relatively abundant at the time of the experiment (40 ind. m⁻² on the Mersey vs. 400 ind. m⁻² on the Dee). Control plots (no cage, no transplanted individuals) and procedural control plots (cage, no transplanted individuals) were also used.

A randomised block design was used (Hurlbert 1984), in which 4 blocks were spaced at 10 m intervals across the shore at an elevation of 1 m above mean sea level. Each block consisted of 15 plots—1 replicate of each of the 5 treatments, for 3 experiment durations (1, 5 and 9 wk)—randomly allocated within a 5 × 3 grid (Block 1 is shown in Fig. 2). A total of 60 plots were used.

S. plana were collected from the donor site on 29 July 2013, transported to the laboratory and incubated in aerated aquaria at the approximate temperature and salinity of the study site (15°C; 30 psu). Water in the aquaria was partially replaced each day to prevent the build-up of excreted toxins. Immediately prior to transplantation, *S. plana* were weighed in the laboratory and transported to the study site in containers filled with water from the aquaria.

The 4 blocks were set up on consecutive days, one block each day, from 30 July to 2 August 2013. Transplanted individuals were placed onto the sediment surface in an even distribution over the plot area. Stainless steel cages (1 cm² mesh) with wire net roofs (1 cm² mesh), were fitted to plastic corers (20 cm

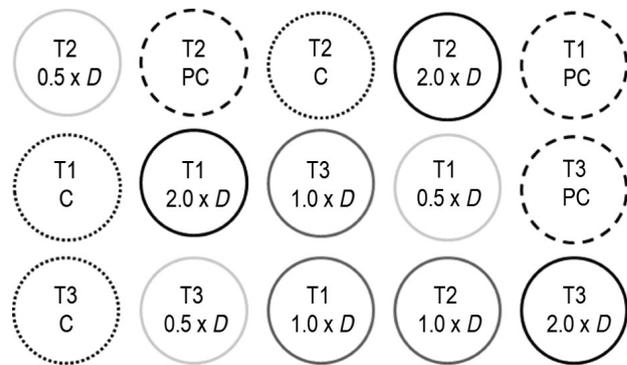


Fig. 2. Experimental plots (20 cm diameter) within Block 1 (not to scale; plots spaced 2 m apart). The density of *Scrobicularia plana* transplanted into each plot is expressed in terms of the mean density of the species at the donor site (*D*): 1.0 × *D* (dark grey ring), 0.5 × *D* (light grey) and 2.0 × *D* (black). Control plots (no cage, no transplanted individuals; dotted ring) and procedural control plots (cage, no transplanted individuals; dashed ring) are shown. Each treatment was replicated for 3 sampling occasions: 1 wk (T1), 5 wk (T2) and 9 wk (T3)

diameter × 30 cm depth) and pushed into the mud to contain the plots, such that the top of the corer was level with the sediment surface and the cage extended 20 cm above the seabed (Fig. 3). This prevented transplanted *S. plana* from being swept away by currents or being depredated by birds while allowing other, comparatively small, invertebrates to relocate in response to the experimental treatments.

Data collection

One replicate of each of the 5 treatments was sampled from each block at 1 wk (T1), 5 wk (T2) and 9 wk (T3) after the block was laid. Each sampling occasion

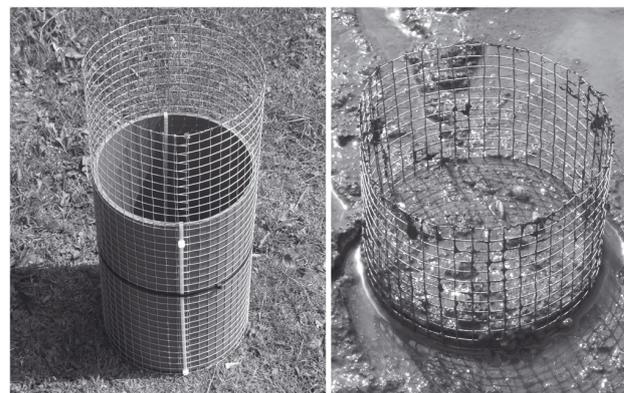


Fig. 3. Cages before and after being inserted into the sediment to contain *Scrobicularia plana* within plots at the experiment site on the Mersey Estuary, Liverpool

coincided with the 'spring' period of the tidal cycle. Pools developed within the plots during the experiment as corers experienced a net loss of sediment. This was not yet apparent at T1. At T2, all pools were <1 cm deep. By T3, pools had reached an average (\pm SE) depth of 6 ± 0.2 cm.

At sampling, intact cores were lifted from the seabed, capped at the bottom and placed upright onto plastic trays. This maintained the sediment profile and prevented contained animals from escaping during transportation back to the laboratory. For control plots, corers with the same dimensions as those used to contain the experimental plots were pushed down into the sediment until the upper edge was level with the sediment surface. Sampling then proceeded in the same way as described for experimental plots.

Upon returning to the laboratory (<1 h after sampling) a transparent corer (5 cm diameter) was used to extract sediment from the centre of each plot, such that the entire vertical section was removed. A ruler was used to measure RPD depth through the corer to the nearest 0.5 cm (*sensu* Gerwing et al. 2013), with 4 measurements taken at 90° to each other and averaged to give the mean value for each plot. Additional measurements of RPD depth taken from the remaining sediment in T1 plots confirmed that this sub-sampling procedure gave an accurate representation of the mean values for the plots.

Sediment extracted using the transparent corer was sliced into 2 equal vertical sections, and any *S. plana* individuals were removed using forceps. Surface sediment (0 to 1 cm) was retained from one section and the bulk core (0 to 20 cm) was retained from the other. These samples were frozen at -15°C and later dried at 90°C , homogenised and percent organic matter content calculated by weighing before and after incineration at 550°C (Buchanan & Kain 1984). Because *S. plana* both feeds and deposits pseudofaeces on the sediment surface, we focused on surface sediment organic matter content in our analyses; however, the relationship between surface and bulk organic matter content is shown in Table S1 and Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m547p149_supp.pdf.

The sediment remaining in each plot was sieved through 0.5 mm mesh and the residue preserved in 70% ethanol. After 1 to 2 mo, to allow biomass to stabilise following preservation (Wetzel et al. 2005), the retained fauna (including *S. plana* removed from sediment used to calculate organic matter content) were identified to the lowest taxonomic level possible, enumerated and weighed after soaking in fresh-

water and draining through filter paper for 30 min (fresh biomass). We based densities on biomass rather than abundance as the former is a particularly good predictor of a species' relative contribution to their associated functions (Garnier et al. 2004). For bivalves, biomass was taken as the flesh weight only. With the exception of *S. plana*, for which all individuals were retained, densities were corrected to account for the loss of biomass contained within the sediment used for organic matter content analysis. Densities were standardised to g m^{-2} .

Data analysis

Analyses were performed using R statistical software (v.3.1.2; R Development Core Team 2014). Type II sums of squares were used in all general linear models (LMs) and null hypotheses were rejected at $p < 0.05$. Assumptions of homoscedasticity and normality of residuals were checked by inspection of plots of residuals against fits and normal quantile plots, respectively.

Cage effect

LMs were used to test whether the density of each species and sediment organic matter content differed between control and procedural control plots (i.e. whether there was an experimental artefact associated with the cage). 'Block' and 'time' were included in the models; each explanatory variable was treated as a factor. Since there was a significant cage effect, control plots were removed from further analyses and the procedural control plots were used as the 'no *S. plana* addition' baseline.

S. plana density vs. RPD depth

A LM was used to test the relationship between RPD depth and *S. plana* density, with time and block included as explanatory variables. Since we predicted that species-specific density effects would weaken over time as biological interactions became more important, the interaction between *S. plana* density and time was also tested. *S. plana* density reflected a gradient from low to high biomass among plots at each sampling occasion (i.e. did not divide into the initial density categories) and was therefore treated as a quantitative explanatory variable; time and block were treated as factors.

Conditional on a significant relationship between RPD depth and *S. plana* density (or a significant *S. plana* density \times time interaction), analogous LMs were used to test the relationship between *S. plana* density and the hypothesised mediators of the effect of *S. plana* density on RPD depth: sediment organic matter content and *C. volutator* density. If relationships were significant then the relevant mediators were retained. If relationships were not significant then the relevant mediators were dropped from further analyses as they did not constitute potential mechanisms through which *S. plana* density could affect RPD depth.

Causal mediation analysis, a generalization of linear structural equation modelling (Imai et al. 2010), was used to partition any effect of *S. plana* density on RPD depth into direct and indirect paths (via the retained mediator). Two regression models were created as the basis of the mediation analysis: the first expressing variation in the mediator in relation to *S. plana* density (the 'mediator model'), and the second expressing variation in RPD depth in relation to the mediator and *S. plana* density (the 'outcome model'). Block and time were included in the models as pre-treatment explanatory variables. Temporal interactions were not incorporated since they were statistically insignificant in the preceding LMs (see Table 2). The objects of the mediator and outcome models were then fed into the 'mediate' function in the R package 'mediation' (Tingley et al. 2014), which computes the average direct effect (ADE; i.e. the direct effect of *S. plana* density), the average causal mediation effect (ACME; i.e. the indirect effect of *S. plana* density via the mediator) and the total effect (i.e. the sum of ADE and ACME) using the general algorithms described in Imai et al. (2010). A non-parametric bootstrap approach was used to estimate bias-corrected 95% confidence intervals for the ADE, ACME and total effect from 10 000 iterations (Tingley et al. 2014).

Sensitivity analysis was used to assess the robustness of the ACME to the violation of 'sequential ignorability' (Imai et al. 2010). Sequential ignorability is the assumption that relationships between the treatment (i.e. *S. plana* density), the mediator and the outcome (RPD depth) are not confounded by other potential explanatory variables. Robustness was determined with respect to the range of ρ (the correlation between the residuals for the mediator and outcome models) under which the confidence intervals for ACME overlapped with zero. Relationships between the mediator and the densities of sampled taxa were inspected to identify any potential con-

founders of the relationship between the mediator and the outcome. Treatment assignment was assumed to be independent of potential outcomes and mediators, given the randomised experimental design.

Density-dependent intra- and interspecific interactions vs. RPD depth

To assess whether antagonism and/or facilitation among macroinfauna influenced RPD depth, indices representing the potential for density-dependent intraspecific interactions (the sum of squared biomasses; 'Intra') and density-dependent interspecific interactions (the sum of products of biomasses for pairs of different species; 'Inter') were calculated for each plot. To obtain these indices, it was assumed that the expected RPD depth at a given time and in a given block is an unknown function of the biomasses of each of the species within the assemblage. If this function is continuous and sufficiently smooth, it can be approximated using a second-order Taylor polynomial (e.g. Riley et al. 2002, section 5.7). With the simplifying assumptions that density-dependent intra- and interspecific interactions act in the same way for all species or pairs of species, respectively, the following regression model was obtained:

$$\text{RPD depth} = \alpha + \sum_{i=1}^s \beta_i b_i + \gamma \sum_{i=1}^s b_i^2 + \delta \sum_{i=1}^s \sum_{j=i+1}^s b_i b_j + \varepsilon \quad (1)$$

where α is an intercept, β_i is the effect of the biomass of the i^{th} species on RPD depth, γ is the effect of the intraspecific index, Intra = $\sum_{i=1}^s b_i^2$ (the sum of squared biomasses; $\text{g}^2 \text{m}^{-4}$) on RPD depth, δ is the effect of the interspecific index, Inter = $\sum_{i=1}^s \sum_{j=i+1}^s b_i b_j$ (the sum of products of biomasses for pairs of different species; $\text{g}^2 \text{m}^{-4}$) on RPD depth, and ε is a normally-distributed error term.

The densities of each taxon were included in the model in order to account for the individual effects of species densities before testing for the effects of their interactions. The factors block and time were also included in the model, and Intra \times time and Inter \times time were tested as per the predictions that the effects of intra- and interspecific interactions on RPD depth would become increasingly negative (antagonism) and positive (facilitation) over time, respectively. The effects of taxa densities on RPD depth were assumed not to vary over time on the premise that any changes in activity will result from interactions among organisms and thus will be picked up by Intra and Inter.

RESULTS

Cage effect

Sediment organic matter content and the densities of all but 2 taxa did not differ significantly between control and procedural control plots (Table 1). The cage effect consisted of a significant increase in *Corophium volutator* density and a significant decrease in *Tubificoides* spp. density (Table 1, Fig. 4).

Scrobicularia plana density vs. RPD depth

There was a significant negative linear relationship between RPD depth and *Scrobicularia plana* density

Table 1. General linear model summary of variation in taxa densities and sediment organic matter content across experimental blocks, over time (1, 5 and 9 wk) and in response to the experimental cage. Significant p-values ($p < 0.05$) are in **bold**

Source	df	F	p
<i>Corophium volutator</i>			
Block	3	2.316	0.1122
Time	2	16.520	0.0001
Cage	1	32.797	<0.0001
Residuals	17		
<i>Hediste diversicolor</i>			
Block	3	1.798	0.1858
Time	2	8.987	0.0022
Cage	1	0.036	0.8512
Residuals	17		
<i>Tubificoides</i> spp.			
Block	3	1.351	0.2911
Time	2	2.460	0.1153
Cage	1	15.447	0.0011
Residuals	17		
<i>Macoma balthica</i>			
Block	3	0.525	0.6710
Time	2	1.067	0.3659
Cage	1	0.576	0.4585
Residuals	17		
<i>Eteone longa</i>			
Block	3	1.320	0.3007
Time	2	2.099	0.1532
Cage	1	3.381	0.0835
Residuals	17		
<i>Scrobicularia plana</i>			
Block	3	1.673	0.2104
Time	2	0.638	0.5406
Cage	1	1.218	0.2852
Residuals	17		
Organic matter content			
Block	3	2.150	0.1316
Time	2	7.038	0.0059
Cage	1	0.031	0.8619
Residuals	17		

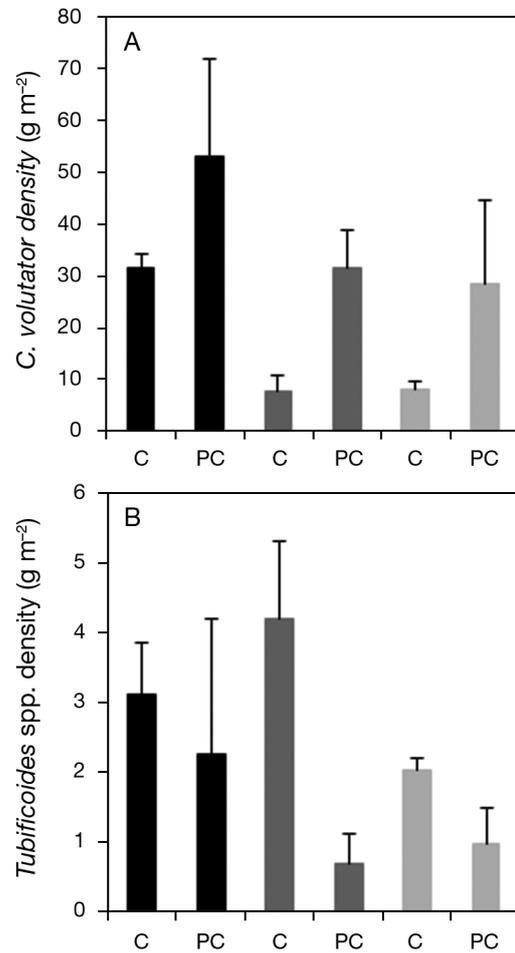


Fig. 4. Variation in the densities of (A) *Corophium volutator* and (B) *Tubificoides* spp. (with 95% CI) in response to the experimental cage (C: control, i.e. no cage; PC: procedural control, i.e. cage) at T1 (1 wk; black), T2 (5 wk; dark grey) and T3 (9 wk; light grey)

(Table 2, Fig. 5a). RPD depth varied significantly over time and was deepest at T1 and shallowest at T2, but *S. plana* density \times time was not significant (Table 2, Fig. 5a).

C. volutator density decreased linearly with increasing density of *S. plana* and was higher at T1 than at T2 and T3 (Table 2, Fig. 5b). *S. plana* density \times time was not significant (Table 2, Fig. 5b). Sediment organic matter content did not vary significantly with respect to *S. plana* density (Table 2). Therefore, only *C. volutator* density was retained as a potential mediator of the effect of *S. plana* density on RPD depth.

Causal mediation analysis revealed no significant ADE; however, the ACME and the total effect were statistically significant (Table 3). The linear decline in RPD depth with increasing *S. plana* density

Table 2. General linear model summary of variation in redox potential discontinuity (RPD) depth, sediment organic matter content and *Corophium volutator* density across experimental blocks, over time (1, 5 and 9 wk) and in relation to *Scrobicularia plana* density. Significant p-values ($p < 0.05$) are in **bold**

Source	df	F	p
RPD depth			
Block	3	0.357	0.7842
Time	2	11.128	0.0002
<i>S. plana</i>	1	6.421	0.0154
<i>S. plana</i> × time	2	1.139	0.3306
Residuals	39		
Organic matter content			
Block	3	1.664	0.1905
Time	2	6.031	0.0052
<i>S. plana</i>	1	1.030	0.3163
<i>S. plana</i> × time	2	1.756	0.1861
Residuals	39		
<i>Corophium volutator</i>			
Block	3	3.842	0.0168
Time	2	27.343	<0.0001
<i>S. plana</i>	1	17.271	0.0002
<i>S. plana</i> × time	2	0.415	0.6635
Residuals	39		

(Fig. 5a) was therefore fully mediated by a negative impact on the density of *C. volutator* (Fig. 5b), which had a positive influence over RPD depth (Fig. 5c) (i.e. P4; Fig. 1).

Sensitivity analysis revealed that the confidence intervals for ACME overlapped zero when $0.13 < \rho < 0.60$, which makes the ACME robust to small violations of sequential ignorability. Inspection of the relationships between the densities of *C. volutator* and other taxa revealed only one potential confounder in *Hediste diversicolor*, to which *C. volutator* was negatively related (see Table S2 and Fig. S2 in the Supplement at www.int-res.com/articles/suppl/m547p149_supp.pdf). This correlation was deemed unlikely to undermine the ACME (see 'Discussion').

Table 3. Average direct effect (ADE), average causal mediation effect (ACME, i.e. the effect mediated by *Corophium volutator* density) and total effect of *Scrobicularia plana* density on redox potential discontinuity (RPD) depth. Estimated effects (with 95% confidence intervals) are presented. Significant p-values ($p < 0.05$) are in **bold**

	Estimate	95% CI	p
ADE	-0.0016	-0.0056, 0.0025	0.4416
ACME	-0.0033	-0.0067, -0.0010	0.0044
Total effect	-0.0049	-0.0089, -0.0010	0.0168

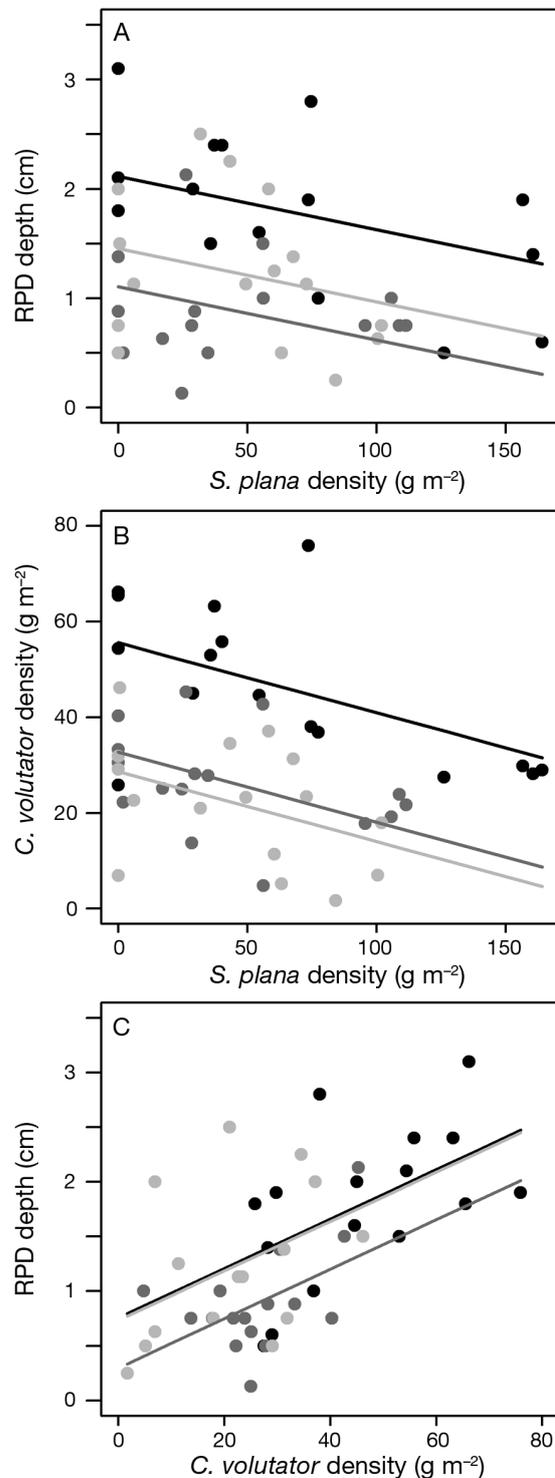


Fig. 5. Relationships between (A) redox potential discontinuity (RPD) depth and *Scrobicularia plana* density, (B) *Corophium volutator* density and *S. plana* density, and (C) RPD depth and *C. volutator* density at T1 (1 wk; black), T2 (5 wk; dark grey) and T3 (9 wk; light grey). Trend lines were predicted from models which had block fixed to 'A' and did not include interactions between the explanatory variables and time, as these terms were statistically insignificant

Density-dependent intra- and interspecific interactions vs. RPD depth

The relationship between RPD depth and Intra was initially slightly positive and became increasingly negative over time (Fig. 6a). While this result is consistent with a temporal increase in the effect of intraspecific antagonism on function delivery, the null hypothesis of no Intra \times time interaction could not be rejected ($p = 0.079$; Table 4).

The effect of Inter on RPD depth varied significantly over time (Table 4, Inter \times time). The relationship between RPD depth and Inter was initially

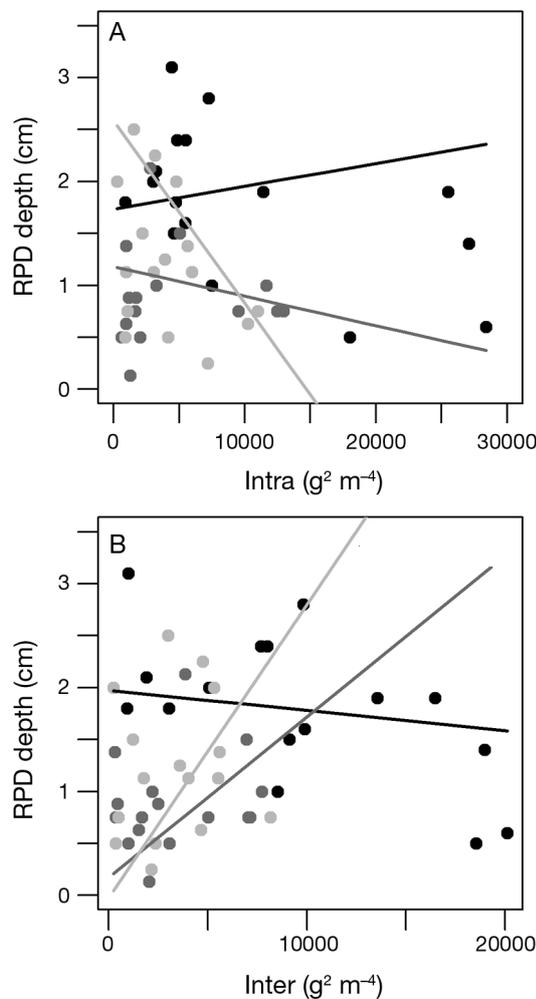


Fig. 6. Relationships between redox potential discontinuity (RPD) depth and (A) density-dependent intraspecific interactions (Intra), and (B) density-dependent interspecific interactions (Inter). Data are presented for sampling occasions T1 (1 wk; black), T2 (5 wk; dark grey) and T3 (9 wk; light grey). Trend lines were predicted from models which had block fixed to 'A' and all other explanatory variables (except the term of interest) fixed to the mean values across sampling occasions

Table 4. General linear model summary of variation in redox potential discontinuity (RPD) depth across experimental blocks, over time (1, 5 and 9 wk), in relation to taxa densities and in relation to indices for density-dependent intraspecific interactions (Intra) and interspecific interactions (Inter). Significant p -values ($p < 0.05$) are in **bold**

Term	df	F	p
Block	3	0.197	0.8974
Time	2	3.796	0.0339
<i>Scrobicularia plana</i>	1	0.130	0.7208
<i>Corophium volutator</i>	1	0.119	0.7322
<i>Hediste diversicolor</i>	1	1.549	0.2229
<i>Tubificoides</i> spp.	1	2.151	0.1529
<i>Macoma balthica</i>	1	1.344	0.2555
<i>Eteone longa</i>	1	0.446	0.5094
Intra	1	1.194	0.2833
Inter	1	0.199	0.6585
Intra \times time	2	2.766	0.0790
Inter \times time	2	4.562	0.0186
Residuals	30		

slightly negative, and as predicted, became increasingly positive as the experiment progressed, indicating a temporal increase in the effect of interspecific facilitation on function delivery (Fig. 6b).

DISCUSSION

Previous BEF experiments conducted within marine sediments have suggested that biotic influence over function delivery is driven by individual, functionally dominant species (Gamfeldt et al. 2015). Complementarity among species, although prevalent in terrestrial plant assemblages (Loreau & Hector 2001, Tilman et al. 2014) and possibly freshwater sediments (Mermillod-Blondin et al. 2003, Caliman et al. 2011), has not previously been identified as an important driver of marine sediment functioning (Gamfeldt et al. 2015, Strong et al. 2015). Our results reaffirm the direct functional importance of certain marine benthic species, but also point to the indirect functional importance of other species to which their density is tightly coupled. Additionally, our results suggest that interspecific facilitation and possibly intraspecific antagonism play roles in benthic ecosystem functioning, and that these effects increase over time (on the scale of weeks). This study therefore provides some evidence in marine sediments for BEF mechanisms that previously had little support.

In accordance with other studies that manipulated the density of large bivalves (e.g. Jensen 1985, Beadman et al. 2004), the density of the mud shrimp *Corophium volutator* decreased with increasing den-

sity of *Scrobicularia plana* (Fig. 5b). Such declines in *Corophium* density have previously been attributed to disturbance of its burrows due to sediment reworking by the common cockle *Cerastoderma edule* (Jensen 1985) and surface organic matter deposition (faeces and pseudofaeces) by the blue mussel *Mytilus edulis* (Beadman et al. 2004). Since *S. plana* disturbs the sediment both through feeding and organic matter deposition, it is possible that either or both of these activities explain the negative impact on *C. volutator* density. Indeed, we found no evidence for net organic matter deposition or consumption by *S. plana*, suggesting that both processes may have contributed to a similar degree. While *S. plana* density had a negative impact on the *C. volutator* population, *C. volutator* density increased as a result of a cage effect in our study, but not beyond the range of abundance observed in natural populations in the UK (a max. of 75 000 ind. m⁻² were recorded here, compared to >100 000 ind. m⁻² recorded in the Stour Estuary, Suffolk; Hughes 1988). This experimental artefact was therefore deemed not to have created unrealistic conditions.

The reduction in RPD depth with decreasing *C. volutator* density (Fig. 5c) is consistent with previous observations (Limia & Raffaelli 1997) and can be explained by a reduced capacity of *Corophium* populations to rework the sediment as density declines (De Backer et al. 2011). Reduced densities of 'bioturbator' species have been found to negatively impact marine sediment functioning in various studies, including those conducted in the field (e.g. Lohrer et al. 2004, Norkko et al. 2013). While the ACME (the impact of *S. plana* density on RPD depth via *C. volutator* density) in our analysis was robust only to small violations of sequential ignorability (i.e. the non-existence of confounders; see Imai et al. 2010), the only potential confounder identified within the macroinfaunal assemblage was the density of the ragworm *Hediste diversicolor*; to which *C. volutator* density was negatively related. Since *H. diversicolor* reworks the sediment and ventilates burrows (Christensen et al. 2000, Hale et al. 2014), and thus any influence of this species on RPD depth would be expected to be positive, it was deemed unlikely that the negative correlation with *C. volutator* density undermined the ACME. The absence of a direct influence of *S. plana* density on RPD depth could be due to its low mobility and high per capita biomass, as ventilation of vertical burrows by relatively few individuals may be insufficient to increase oxygen penetration at the patch scale.

Although the influence of *S. plana* density (via *C. volutator* density) on RPD depth did not weaken

temporally, the influence of density-dependent interspecific interactions on RPD depth did become increasingly positive over time, as predicted (Fig. 6b). This result suggests that interspecific facilitation becomes an increasingly important driver of benthic ecosystem functioning as time progresses, which is consistent with the temporal emergence of complementarity among marine macroalgae in natural, environmentally heterogeneous systems (e.g. Stachowicz et al. 2008). To date, most marine sediment BEF experiments have been conducted within homogeneous laboratory microcosms. Others have been conducted for short durations (≤ 2 wk) in field mesocosms (e.g. Godbold et al. 2009b) or environmentally heterogeneous laboratory microcosms (e.g. Godbold et al. 2011). The results of our field experiment suggest that the same meticulously-designed experiments, if conducted for longer durations and in natural heterogeneous environments, may show complementarity to be a more important driver of ecosystem functioning than previously suggested. That said, short-term microcosm experiments using freshwater sediments have shown consistent complementarity effects among macroinfauna (e.g. Mermillod-Blondin et al. 2003, Caliman et al. 2011), which appear to increase in strength for more integrative measures of ecosystem functioning (Caliman et al. 2012, 2013). It is therefore possible that the apparent dominance of individual species over marine sediment functioning is biased by a tendency for previous studies to focus on a few simple ecosystem functions (e.g. benthic–pelagic nutrient flux). Here, our focus on RPD depth, a sedimentary property that influences multiple benthic ecosystem functions (Solan et al. 2004, Teal et al. 2010), may have left us well-placed to detect biotic effects relating to complementarity. Therefore, as well as conducting longer experiments in the field, future BEF studies may be more likely to detect complementarity by considering more integrative measures of ecosystem functioning.

Unlike most BEF studies, we considered the influence of intraspecific interactions on ecosystem functioning in our analyses. Whereas any trait dissimilarities among species will aid their ability to partition niches and may promote facilitation (Hooper et al. 2005), high densities of a single species may lead to reduced individual performance, if, for example, space limitation causes territorial behaviour and reduced activity (Bridges et al. 1996). Consistent with an increasing effect of intraspecific antagonism over time, we found that the relationship between RPD depth and density-dependent intraspecific interac-

tions became increasingly negative as the experiment progressed (Fig. 6a); however, we could not categorically reject the null hypothesis ($p = 0.079$). Our results therefore provide weaker evidence for intraspecific antagonism than interspecific facilitation as a driver of ecosystem functioning. We also note here that while the development of pools during the experiment represents a potential confounder of the relationships between intra- and interspecific interactions and RPD depth, the consistent relationship between *S. plana* density and RPD depth (via *C. volutator* density) over time suggests that the pools did not have a major influence on relationships between the macroinfaunal assemblage and benthic functioning.

In line with calls to increase realism in BEF research (e.g. Hillebrand & Matthiessen 2009) we used an *in situ* experiment to assess how the functioning of an intertidal mudflat was impacted after simulating a realistic pattern of biodiversity change. While some consistency was found with laboratory experiments that have assessed marine sediment BEF relationships (i.e. the importance of the density of functionally dominant species), the suggestion that additional mechanisms are in operation reiterates the need to manipulate natural assemblages in order to understand how ongoing biodiversity change is likely to impact ecosystem functioning (see Gamfeldt et al. 2015). Contrary to the modern paradigm of biodiversity conservation, our results imply that a decline or loss of a population can enhance function delivery if it causes the population of a functionally dominant species to increase. Likewise, a non-native species introduction could reduce function delivery if it causes the population of a functionally dominant native species to decline or go extinct (see Gurevitch & Padilla 2004). Perhaps most noteworthy from a conservation perspective, however, is our finding that the effects of interspecific facilitation, and possibly intraspecific antagonism, increased as the experiment progressed, which suggests that diverse species assemblages may generally promote high function delivery.

This study provides an incremental step forward in developing our understanding of the impact of real-world biodiversity change on ecosystem functioning. While we have built on previous studies by conducting our experiment in the field and for a longer duration, as with all small-scale experiments there remain issues regarding the wider implications of the results. We selected a low diversity system for our study site, which is typical of intertidal mudflats, and it is under these conditions that we are most confident our findings will generally apply. The evidence presented

here, and elsewhere for low diversity marine systems (see Gamfeldt et al. 2015, Strong et al. 2015), highlights the importance of protecting species whose associated functions underpin ecosystem services (e.g. waste assimilation is underpinned by decomposition, which mainly occurs above the RPD depth; Teal et al. 2010). Our results also suggest that those charged with environmental and biodiversity protection need to consider the potential importance of species that have no apparent direct influence over functioning, but may influence the densities of species that do. The degree to which our results apply to more diverse marine sediments (and other ecosystems) with high functional redundancy remains unclear. Individual species may be of lesser importance under these conditions, but species losses could potentially induce changes to many populations and thus have large indirect impacts on functioning. Studies have yet to experimentally assess how changes to biodiversity affect ecosystem functioning in species-rich benthos, but methods are being proposed to integrate the design of such experiments with broad-scale patterns in seafloor biodiversity (e.g. Snelgrove et al. 2014). For now, the evidence presented here for facilitative interactions among species supports the general principle of maintaining diverse species assemblages so as to avoid potential declines in function delivery associated with biotic impoverishment.

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LITERATURE CITED

- Beadman HA, Kaiser MJ, Galanidi M, Shucksmith R, Willows RI (2004) Changes in species richness with stocking density of marine bivalves. *J Anim Ecol* 41:464–475
- Benedetti-Cecchi L, Maggi E (2012) The analysis of biodiversity-ecosystem function experiments: partitioning richness and density-dependent effects. In: Solan M, Aspiden RJ, Paterson DM (eds) *Marine biodiversity and ecosystem functioning: frameworks, methodologies and integration*. Oxford University Press, Oxford, p 73–195
- Beukema JJ (1979) Biomass and species richness of the macrobenthic animals living on a tidal flat area in the Dutch Wadden Sea: effects of a severe winter. *Neth J Sea Res* 13:203–223
- Birchough SNR, Parker RE, McManus E, Barry J (2012) Combining bioturbation and redox metrics: potential tools for assessing seabed function. *Ecol Indic* 12:8–16
- Boldina-Cosqueric I, Amiard JC, Amiard-Triquet C, Dédourge-Geffard O and others (2010) Biochemical, physiological and behavioural markers in the endobenthic bivalve *Scrobicularia plana* as tools for the assessment of

- estuarine sediment quality. *Ecotoxicol Environ Saf* 73: 1733–1741
- Bouma TJ, Olenin S, Reise K, Ysebaert T (2009) Ecosystem engineering and biodiversity in coastal sediments: posing hypotheses. *Helgol Mar Res* 63:95–106
- Bracken MES, Low NHN (2012) Realistic losses of rare species disproportionately impact higher trophic levels. *Ecol Lett* 15:461–467
- 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
- Braeckman U, Provoost P, Gribsholt B, van Gansbeke D and others (2010) Role of macrofauna functional traits and density in biogeochemical fluxes and bioturbation. *Mar Ecol Prog Ser* 399:173–186
- Bridges TS, Farrar JD, Gamble EV, Dillon TM (1996) Intraspecific density effects in *Nereis (Neanthes) arenaceodentata* Moore (Polychaeta: Nereidae). *J Exp Mar Biol Ecol* 195:221–235
- Buchanan JD, Kain JM (1984) Measurement of the physical and chemical environment. In: Holme NA, McIntyre AD (eds) *Methods for the study of marine benthos*. Blackwell Scientific Publications, Oxford, p 30–50
- Caliman A, Carneiro LS, Bozelli RL, Farjalla F, Esteves FA (2011) Bioturbating space enhances the effects of non-additive interactions among benthic ecosystem engineers on cross-habitat nutrient regeneration. *Oikos* 120: 1639–1648
- Caliman A, Carneiro LS, Leal JFF, Farjalla F, Bozelli RL, Esteves FA (2012) Community biomass and bottom up multivariate nutrient complementarity mediate the effects of bioturbator diversity on pelagic production. *PLoS One* 7:e44925
- Caliman A, Carneiro LS, Leal JFF, Farjalla F, Bozelli RL, Esteves FA (2013) Biodiversity effects of ecosystem engineers are stronger on more complex ecosystem processes. *Ecology* 94:1977–1985
- Cardinale BJ (2011) Biodiversity improves water quality through niche partitioning. *Nature* 472:86–89
- Cardinale BJ, Palmer MA, Collins SL (2002) Species diversity enhances ecosystem functioning through interspecific facilitation. *Nature* 415:426–429
- Chapin FS III, Walker BH, Hobbs RJ, Hooper DU, Lawton JH, Sala OE, Tilman D (1997) Biotic control over the functioning of ecosystems. *Science* 277:500–504
- Christensen B, Vedel A, Kristensen E (2000) Carbon and nitrogen fluxes in sediment inhabited by suspension-feeding (*Nereis diversicolor*) and non-suspension-feeding (*N. virens*) polychaetes. *Mar Ecol Prog Ser* 192: 203–217
- De Backer A, Van Coillie F, Montserrat F, Provoost P, Van Colen C, Vincx M, Degraer S (2011) Bioturbation effects of *Corophium volutator*: importance of density and behavioural activity. *Estuar Coast Shelf Sci* 91:306–313
- Diaz S, Cabido M (2001) Vive la différence: plant functional diversity matters to ecosystem processes. *Trends Ecol Evol* 16:646–655
- Duport E, Stora G, Tremblay P, Gilbert F (2006) Effects of population density on the sediment mixing induced by the gallery-diffuser *Hediste (Nereis) diversicolor* O.F. Müller, 1776. *J Exp Mar Biol Ecol* 336:33–41
- Emmerson MC, Solan M, Emes C, Paterson DM, Raffaelli D (2001) Consistent patterns and the idiosyncratic effects of biodiversity in marine ecosystems. *Nature* 411:73–77
- Essink K, Beukema JJ, Coosen J, Craeymeersch JA, Ducrotoy JP, Michaelis H, Robineau B (1991) Population dynamics of the bivalve mollusc *Scrobicularia plana* da Costa: comparisons in time and space. In: Elliott M, Ducrotoy JP (eds) *Estuaries and coasts: spatial and temporal intercomparisons*. Proc ECSA 19 Symp. Olsen & Olsen, Fredensborg, p 167–72
- Freeman RFH, Rigler FH (1957) The responses of *Scrobicularia plana* (da Costa) to osmotic pressure changes. *J Mar Biol Assoc UK* 36:553–567
- 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
- Garnier E, Cortez J, Billès G, Navas ME and others (2004) Plant functional markers capture ecosystem properties during secondary succession. *Ecology* 85:2630–2637
- Gerwing TG, Gerwing AMM, Drolet D, Hamilton DJ, Barbeau MA (2013) Comparison of two methods of measuring the depth of the redox potential discontinuity in intertidal mudflat sediments. *Mar Ecol Prog Ser* 487:7–13
- Gerwing TG, Gerwing AMM, Hamilton DJ, Barbeau M (2015) Apparent redox potential discontinuity (aRPD) depth as a relative measure of sediment oxygen content and habitat quality. *Int J Sediment Res* 30:74–80
- Godbold JA, Rosenberg R, Solan M (2009a) Species-specific traits rather than resource partitioning mediate diversity effects on resource use. *PLoS One* 4:e7423
- Godbold JA, Solan M, Killham K (2009b) Consumer and resource diversity effects on marine macroalgal decomposition. *Oikos* 118:77–86
- Godbold JA, Bulling MT, Solan M (2011) Habitat structure mediates biodiversity effects on ecosystem properties. *Proc R Soc B* 278:2510–2518
- Gray JS, Wu RS, Or YY (2002) Effects of hypoxia and organic enrichment on the coastal marine environment. *Mar Ecol Prog Ser* 238:249–279
- Green J (1967) Activities of the siphons of *Scrobicularia plana* (da Costa). *Proc Malac Soc Lond* 37:339–341
- Gurevitch J, Padilla DK (2004) Are invasive species a major cause of extinctions? *Trends Ecol Evol* 19:470–474
- Hale R, Mavrogordato MN, Tolhurst TJ, Solan M (2014) Characterizations of how species mediate ecosystem properties require more comprehensive functional effect descriptors. *Sci Rep* 4:6463
- Hillebrand H, Matthiessen B (2009) Biodiversity in a complex world: consolidation and progress in functional biodiversity research. *Ecol Lett* 12:1405–1419
- Hooper DU, Chapin FS, Ewel JJ, Hector A and others (2005) Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecol Monogr* 75:3–35
- Hughes RN (1969) A study of feeding in *Scrobicularia plana*. *J Mar Biol Assoc UK* 49:805–823
- Hughes RG (1988) Dispersal by benthic invertebrates: the *in situ* swimming behaviour of the amphipod *Corophium volutator*. *J Mar Biol Assoc UK* 68:565–579
- Hulthe G, Hulth S, Hall POJ (1998) Effect of oxygen on degradation rate of refractory and labile organic matter in continental margin sediments. *Geochim Cosmochim Acta* 62:1319–1328
- Hurlbert SH (1984) Pseudoreplication and the design of ecological field experiments. *Ecol Monogr* 54:187–211
- Ieno EN, Solan M, Batty P, Pierce GJ (2006) How biodiversity affects ecosystem functioning: roles of infaunal spe-

- cies richness, identity and density in the marine benthos. *Mar Ecol Prog Ser* 311:263–271
- Imai K, Tingley D, Keele L (2010) A general approach to causal mediation analysis. *Psychol Methods* 15:309–334
 - Jensen KT (1985) The presence of the bivalve *Cerastoderma edule* affects migration, survival and reproduction of the amphipod *Corophium volutator*. *Mar Ecol Prog Ser* 25: 269–277
 - Karlson AML, Nascimento FJA, Näslund J, Elmgren R (2010) Higher diversity of deposit-feeding macrofauna enhances phytodetritus processing. *Ecology* 91:1414–1423
 - Larsen TH, Williams NM, Kremen C (2005) Extinction order and altered community structure rapidly disrupt ecosystem functioning. *Ecol Lett* 8:538–547
 - Limia J, Raffaelli D (1997) The effects of burrowing by the amphipod *Corophium volutator* on the ecology of intertidal sediments. *J Mar Biol Assoc UK* 77:409–423
 - Lohrer AM, Thrush SF, Gibbs MM (2004) Bioturbators enhance ecosystem function through complex biogeochemical interactions. *Nature* 431:1092–1095
 - Loreau M, Hector A (2001) Partitioning selection and complementarity in biodiversity experiments. *Nature* 412: 72–76
 - Mermillod-Blondin F, des Chatelliers MC, Gerino M (2003) Effects of the interactions between tubificid worms on the functioning of hyporheic sediments: an experimental study. *Arch Hydrobiol* 156:203–223
 - Naem S, Duffy JE, Zavaleta E (2012) The functions of biological diversity in an age of extinction. *Science* 336: 1401–1406
 - 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
 - Norling K, Rosenberg R, Hulth S, Grémare A, Bonsdorff E (2007) Importance of functional biodiversity and species-specific traits of benthic fauna for ecosystem functions in marine sediment. *Mar Ecol Prog Ser* 332:11–23
 - O'Connor NE, Crowe TP (2005) Biodiversity loss and ecosystem functioning: distinguishing between species number and identity of species. *Ecology* 86:1783–1796
 - R Development Core Team (2014) R: a language and environment for statistical computing. R Foundation for Statistical Computing. www.r-project.org
 - Riley KF, Hobson MP, Bence SJ (2002) *Mathematical methods for physics and engineering*, 2nd edn. Cambridge University Press, Cambridge
 - Ruardij P, van Raaphorst W (1995) Benthic nutrient regeneration in the ERSEM ecosystem model of the North Sea. *Neth J Sea Res* 33:453–483
 - Ruesink JL, Srivastava DS (2001) Numerical and per capita responses to species loss: mechanisms maintaining ecosystem function in a community of stream insect detritivores. *Oikos* 93:221–232
 - Santos S, Luttikhuisen PC, Campos J, Heip CHR, van der Veer HW (2011) Spatial distribution of the peppery furrow shell *Scrobicularia plana* (da Costa, 1778) along the European coast: a review. *J Sea Res* 66:238–247
 - Silvertown J (2004) Plant coexistence and the niche. *Trends Ecol Evol* 19:605–611
 - Snelgrove PVR, Thrush SF, Wall DH, Norkko A (2014) Real world biodiversity-ecosystem functioning: a seafloor perspective. *Trends Ecol Evol* 29:398–405
 - Solan M, Cardinale BJ, Downing AL, Engelhardt KAM, Ruesink JL, Srivastava DS (2004) Extinction and ecosystem function in the marine benthos. *Science* 306: 1177–1180
 - Srivastava DS, Vellend M (2005) Biodiversity-ecosystem function research: Is it relevant to conservation? *Annu Rev Ecol Syst* 36:267–294
 - Stachowicz JJ, Best RJ, Bracken MES, Graham MH (2008) Complementarity in marine biodiversity manipulations: reconciling divergent evidence from field and mesocosm experiments. *Proc Natl Acad Sci USA* 105:18842–18847
 - Strong JA, Andonegi E, Bizsel KC, Danovaro R and others (2015) Marine biodiversity and ecosystem function relationships: the potential for practical monitoring applications. *Estuar Coast Shelf Sci* 161:46–64
 - Teal LR, Parker ER, Solan M (2010) Sediment mixed layer as a proxy for benthic ecosystem process and function. *Mar Ecol Prog Ser* 414:27–40
 - 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
 - Tilman D, Isbell F, Cowles JM (2014) Biodiversity and ecosystem functioning. *Annu Rev Ecol Syst* 45:471–493
 - Tingley D, Yamamoto T, Hirose K, Keele L, Imai K (2014) mediation: R package for causal mediation analysis. *J Stat Softw* 59:1–38
 - Tiunov AV, Scheu S (2005) Facilitative interactions rather than resource partitioning drive diversity-functioning relationships in laboratory fungal communities. *Ecol Lett* 8:618–625
 - Wacker L, Baudois O, Eichenberger-Glinz S, Schmid B (2008) Environmental heterogeneity increases complementarity in experimental grassland communities. *Basic Appl Ecol* 9:467–474
 - Wetzel MA, Leuchs H, Koop JHE (2005) Preservation effects on wet weight, dry weight, and ash-free dry weight biomass estimates of four common estuarine macroinvertebrates: no difference between ethanol and formalin. *Helgol Mar Res* 59:206–213
 - Ysebaert T, Herman PMJ (2002) Spatial and temporal variation in benthic macrofauna and relationships with environmental variables in an estuarine, intertidal soft-sediment environment. *Mar Ecol Prog Ser* 244:105–124

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