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Assessing the consequences of environmental impacts: variation in species responses has unpredictable functional effects

Fiona Murray[1,3,*], Stephen Widdicombe[2], C. Louise McNeill[2], Alex Douglas[1]

[1]Institute of Biological and Environmental Sciences, University of Aberdeen, Zoology Building, Tillydrone Avenue, Aberdeen AB24 2TZ, UK

[2]Plymouth Marine Laboratory, Prospect Place, West Hoe, Plymouth PL1 3DH, UK

[3]*Present address:* School of Geosciences, The Grant Institute, University of Edinburgh, King's Buildings, James Hutton Road, Edinburgh EH9 3FE, UK

ABSTRACT: Many biological processes underpin ecosystem functioning and health. Determining changes in these processes following disturbance is crucial in assessing the wider impacts on ecosystem function and ultimately ecosystem services. Whilst the focus is often on whether disturbance drives changes in ecosystem function through mortality, sub-lethal effects on the physiology and behaviour of organisms may also have cascading effects on ecosystem processes, functions and services. In this mesocosm study, we investigated the effects of a severe short-term exposure (8 d) to a simulated environmental impact — a leak of a subsea geological CO_2 capture and storage reservoir — on key biological processes (bioturbation), an ecosystem function (nutrient cycling) and on the functional group composition for 7 common benthic invertebrate species. We statistically allocated species to functional effect groups based on their measured functional effect relative to other species. Following exposure, we observed behavioural responses driving changes in bioturbation for several species and altered nutrient cycling. Responses were species specific and resulted in shifts in functional effect group composition for some key nutrients (nitrate and silicate). We show that the allocation of species to functional groups by measuring specified ecosystem processes and functions can change following environmental perturbations. This implies that whilst biodiversity and ecosystem functioning are intricately linked, maintaining species identities and abundances after environmental perturbation is no guarantee to maintaining ecosystem functions, as species alter their rate and mode of activity following an environmental stress.

KEY WORDS: Benthic invertebrates · Bioturbation · Carbon dioxide capture and storage · CCS · Ecosystem function · Functional diversity · Functional groups · Ocean acidification

INTRODUCTION

Ecosystem functioning is widely recognised to be an important component of ecosystem health (Tett et al. 2013), and there is strong evidence that changes in biodiversity lead to shifts in ecosystem functioning (Cardinale et al. 2012). Such is the strength of evidence supporting this assertion that many marine environmental policies explicitly acknowledge the need to ensure the sustainable functioning of aquatic ecosystems (e.g. the European Marine Strategy Framework Directive 2008/56/EU). The potential for communities and ecosystems to recover from perturbations will depend largely on the functional resilience of a given ecosystem (Hawkes & Keitt 2015), which, in turn, depends on the responses of individ-

*Corresponding author: fiona.murray25@gmail.com

ual species. However, the sub-lethal behavioural and physiological responses of organisms that pre-empt changes in community structure are rarely considered following acute environmental impact events, and taxonomic inventories are widely used as proxies to assess the effects on ecosystem function (e.g. oil spills, Law et al. 2011; fires, Smith et al. 2014). Consequently, studies into the functional effects of environmental changes tend to rely on assessing shifts in community composition, either directly through the loss of species (e.g. de Juan et al. 2007) or indirectly through the loss of traits (Papageorgiou et al. 2009).

There are 2 difficulties with this approach. Firstly, the relevance of species, groups of species or traits to specific ecosystem functions is not always known and is often assumed rather than examined empirically (Petchey & Gaston 2006, Teal et al. 2008). Consequently, the mechanistic link between species and ecosystem properties may be weak or invalid (Luck et al. 2012). Secondly, an underlying assumption is that as long as a given set of species or traits remain present within a community, levels of ecosystem functioning will remain constant; however, this assumption is not supported by studies where the functional performance of a species has been shown to vary with context (Wardle et al. 2008, Needham et al. 2011). This has implications for estimating functional redundancy and understanding the role biodiversity plays in underpinning ecosystem functioning, especially when supporting evidence-based policy making (Svancara et al. 2005, Hodapp et al. 2014).

This issue is of particular concern in marine ecosystems where there is a well-established precedent for using functional group and functional trait approaches in assessing ecosystem health (for a summary, see Murray et al. 2014). However, the application of these approaches often fails to link the functional groups identified to explicit individual ecosystem functions and is incapable of accounting for the strong behavioural (e.g. de la Haye et al. 2011), physiological (e.g. Dissanayake & Ishimatsu 2011) and phenological (Yang & Rudolf 2010) responses to environmental stressors seen in marine organisms. As such, it has been argued elsewhere that assigning species to functional groups based on traits alone is insufficient and that establishing the contributions of individual species to specified ecosystem functions is required (Murray et al. 2014).

In marine benthic systems, the process known as bioturbation (particle reworking and bioirrigation due to the activities of benthic fauna) has often been used to demonstrate a link between species composition and ecosystem function (e.g. bioturbation and nutrient cycling, Solan et al. 2008; benthic–pelagic coupling, Kristensen et al. 2014). Consequently, bioturbation activity is often used as proxy of wider ecosystem functioning (e.g. nutrient cycling), both for classifying species into functional groups (e.g. Solan & Wigham 2005) and as an as indicator of environmental change (Thrush et al. 2006; for a review, see Queirós et al. 2015).

Soft sediment benthic communities are particularly vulnerable to anthropogenic impacts, such as pollution (Lee & Cundy 2001) and hypoxia due to organic enrichment (Gray et al. 2002). When extreme, these impacts can cause mortalities in the benthic community, but often they will stress rather than kill individual organisms. This will impact on these organism's behaviours and therefore their contributions to ecosystem functioning (i.e. they will have sub-lethal effects). These communities can support biodiversity, ecosystem functioning and ecosystem services; therefore, an understanding of the sub-lethal effects of environmental stresses on benthic organisms is important.

Here we simulated a CO_2 leak from a geological carbon dioxide capture and storage (CCS) reservoir as an example of a perturbation event to investigate effects of environmental stress on bioturbation and the consequences for ecosystem functioning and functional group composition. In many countries, plans to implement CCS are primarily offshore and likely beneath biologically diverse sediments on the continental shelf (Widdicombe et al. 2015). Whilst the risk of a leak from CCS infrastructure is likely to be small, it is generally acknowledged that leakage will occur over time, potentially affecting benthic ecosystems (Blackford et al. 2014). Short-term exposure to acidified seawater does not always kill adult organisms outright, yet sub-lethal effects have been reported (for a review, see Kroeker et al. 2011), and the consequences for ecosystem functioning are rarely examined. Such attributes make high CO_2 exposure an ideal, real-world vector for delivering environmental stress to our experimental system.

Our overall objective was to characterise the functional contributions of 7 common benthic invertebrate species to 2 benthic ecosystem processes (particle reworking and bioirrigation). These processes have strong links to the provision of ecosystem functioning (nutrient cycling inferred from concentrations of NH_4^+-N, NO_X^--N, PO_4^{3-}-P and SiO_2-Si). The nature and rate of these processes were examined under 2 environmental regimes: (1) ambient seawater with natural levels of CO_2 and (2) seawater

Table 1. Selected species attributes traditionally ascribed as functional traits (bioturbation mode and appropriate traits from Bremner et al. 2003)

Species	Feeding habit	Bioturbation mode	Adult mobility	Adult life habit
Amphiura filiformis (O.F. Müller, 1776) (Brittle star)	Suspension feeder	Upward conveyer	High	Burrow
Anapagurus laevis (Bell, 1846) (Hermit crab)	Opportunist/scavenger	Epifaunal	High	Crawl
Aporrhais pespelecani (Linnaeus, 1758) (Pelican's foot shell)	Deposit feeder	Epifaunal	High	Crawl
Chamelea gallina (Linnaeus, 1758) (Venus clam)	Suspension feeder	Biodiffuser	High	Burrow
Nucula hanleyi Winckworth, 1931 (Nut clam)	Deposit feeder	Biodiffuser	High	Burrow
Sternaspis scutata (Ranzani, 1817) (Bristleworm)	Deposit feeder	Biodiffuser	High	Burrow
Turritella communis Risso, 1826 (Tower shell)	Suspension feeder	Biodiffuser	High	Burrow/crawl

with elevated levels of CO_2 representative of a CCS leak. The data generated were then used to statistically allocate species to functional effect groups based on their measured functional effect, i.e. for bioturbation and nutrient cycling, relative to the other species. Species were selected from 4 phyla (Arthropoda, Mollusca, Echinodermata and Annelida) and represented species that would have been classified into different functional groups using traditional methodologies (see Table 1). We hypothesised that the stress of exposure to seawater with elevated levels of CO_2 would affect particle reworking and bioirrigation, which in turn would influence ecosystem functioning (nutrient cycling). We further hypothesised that functional effect groupings would be identifiable but that these would vary between response variables and change following exposure to the high CO_2 seawater.

MATERIALS AND METHODS

Sediment and fauna collection

Sediment was collected from Cawsand Bay, Plymouth Sound (approximately 15 m water depth, 50° 19.8′ N, 04° 11.5′ W) using an anchor dredge. It was sieved (500 µm mesh) in a seawater bath to remove macrofauna, allowed to settle for 24 h to retain the fine fraction and homogenised by stirring, before being added to individual cores (capped PVC cores, 100 mm diameter, 200 mm tall) to a depth of 150 mm and overlain by 50 mm seawater. Macrofauna representing 4 common benthic phyla (Mollusca, Annelida, Arthropoda and Echinodermata, Table 1) were obtained from Plymouth Sound using an anchor dredge (*Amphiura filiformis* and *Sternaspis scutata*) or a beam trawl (*Aporrhais pespelecani, Chamelea gallina, Nucula hanleyi, Turritella communis* and *Anapagurus laevis*). Specimens were separated by species and kept in seawater on deck before being transferred to Plymouth Marine Laboratory, where they were held in a recirculating seawater system until they were used in the exposure trials. Sediment cores were kept for 8 wk prior to use in the experimental runs. This allowed time for the natural sediment stratification and microbial community to recover from the defaunation process (Stocum & Plante 2006).

Seawater acidification and exposure

Carbon dioxide (CO_2) gas was bubbled through natural seawater (salinity 35). Gas flow was controlled via a solenoid valve connected to the gas cylinder to maintain the pH (following methods described by Widdicombe & Needham 2007). Seawater acidification was monitored using a pH controller (Aqua Digital pH-201, accuracy 0.1 ± 0.02%), which was crosschecked weekly against values given by a regularly calibrated pH meter (NBS scale, InLab® 413SG, Mettler-Toledo). Two 1 m^3 acclimatisation tanks, one containing the acidified seawater (pH = 6.5) and one containing ambient seawater (pH = 8.1), were used to acclimatise both the invertebrates and the sediment (with meiofauna and microorganisms) prior to the experiment. The pH level for the acidifi-

cation treatment was determined from modelled simulations (pipeline scenario: Blackford et al. 2013). Cores containing individuals of a single species (*A. filiformis, A. pespelecani, C. gallina, N. hanleyi, S. scutata* and *T. communis*: at 5 ind. core^{-1}, density equivalent to 640 ind. m^{-2}; *A. laevis*: at 1 ind. core^{-1}, density equivalent to 127 ind. m^{-2}) or no macrofauna were positioned randomly in the recirculating sea water system for 96 h prior to the start of the experiment (5 cores species^{-1} treatment^{-1}, n = 80, Fig. 1). These densities were similar to natural densities for *A. filiformis, A. laevis* and *S. scutata* but higher for the other species. Cores containing no macrofaunal species (n = 10) were maintained to determine individual species effects from the background levels created by resident micro- and meiofaunal organisms. Salinity, temperature and alkalinity (using an Apollo Scitech total alkalinity titrator) were monitored in both acclimatisation tanks 3 times wk^{-1} (Monday, Wednesday, Friday) throughout the duration of the experiment. Unmeasured carbonate parameters were calculated from these data using constants supplied by Lueker et al. (2000) and Millero (2010) with *CO_2Calc*, an application developed by the US Geological Survey Florida Shelf Ecosystems Response to Climate Change Project (Robbins et al. 2010).

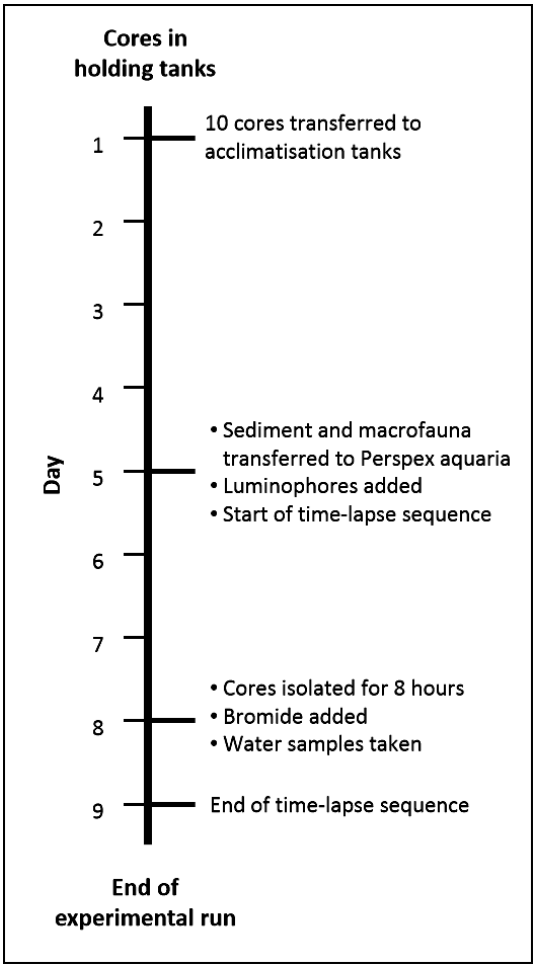

Fig. 1. Timeline of the experimental procedure

Experimental setup

Following the 5 d acclimatisation period, sediment and macrofauna from individual cores were transferred into individual rectangular thin-walled (5 mm) Perspex aquaria (1 core aquarium^{-1}, 33 × 10 × 10 cm, *A. filiformis, A. pespelecani, C. gallina, N. hanleyi, S. scutata* and *T. communis*: density equivalent to 500 ind. m^{-2}; *A. laevis*: density equivalent to 100 ind. m^{-2}). Cores were uncapped and the sediment pushed through the core into the aquarium maintaining the sediment stratification to minimise disturbance. Water was added slowly to each aquarium using a polystyrene disc to avoid resuspension of particles. Each aquarium was maintained in a temperature-controlled room (10°C) and supplied with seawater (using a flow-through system from the acclimatisation tanks) at the appropriate pH level and at a rate of 10 ml min^{-1} using a peristaltic pump (Watson-Marlow 323, following Widdicombe & Needham 2007). Due to space limitations, aquaria were randomly allocated to one of 8 consecutive experimental runs, ensuring that species replicates were mixed across multiple runs. Each run took 9 d to complete (Fig. 1). Ten aquaria were used in each run: 5 treatment aquaria and 5 controls.

Measures of particle reworking and bioirrigation

Particle reworking was measured non-invasively using fluorescent sediment profile imaging (Solan et al. 2004) and fluorescent-dyed sediment particles (luminophores, 20 g aquarium^{-1}; Partrac Tracer 2290 pink, size 125–355 µm). Aquaria were housed in a UV imaging box to determine the distribution of luminophores at high spatial resolution (67 µm). Luminophores were distributed across the sediment surface of each aquarium and imaged at 0 and 72 h using a Canon 400D, 10.14-megapixel camera (set to a shutter speed of 0.25 s, aperture f = 5.6, sensitivity equivalent to ISO 400) controlled using third party software (GB Timelapse, v2.0.20.0). Image analysis was conducted using a custom-made semi-automatic

macro in ImageJ (v1.44, http://rsbweb.nih.gov/ij/download.html). From these images (n = 70, following Murray et al. 2014) the maximum (Lum_{max}), mean (Lum_{mean}) and median (Lum_{med}) vertical distributions of luminophores were identified after 72 h. The rugosity of the lower extent of the mixed layer (Lum_{rug}) was calculated as the sum of the Euclidian distances between the deepest luminophores in adjacent pixel columns across the width of the image. These descriptors provide an indication of the maximum (Lum_{max}) and typical (Lum_{mean}, Lum_{med}) extent of vertical particle redistribution, as well as the lateral extent of surficial infaunal activity (Lum_{rug}).

Bioirrigation activity was estimated from changes in water column concentrations of an inert tracer (sodium bromide, NaBr, dissolved in seawater [Br^-] = 800 ppm, 5 mmol l^{-1}, stirred into the overlying seawater) on Day 8 of each experimental run, during which time the aquaria were isolated from the seawater supply for 8 h. Water samples (5 ml) were taken at 0, 1, 2, 4 and 8 h and immediately filtered (47 mm ∅ GF/F filter) and frozen (−18°C). Br^- concentration was analysed using colorimetric analysis (FIAstar 5000 flow injection analyser, FOSS Tecator). From these data, the maximal time for the redistribution of tracer prior to reaching equilibrium was determined to be 4 h (consistent with Forster et al. 1999), and therefore, the change in the relative concentration of Br^- ($\Delta[Br^-]$) was calculated over 4 h.

Measures of ecosystem functioning (nutrient sediment fluxes)

Water samples (50 ml, 47 mm ∅ GF/F filter, at $t = 0$ and $t = 8$ h) were taken to determine the change in nutrient concentrations (NH_4^+-N, NO_x^--N, PO_4^{3-}-P and SiO_2-Si) over 8 h whilst the aquaria were isolated from the seawater supply and analysed using a nutrient autoanalyser (Branne & Luebbe, AAIII).

Statistical analysis

Species-specific differences and the effects of elevated CO_2 levels on particle reworking, bioirrigation and changes in nutrient concentrations were investigated using mixed effects models. Individual models were developed for each of the response variables examined (Lum_{max}, Lum_{mean}, Lum_{med} and Lum_{rug}, each based on data extracted from the 72 h images, and $\Delta[Br^-]$, $\Delta[NH_4^+$-N], $\Delta[NO_x^-$-N], $\Delta[PO_4^{3-}$-P] and $\Delta[SiO_2$-Si]), with species identity and acidification treatment used as explanatory variables. Models for changes in nutrient concentrations were run using data from all aquaria including the no-macrofauna aquaria to establish the effects of elevated seawater CO_2 levels on nutrient changes associated with the meio- and microfauna in the sediment. No-macrofauna aquaria were excluded from models on particle reworking and bioirrigation. Following Murray et al. (2014), species were grouped by functional effect size. Subsets of species that did not significantly differ ($p > 0.05$) were grouped together with respect to their contribution (relative to each other, in absolute terms) to ecosystem processes (particle reworking and bioirrigation) and functions (nutrient concentrations) under study. Species were only grouped together if every species did not significantly differ from every other species in that group. Where a significant interaction was detected between species identity and acidification treatment, functional differences between species were determined separately for each treatment to determine any changes in functional group composition.

In all of our models, species identity and seawater acidification (elevated CO_2 levels) were included as fixed effects and experimental run was included as a random effect to estimate and account for inter-run variance. During the initial model-building phase of the analysis, diagnostic residual plots indicated the presence of heteroscedasticity due to differences in species-specific variances for Lum_{max}, Lum_{mean}, Lum_{med}, Lum_{rug}, $\Delta[NH_4^+$-N], $\Delta[PO_4^{3-}$-P] and $\Delta[SiO_2$-Si] and differences in acidification treatment-specific variances for Lum_{max}, Lum_{rug} and $\Delta[Br^-]$, not accounted for by the fixed and random effects. Where appropriate, a species-specific or a pH level-specific variance covariate was included to model the variance (Pinheiro & Bates 2000). Following the inclusion of variance covariates, diagnostic residual plots indicated homoscedasticity. Significance of the fixed effects was assessed by comparing nested models fitted using maximum likelihood (ML) followed by likelihood ratio tests. Parameters in the final models were estimated using restricted maximum likelihood (REML, following West et al. 2007). Details of the initial and minimum adequate models for each variable (Models S1–S8, Figs. S1–S8) are included in the Supplement at www.int-res.com/articles/suppl/m583p035_supp.pdf.

All mixed modelling analyses were carried out using the nlme package, within the R statistical and programming environment (v2.15.0, R Development Core Team 2011). Figures were produced using the ggplot2 (Wickham 2009) package within R.

Table 2. Chemical and physical properties of seawater tanks (mean ± SD) during the experimental period. pH, salinity, temperature and alkalinity were measured. All other values (pCO_2; DIC: dissolved inorganic carbon; $\Omega_{aragonite}$: aragonite saturation state; $\Omega_{calcite}$: calcite saturation state) were calculated

	Ambient	Acidified
pH (NBS)	8.05 ± 0.07	6.51 ± 0.06
Temperature (°C)	11.29 ± 0.46	11.52 ± 0.55
Salinity	35.61 ± 0.23	35.29 ± 0.34
Alkalinity (mmol l^{-1})	2.63 ± 0.13	2.43 ± 0.03
pCO_2 (µatm)	418.36 ± 114.24	18 325.05 ± 2421.70
DIC (µmol kg^{-1})	2048.92 ± 72.28	2615.51 ± 129.11
$\Omega_{aragonite}$	2.33 ± 0.26	0.09 ± 0.01
$\Omega_{calcite}$	3.64 ± 0.41	0.13 ± 0.02

RESULTS

Observations

Seawater carbonate chemistry parameters (Table 2) within the recirculating seawater tanks were stable and species survival was 100% throughout the experiment. Dissolved oxygen concentrations in individual aquaria were not measured; however, visual examination of the sediment profile did not reveal any evidence of enhanced reduction below the sediment–water interface (e.g. changes in sediment colour, elevation of redox boundary; Lyle 1983) over the course of the experiment. Following exposure to acidified seawater, 3 species displayed behaviours that were not apparent during the acclimatisation period or in the ambient treatment aquaria. All individuals of the brittlestar *Amphiura filiformis* displayed emergent behaviour within minutes of exposure, typical of a stress response to hypoxia (Nilsson 1999), consistent with previous studies in which concentrations of dissolved oxygen were monitored and echinoderms displayed emergent behaviour in response to hypercapnia (Wood et al. 2009). The sea snail *Turritella communis* also spent more time emerged from the sediment under acidified conditions. Hermit crabs *Anapagurus laevis* were less responsive and would lean out of, or leave, their gastropod shells in acidified seawater (consistent with de la Haye et al. 2011), with one individual autotomising a cheliped. We observed no clear behavioural changes in *Aporrhais pespelecani, Chamelea gallina*, *Nucula hanleyi* and *Sternaspis scutata.*

Ecosystem processes (particle reworking and bioirrigation)

The 72 h images showed active particle reworking in both acidified and ambient treatments, and species-specific differences in vertical and lateral particle reworking. There was an interaction between species identity and seawater acidification effects on the maximum depth of particle reworking (Lum_{max}, Table 3, Fig. 2a). Lum_{max} was reduced in aquaria containing individuals of *A. filiformis* (mean ± SD: 1.48 ± 1.11 cm in ambient seawater, 0.40 ± 0.25 cm in acidified seawater), *N. hanleyi* (ambient: 1.05 ± 0.21 cm; acidified: 0.65 ± 0.26 cm) and *T. communis* (ambient: 1.03 ± 1.03 cm; acidified: 0.51 ± 0.27 cm) in contrast to *C. gallina* which had an increased Lum_{max} in acidified aquaria (0.54 ± 0.28 cm) compared to ambient seawater (0.42 ± 0.22 cm).

Analysis of Lum_{mean}, Lum_{med} and Lum_{rug} revealed no interactions between seawater acidification and

Table 3. Results from significant statistical models including test statistics (F), nominator and denominator degrees of freedom (df) and probability (p) and the corresponding model and tables provided in the Supplement at www.int-res.com/articles/suppl/m583p035_supp.pdf. Lum_{max}/Lum_{mean}/Lum_{med}: maximum/mean/median vertical distributions of luminophores; Lum_{rug}: rugosity of the lower extent of the mixed layer

Response	Explanatory variable	F	df	p	Model	Table
Lum_{max}	Species identity:treatment interaction	2.407	6, 49	0.041	S1	S1
Lum_{mean}	Species identity	5.352	6, 56	<0.001	S2	S4
Lum_{med}	Species identity	2.628	6, 56	0.026	S3	S6
Lum_{rug}	Species identity	3.776	6, 56	0.003	S4	S8
$\Delta[NH_4^+\text{-N}]$	Species identity	2.575	7, 63	0.021	S5	S10
	Treatment	18.929	1, 63	<0.001	S5	S10
$\Delta[NO_X^-\text{-N}]$	Species identity:treatment interaction	2.312	7, 56	0.050	S6	S12
$\Delta[PO_4^{3-}\text{-P}]$	Species identity	4.623	7, 63	<0.001	S7	S15
	Treatment	17.927	1, 63	<0.001	S7	S15
$\Delta[SiO_2\text{-Si}]$	Species identity:treatment interaction	3.277	7, 56	0.006	S8	S17

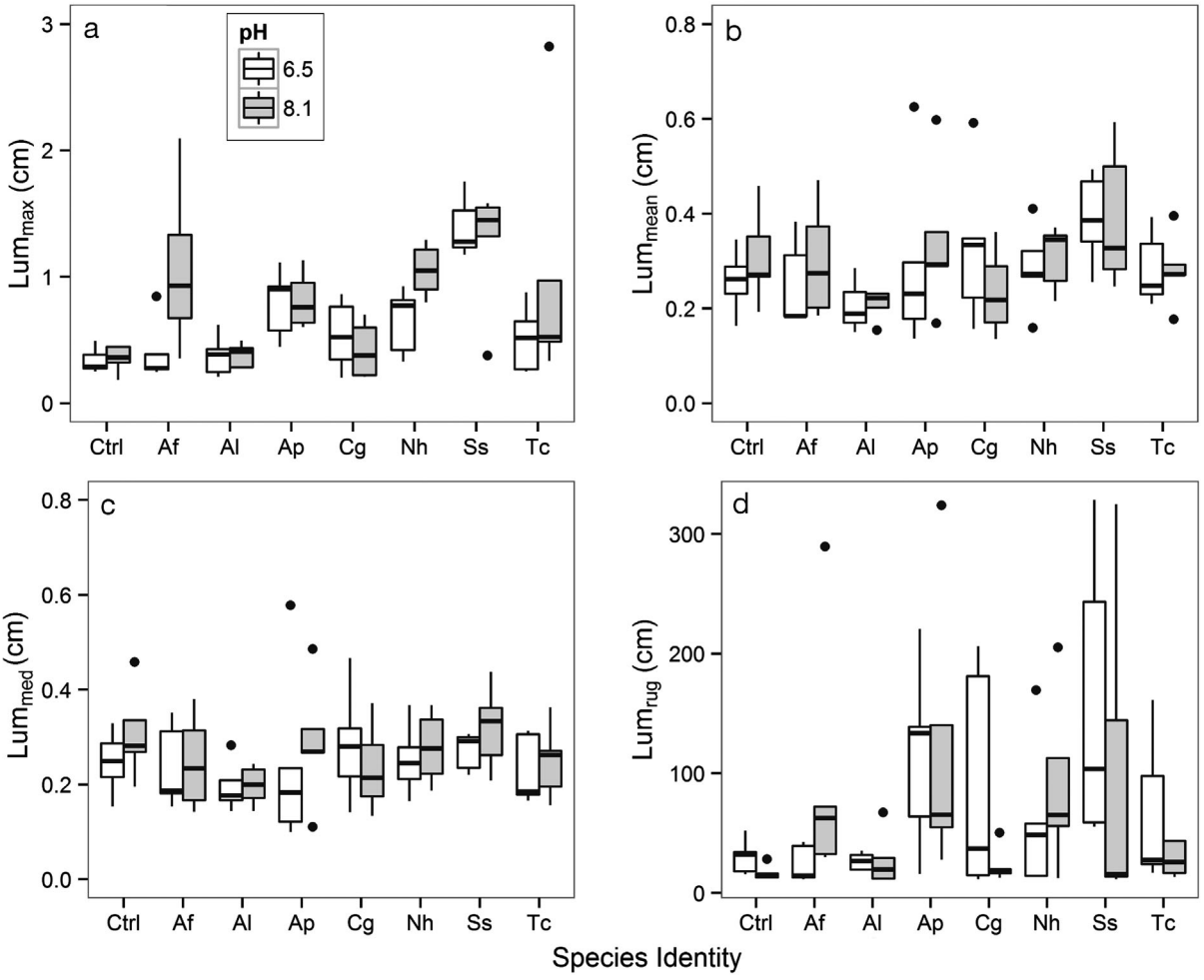

Fig. 2. Sediment reworking associated with benthic invertebrates. Species-specific effects on the particle reworking metrics: (a) maximum (Lum_{max}), (b) mean (Lum_{mean}) and (c) median (Lum_{med}) vertical distributions of luminophores, and (d) the rugosity of the lower extent of the mixed layer (Lum_{rug}). Boxes are upper and lower quartiles; median is indicated at the midpoint; whiskers represent the spread; circles are outliers defined as >1.75 × interquartile range. Ctrl: no macrofauna; Af: *Amphiura filiformis*; Al: *Anapagurus laevis*; Ap: *Aporrhais pespelecani*; Cg: *Chamelea gallina*; Nh: *Nucula hanleyi*; Ss: *Sternaspis scutata*; Tc: *Turritella communis*

species identity, nor significant main effects of seawater acidification. There were, however, clear differences in the average extent of vertical (Lum_{mean}: Table 3, Fig. 2b; Lum_{med}: Table 3, Fig. 2c) and lateral (Lum_{rug}: Table 3, Fig. 2d) sediment reworking between species. There was no effect of either seawater acidification or species identity on bioirrigation activity.

Ecosystem functions (nutrient concentrations)

Both species identity and seawater acidification influenced $\Delta[NH_4^+\text{-N}]$ (Table 3, Fig. 3a), but there was no interaction. Acidified aquaria showed an increase in NH_4^+-N concentrations, with the greatest increases observed in aquaria containing *A. filiformis* (0.42 ± 0.62 µmol l^{-1} in ambient seawater, 3.60 ± 2.33 µmol l^{-1} in acidified seawater), *A. pespelecani* (ambient: 1.11 ± 2.27 µmol l^{-1}; acidified: 4.59 ± 3.53 µmol l^{-1}), *C. gallina* (ambient: 0.04 ± 0.39 µmol l^{-1}; acidified: 5.68 ± 3.38 µmol l^{-1}) and *T. communis* (ambient: 3.09 ± 6.82 µmol l^{-1}; acidified: 3.12 ± 1.51 µmol l^{-1}; Fig. 3a, Table S11). There was an interaction between the effects of species identity and seawater acidification on changes in $[NO_X^-\text{-N}]$ (Table 3, Fig. 3b). Small changes in $[NO_X^-\text{-N}]$ were detected in ambient seawater aquaria, and no interspecies differences were detected. Exposure to acidified seawater resulted in a significantly higher increase in $[NO_X^-\text{-N}]$ in aquaria containing *A. pespelecani* (ambient: 0.05 ± 0.13 µmol l^{-1}; acidified: 0.76 ± 0.53 µmol l^{-1}), in contrast to a reduced increase in $[NO_X^-\text{-N}]$ in aquaria containing *A.*

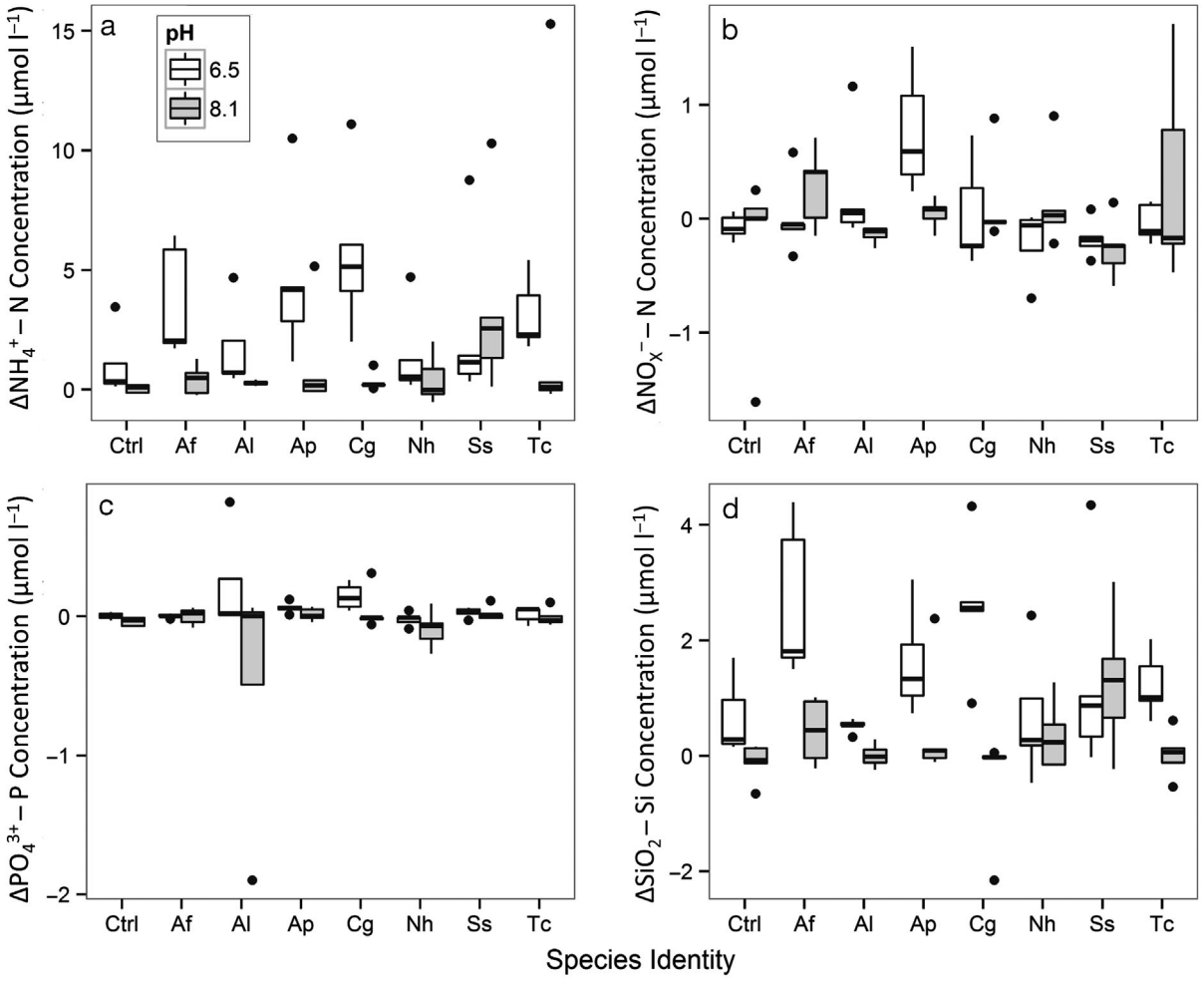

Fig. 3. Benthic invertebrate effects on (a) ammonium, (b) nitrate and nitrite, (c) phosphate and (d) silicate concentrations. Parameters of boxplots and full species names as in Fig. 2

filiformis (ambient: 0.28 ± 0.35 µmol l^{-1}; acidified: 0.01 ± 0.34 µmol l^{-1}) and a decrease for *N. hanleyi* (ambient: 0.15 ± 0.43 µmol l^{-1}; acidified: −0.21 ± 0.30 µmol l^{-1}) when compared to ambient conditions (Fig. 3b, Tables S13 & S14).

Species identity and seawater acidification both had an impact on Δ[PO_4^{3-}-P] (Table 3, Fig. 3c), although no significant interaction was detected. In ambient seawater, small decreases in PO_4^{3-}-P concentrations were observed in contrast to the acidified aquaria, where concentrations increased. *A. pespelecani* (ambient: 0.01 ± 0.05 µmol l^{-1}; acidified: 0.06 ± 0.04 µmol l^{-1}) and *C. gallina* (ambient: 0.04 ± 0.15 µmol l^{-1}; acidified: 0.14 ± 0.09 µmol l^{-1}) mediated small increases which were significantly different from the small decreases recorded in aquaria containing *N. hanleyi* (ambient: −0.09 ± 0.13 µmol l^{-1}; acidified: −0.02 ± 0.05 µmol l^{-1}; Table S16). *C. gallina* also mediated a significantly greater increase in [PO_4^{3-}-P] than *A. filiformis* (ambient: 0.000 ± 0.058 µmol l^{-1}; acidified: 0.002 ± 0.015 µmol l^{-1}; Table S16).

Seawater acidification and species identity had a significant interactive effect on changing SiO_2-Si concentrations (Table 3, Fig. 3d). In ambient seawater there was a greater increase in [SiO_2-Si] in aquaria containing *S. scutata* (ambient: 1.29 ± 1.21 µmol l^{-1}; acidified: 1.31 ± 1.75 µmol l^{-1}) than *C. gallina* (ambient: −0.44 ± 0.96 µmol l^{-1}; acidified: 2.59 ± 1.21 µmol l^{-1}; Table S17) which was the only inter-species difference detected. Species specific effects diverged in acidified aquaria where *A. filiformis* (ambient: 0.43 ± 0.56 µmol l^{-1}; acidified: 2.63 ± 1.34 µmol l^{-1}), *A. pespelecani* (ambient: 0.48 ± 1.06 µmol l^{-1}; acidified: 2.63 ± 0.92 µmol l^{-1}) and *C. gallina* mediated a greater increase in [SiO_2-Si] than aquaria containing *A. laevis* (ambient: 0.003 ± 0.22 µmol l^{-1}; acidified: 0.52 ± 0.12 µmol l^{-1}), *N. hanleyi* (ambient: 0.35 ± 0.59 µmol l^{-1}; acidified: 0.68 ± 1.11 µmol l^{-1}) or *T. communis*

(ambient: 0.03 ± 0.42 µmol l^{-1}; acidified: 1.23 ± 0.56 µmol l^{-1}; Table S18).

Consequences for functional effect group composition

Where there was a significant interaction between the effects of seawater acidification and species identity on individual ecosystem processes and functions, there was a subsequent change in functional effect group composition (Fig. 4). As a result, functional groups for Lum_{max}, $\Delta[NO_X^-\text{-N}]$ and $\Delta[SiO_2\text{-Si}]$ were different in ambient and acidified seawater. In ambient seawater, 3 Lum_{max} groups were identified: *A. filiformis*, *A. pespelecani*, *N. hanleyi*, *S. scutata* and *T. communis* grouped together (*A. pespelecani* and *N. hanleyi* were significantly different from each other but they did not differ significantly from any other species in this group so have been included here); *C. gallina*, which had a significantly shallower Lum_{max} depth than members of the first group; and *A. laevis*, which had the shallowest depth (Table S2). These groupings shifted in acidified seawater where *S. scutata* maintained a significantly greater Lum_{max} depth than all other species and formed a group by itself. The other species separated into 2 groups: *A. filiformis*, and *A. laevis*, which had the shallowest Lum_{max} depths; and *A. pespelecani*, *C. gallina*, *N. hanleyi* and *T. communis*, which formed an intermediate group. *C. gallina* was not significantly different from species in either group and could be assigned to both groups (Table S3).

Under ambient seawater conditions, there were no between-species differences, and *T. communis* was the only species to mediate a greater increase in

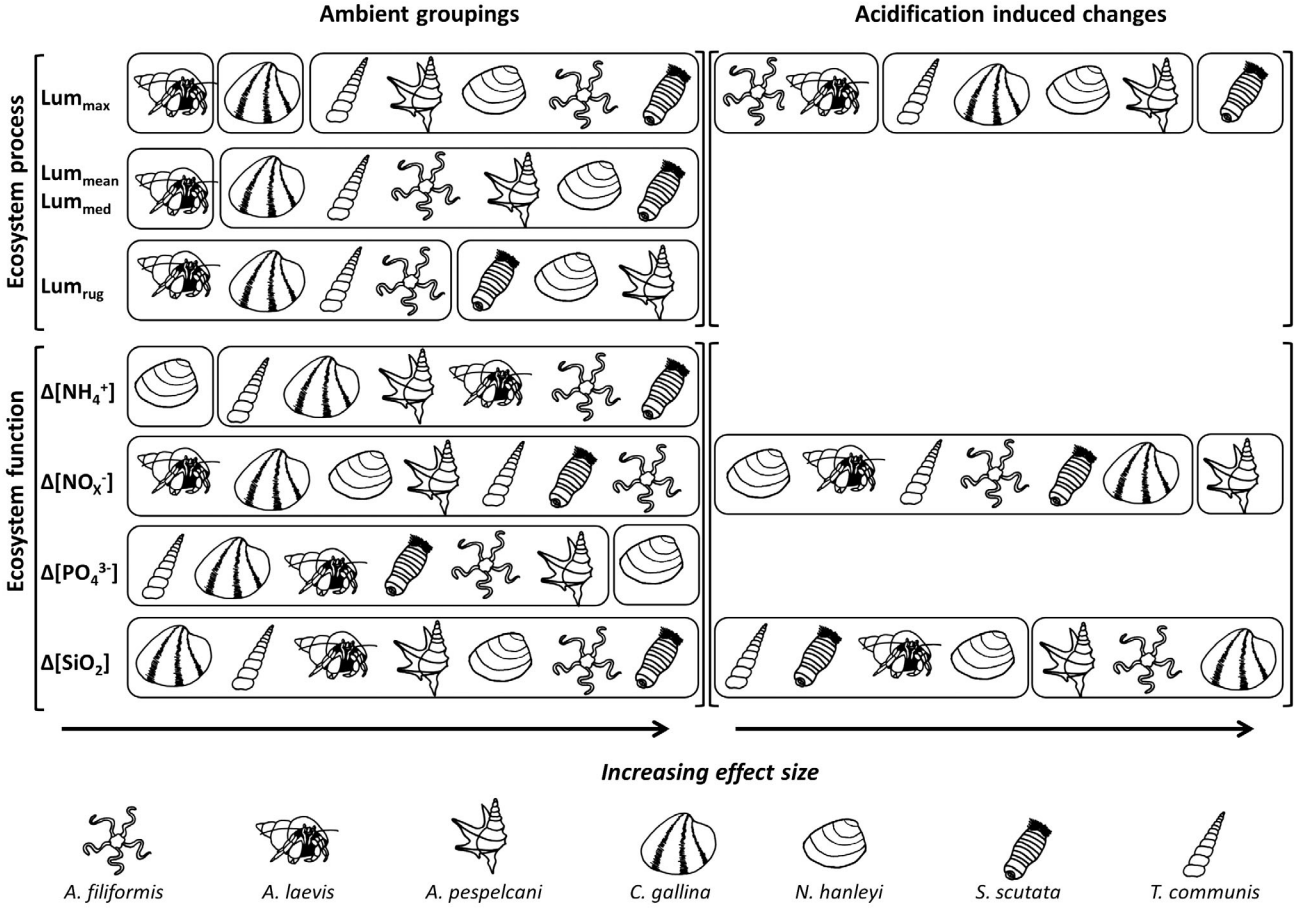

Fig. 4. Summary of functional effect groups identified based on aspects of sediment particle reworking nutrient concentrations. Boxes identify subsets of species that form a functional grouping based on the ecosystem process or function indicated for each row. Species were ascribed to groupings based on statistical differences in response variables and are positioned (from left to right) in order of increasing effect size. Changes in functional group composition due to seawater acidification are indicated. Species details are given in Table 1. Lum_{max}/Lum_{mean}/Lum_{med}: maximum/mean/median vertical distributions of luminophores; Lum_{rug}: rugosity of the lower extent of the mixed layer

[NO_X^--N] than the aquaria containing no macrofauna (Table S13). As such, all species were allocated to the same [NO_X^--N] functional group. In acidified seawater, however, *A. pespelecani* mediated a greater increase in [NO_X^--N] than the no-macrofauna aquaria and aquaria containing *A. filiformis*, *C. gallina*, *N. hanleyi* and *T. communis* (Table S14) and was therefore assigned to a second functional group. There were no other between-species differences. As with [NO_X^--N], all 7 species grouped together for [SiO_2-Si] in ambient seawater (Table S18); however, in contrast to [NO_X^--N] where the response of a single species was responsible for the formation of a new functional group under acidified conditions, 3 species (*A. filiformis, A. pespelecani* and *C. gallina*) mediated a higher release of SiO_2-Si from the sediment and formed a second functional group (Table S19).

Functional group composition was unaffected by seawater acidification for Lum_{mean}, Lum_{med}, Lum_{rug}, $\Delta[Br^-]$, $\Delta[NH_4^+$-N], or $\Delta[PO_4^{3-}$-P] (Tables S5, S7, S9, S11 & S16). Recorded Lum_{mean} and Lum_{med} depths were significantly lower for *A. laevis* than all other species, placing it in its own functional group. All other species were grouped together for both metrics. For Lum_{rug}, 2 functional groups were identified: *A. filiformis*, *A. laevis*, *C. gallina* and *T. communis*; and *A. pespelecani, N. hanleyi* and *S. scutata*, which had a higher level of reworking. As no significant species effect was detected for [Br^-], we concluded that there was 1 functional group containing all species. For both $\Delta[NH_4^+$-N] and $\Delta[PO_4^{3-}$-P], the same 2 functional groups were identified: *N. hanleyi* mediated a significantly lower release of [NH_4^+-N] and a significantly higher release of [PO_4^{3-}-P] than all other species and was the single member of 1 functional group. All other species grouped together in a second group.

DISCUSSION

This study has demonstrated that, whilst benthic invertebrates are capable of surviving short-term exposure to rapidly acidified seawater, consistent with previous studies (Widdicombe & Needham 2007, Small et al. 2010), the contributions of individual species to ecosystem processes and functioning can clearly be altered and, for some species, compromised when exposed to environmental stress. Where species diverge in their responses to seawater acidification, their relative contributions to ecosystem functioning also change, resulting in altered functional group composition and, in some cases, an altered total number of functional groups.

Specific impacts of elevated CO_2 on ecosystem processes and functions

Bioturbation and sediment–seawater nutrient exchange were affected by seawater acidification, and for Lum_{max}, $\Delta[NO_X^-$-N] and $\Delta[SiO_2$-Si], variation in species-specific responses led to a subsequent change in the composition of the functional groups identified. As shown here and previously (Murray et al. 2014), functional groups are function specific, even for related functions such as NH_4^+-N and NO_X^--N and the processes that mediate these functions. Here, changes in behaviour and physiology are likely to lead to changes in the interactions between macrofauna, microbes and the sediment habitat and therefore functional changes within the ecosystem (Gaylord et al. 2015).

It is our contention that understanding how individual species respond to a perturbation is vital to understanding the wider implications for ecosystem processes and functions. However, this study involved single-species aquaria in highly controlled conditions and, as with all laboratory experiments, this is a simplified replication of nature and caution is required when attempting to extrapolate these results to a real-world scenario. Further work testing the importance of the biomass of individuals, species interactions, population densities and natural perturbations (e.g. seasonal effects) will be important in furthering our understanding of biodiversity–ecosystem function relationships. Here, densities were controlled to allow for comparison between species; however, whilst the densities used were appropriate for some species, e.g. *Amphiura filiformis*, they were high for others, e.g. *Aporrhais pespelecani*. Nevertheless, studying species individually is a first step to driving this field beyond taxonomic inventories. Woodin et al. (2016) linked behavioural differences in benthic invertebrates to ecosystem functioning and suggested that spatial and temporal averaging of ecosystem processes and functioning can obscure underlying mechanisms linked to species-specific behaviours. Their study also suggested that sublethal impacts of stressors could have functional consequences that traditional metrics would not be able to detect.

Seawater acidification has been shown to affect physiology, e.g. by inducing muscle wastage (Wood et al. 2008), impeding growth (Chan et al. 2016) and reducing tolerance to other stressors (Lewis et al. 2016); and to affect behaviours including burrowing (Clements et al. 2017) and foraging (Barry et al. 2014). Such changes in behaviour and physiology

alter their interactions with other components of the benthic community, and therefore invertebrate responses are potential indicators of altered ecosystem functioning. Behavioural changes in response to hypercapnia (Christensen et al. 2011, Dissanayake & Ishimatsu 2011) and the onset of acidification can occur rapidly (Dixson et al. 2010, Kim et al. 2016), and there is evidence that altered behaviour may modify organism–sediment and community interactions (Briffa et al. 2012). Whilst the coupling of macrofaunal processes and microfaunal activity is well established (Mermillod-Blondin et al. 2004, Mermillod-Blondin 2011, Yazdani Foshtomi et al. 2015), this coupling may weaken under changing environmental contexts. Our data show a much clearer signal in the nutrient data than the particle reworking data to acidified seawater, and it may be that the microbial responses to change may have been more important in determining ecosystem functioning in this case. Therefore, changes in macrofaunal activity alone cannot necessarily be used to predict biogeochemical changes.

Implications of sub-lethal stress responses for functional group composition

The combined responses of sediment fauna to sublethal stressors have important implications for conclusions on ecosystem functioning based solely on the macrofaunal biodiversity present. Whilst biodiversity–ecosystem function (BEF) relationships are well established (Strong et al. 2015), the context dependence of macrofauna-mediated ecosystem functioning and species-specific responses creates difficulties in estimating changes in ecosystem functioning based on changes in biodiversity. This has potential implications for assessing functional redundancy, where species may remain but their functional role may have been lost following a change in behaviour or physiology. Although the importance of environmental context for ecosystem functioning is acknowledged in the literature (e.g. Godbold et al. 2011), abiotic factors are rarely considered when establishing functional groups or ecosystem functioning within biological communities (e.g. Gilbert et al. 2015). Paradoxically, it is exactly for situations with abrupt or unexpected changes in abiotic factors where practical applications of BEF theory are most likely to be useful. Where functional group frameworks do not have the capacity to account for context dependency, their application is unlikely to give an accurate indication of the functional capacity of remaining populations.

Whilst our study gives an indication that species functional roles can change, there remains a question of where a statistical difference in species activities represents an ecological significance. In our examples, statistically significant differences between species were recorded for very small changes in effect size, e.g. a 1 mm change in the maximum depth of particle reworking was enough to change the functional group to which *Chamelea gallina* belonged in acidified seawater. It is beyond the scope of this study to address whether all significant differences recorded represent true ecological differences between species effects on ecosystem functioning, but where small changes and high variances were detected, the resulting groupings may be statistical artefacts. However, whilst at the individual level these effects are small and could be argued to be inconsequential, when taken in aggregate at the community level, they may have a far greater impact.

Assessing ecosystem resilience following a sublethal stress event clearly requires more than a taxonomic inventory to be effective (also see Douglas et al. 2017). Woodward et al. (2012) highlighted that different conclusions on the health of freshwater streams can be reached depending on the metrics used. They recommended that functional measures, specifically rates of functions such as nutrient cycling, are needed to complement structural measures, including biodiversity and abiotic parameters, to assess ecosystem health. Our findings support this assertion for marine benthic ecosystems. It is fundamental that essential ecosystem functions are maintained (i.e. are resilient) or recover quickly following an impact to prevent longer-term degradation of the ecosystem (Oliver et al. 2015). Monitoring impact and recovery of ecosystem health will only be effective if appropriate measurements are taken.

Where species survive environmental perturbations, their immediate and short-term responses may potentially be useful indicators of the functional consequences and used to direct remedial efforts to prevent irreconcilable shifts. This requires an understanding of the interactions and functional ecology of communities, prior to an environmental impact event, beyond a taxonomic inventory of the species present, which is not currently available for most habitats. Efforts should focus on obtaining high-quality baseline data on ecosystem processes and functioning for areas potentially at risk, such as proposed subsea carbon storage sites, to allow appropriate response strategies to be determined. A more complete understanding of the functional responses of organisms to

changes in their abiotic environment will better equip regulators to assess and monitor ecosystem functioning in efforts to maintain sustainable functioning of marine ecosystems.

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