Downloaded from http://rsbl.royalsocietypublishing.org/ on January 26, 2017

BIOLOGY LETTERS

rsbl.royalsocietypublishing.org

Research

Cite this article: Alsterberg C, Sundbäck K, Gamfeldt L. 2014 Multiple stressors and multifunctionality: limited effects on an illuminated benthic system. *Biol. Lett.* **10**: 20140640.
http://dx.doi.org/10.1098/rsbl.2014.0640

Received: 12 August 2014
Accepted: 19 November 2014

Subject Areas:
ecology

Keywords:
multiple stressors, multifunctionality, shallow-water sediment systems

Author for correspondence:
Christian Alsterberg
e-mail: christian.alsterberg@bioenv.gu.se

Electronic supplementary material is available at http://dx.doi.org/10.1098/rsbl.2014.0640 or via http://rsbl.royalsocietypublishing.org.

THE ROYAL SOCIETY PUBLISHING

Marine biology

Multiple stressors and multifunctionality: limited effects on an illuminated benthic system

Christian Alsterberg, Kristina Sundbäck and Lars Gamfeldt

Department of Biological and Environmental Sciences, University of Gothenburg, PO Box 461, Göteborg 405 30, Sweden

The bulk of experiments that study stressor effects on ecosystem functioning consider only individual functions one at a time, and such narrow focus may well bias our understanding of the overall impact on ecosystem functioning. We used data from six published experiments in which marine illuminated sediment systems were exposed to nutrient enrichment, toxicants, sedimentation and warming, either alone or in combination. Measured functions were primary production, community respiration, inorganic nitrogen and phosphorus fluxes, and autotrophic biomass. We calculated two indices of multifunctionality that simultaneously considered all six functions: (i) a weighted average level of the functions and (ii) the number of functions that simultaneously exceed a critical threshold level. Stressors affected individual functions both positively and negatively, but multifunctionality was generally unaffected by both single and joint stressors. The filtering capacity of coastal illuminated sediment systems thus appears resilient to exposure to moderate levels of multiple stressors, most probably due to the robustness of the benthic microalgal community. We recommend using a multifunctionality approach in future studies on cumulative stressor effects on ecosystem functioning, particularly when considering functions related to ecosystem services.

1. Introduction

Today, ecosystems are simultaneously exposed to several local and global stressors and hence multiple stressor experiments have become common [1]. As individual stressors can interact and generate non-additive effects [2], it is difficult to predict their cumulative impact on ecosystem functions, such as carbon and nutrient cycling. For example, how much does a second or third stressor add to the effect on the overall ecosystem functionality? One approach to this question is to measure multifunctionality, i.e. the simultaneous performance of multiple functions [3] in response to either one or multiple stressors. Here, we do just that, using marine shallow-water sediment as a model system.

Marine shallow-water sediments are located at the interface between land and sea, where they constitute an important component in the 'coastal filtering service' by transforming, storing and removing nutrients [4,5]. In these illuminated ecosystems, benthic microalgae (microphytobenthos, MPB) are at the base of the food web [6]. Through their uptake of nutrients and production of oxygen, MPB control key ecosystem functions, such as productivity, mineralization and nutrient turnover, which are all related to the filtering capacity of coastal ecosystems [4].

Shallow-water sediments are exposed to both local anthropogenic stressors (nutrient loading, toxicants and physical disturbance) [7,8] and global stressors related to climate change [9,10]. Different stressors can have positive,

© 2014 The Author(s) Published by the Royal Society. All rights reserved.

Downloaded from http://rsbl.royalsocietypublishing.org/ on January 26, 2017

Table 1. The six published experiments from which data were used to calculate multiple stressor effects on ecosystem multifunctionality. CPT, copper pyrithione; DW, dry weight.

stressors	level of stressors	reference
copper pyrithione (antifouling toxin)	1 nmol CPT g^{-1} DW sediment	[12]
warming	$\Delta 4^{\circ}C$	
nutrient enrichment	80 mmol N and 20 mmol P m^{-2} d^{-1}	[14]
warming	$\Delta 4^{\circ}C$	
pyrene (polycyclic aromatic hydrocarbon)	2 mg kg^{-1} DW sediment	[15]
nutrient enrichment	400 μmol N and 50 μmol P l^{-1}	
sedimentation	~1.5 mm sediment d^{-1} for 7 days	[16]
warming	$\Delta 4^{\circ}C$	[11]
copper pyrithione (antifouling toxin)	1 nmol CPT g^{-1} DW sediment	[13]
nutrient enrichment	378 g N and 54 g P m^{-2}	

Table 2. The individual functions fitted with a linear mixed model showing 'stressors' and 'time' as fixed factor and the individual standardized functions as response variables. *p*-values in italic are significant at $\alpha = 0.05$.

standardized function	factor	estimate	d.f.	*p*-value
net primary production ($n = 246$)	stressor	0.2448	19	0.1090
	time	0.3760	218	*0.0394*
	stressor × time	−0.2595	218	*0.0030*
community respiration ($n = 246$)	stressor	0.0278	19	0.8586
	time	0.1116	218	0.5771
	stressor × time	−0.0682	218	0.4772
sediment chlorophyll *a* ($n = 246$)	stressor	0.0343	19	0.7662
	time	−0.1308	218	0.4148
	stressor × time	−0.0012	218	0.9873
ammonia flux ($n = 246$)	stressor	−0.1328	19	0.1145
	time	0.0993	218	*0.0000*
	stressor × time	0.0198	218	0.6957
nitrate flux ($n = 246$)	stressor	0.1666	19	0.2076
	time	−0.3331	218	*0.0381*
	stressor × time	−0.1938	218	*0.0116*
phosphorus flux ($n = 246$)	stressor	−0.2411	19	*0.0257*
	time	−0.5656	218	*0.0083*
	stressor × time	0.0457	218	0.6603

negative or interactive effects on individual functions. Thus, studying the overall ability of this ecosystem to simultaneously maintain a diverse set of functions is highly relevant for predicting cumulative stressor impacts. For example, one stressor, such as warming, can modify ecosystem sensitivity to other stressors, such as toxicants and nutrient loading [11–13].

Using data from six published experiments (table 1), we studied whether the multifunctionality of shallow-water sediment systems depended on the number of stressors. Illuminated undisturbed natural sediments were exposed to previously observed realistic levels of local stressors: eutrophication-related nutrient enrichment, toxicants (lowest observed effect concentration), sediment accumulation simulating dredging events and predicted warming (+4°C).

2. Material and methods

(a) Study area

Sediment for all six experiments was collected in cylinders of black acrylonitrile butadiene styrene plastics (height = 25 cm, i.d. = 25 cm) from an Olausson box corer (30 × 30 cm) at a depth of 2 m on the coast of NE Skagerrak. All experiments were run in the same experimental system. The cylinders were placed in a flow-through set-up (water from Gullmar Fjord) in a greenhouse, at close to natural light and temperature conditions (see references in table 1). Depending on experiment, the following stressors were used singly or in combination: nutrient enrichment, sedimentation, the toxicants copper pyrithione (antifouling substance for boats) and pyrene (from, for example, boat engines) and warming (table 1). The stressor

Downloaded from http://rsbl.royalsocietypublishing.org/ on January 26, 2017

levels employed in each experiment were considered ecologically relevant (see original publications for details).

(b) Measurements of ecosystem functions

The six response variables measured were net primary production, community respiration (oxygen fluxes), sediment–water inorganic nitrogen (ammonium and nitrate) and phosphorus fluxes, and sediment chlorophyll *a*. Individual experiments lasted between 23 and 42 days and data from three occasions during each experiment were chosen for the multifunctionality study: 3–4 days after the experiment had started ('start'), after 15–18 days ('middle') and after 23–42 days ('end'). All sediment–water fluxes were measured during day and night by start–stop batch incubations and later calculated as daily fluxes. Oxygen concentrations were determined by the Winkler technique and ammonium, nitrate + nitrite and dissolved inorganic phosphorus (DIP) were analysed using standard colorimetric procedures. Chlorophyll *a* (Chl *a*), proxy for MPB biomass, was measured spectrophotometrically [17]. The complete dataset for all six experiments consisted of 246 data points (one or two stressor treatments, four to five replicates per treatment and sampling date, with independent sampling).

(c) Calculations of multifunctionality and statistics

We used two methods to describe multifunctionality: (i) a weighted average function approach and (ii) a multiple thresholds approach [3,18]. For the averaging approach, all functions were first recalculated and standardized to be positive by adding the lowest value to all data, and standardized by the maximum observed value [3]. The weighted average index was calculated by taking the average of all functions and then subtracting the standard deviation. This index thus takes into account the variation among functions. The relationship between the number of stressors and the weighted average index (as well as all individual functions) was modelled using a linear mixed-effect framework (nlme package) [19]. The six different stressors can potentially have quite different, even opposing, effects on ecosystem functions and multifunctionality. Thus, 'study' and 'stressor treatment' were included as random factors. In addition, we also used analysis of variance (ANOVA) to study how individual stressors and stressor combinations affected the multifunctionality of shallow-water sediment systems.

The multiple threshold approach [3,20] creates an index of the number of functions surpassing the full range of functional thresholds (0–100% of maximum functional values). After identifying the maximum value for each function, we tallied the number of functions above these thresholds for single and multiple stressor scenarios. All statistical analyses and graphical plotting were performed in the R environment using the multifunc and ggplot2 packages [3,21,22].

3. Results

(a) The weighted average approach

Neither the individual effect of stressors (estimate: −0.008, d.f.: 13, *p*-value: 0.6205), time (estimate: −0.005, d.f.: 224, *p*-value: 0.5492), nor the interaction between stressors and time (estimate: −0.009, d.f.: 224, *p*-value: 0.2007) significantly affected multifunctionality. Individual functions, however, responded differently to the stressor treatments (table 2). Net primary production and nitrate fluxes significantly decreased with time during the impacts of multiple stressors (stressor × time interaction) (table 2). DIP fluxes decreased significantly, but independently of time (table 2). Moreover, individual stressors or stressor interactions affected the weighted averaged index either positively or negatively (electronic supplementary material, table S1; figure 1).

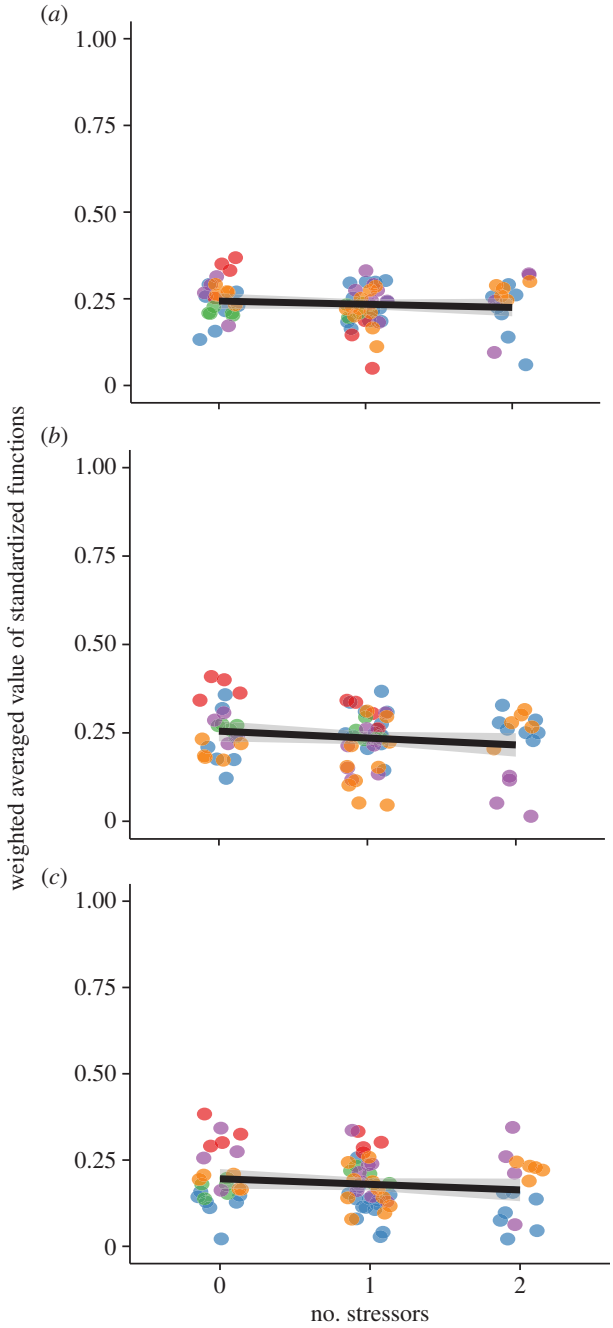

Figure 1. The linear function ±95% confidence interval of the weighted averaged multifunctionality index plotted against the number of stressors during (*a*) start, (*b*) middle and (*c*) end of the six multiple stressor experiments. The coloured dots represent different stressor and or stressor combinations, where red = sedimentation, blue = toxin and nutrients (two experiments), green = warming, purple = warming and nutrients, orange = warming and toxin. (Online version in colour.)

(b) The multiple thresholds approach

The absence of a significant slope of the relationship between the number of stressors and the change in the number of functions per addition of one stressor showed that an increasing number of stressors did not affect ecosystem multifunctionality (figure 2). The high variation of threshold data, and the fact that the 95% confidence interval overlaps zero across the full range of thresholds, indicate that there was no overall significant

Downloaded from http://rsbl.royalsocietypublishing.org/ on January 26, 2017

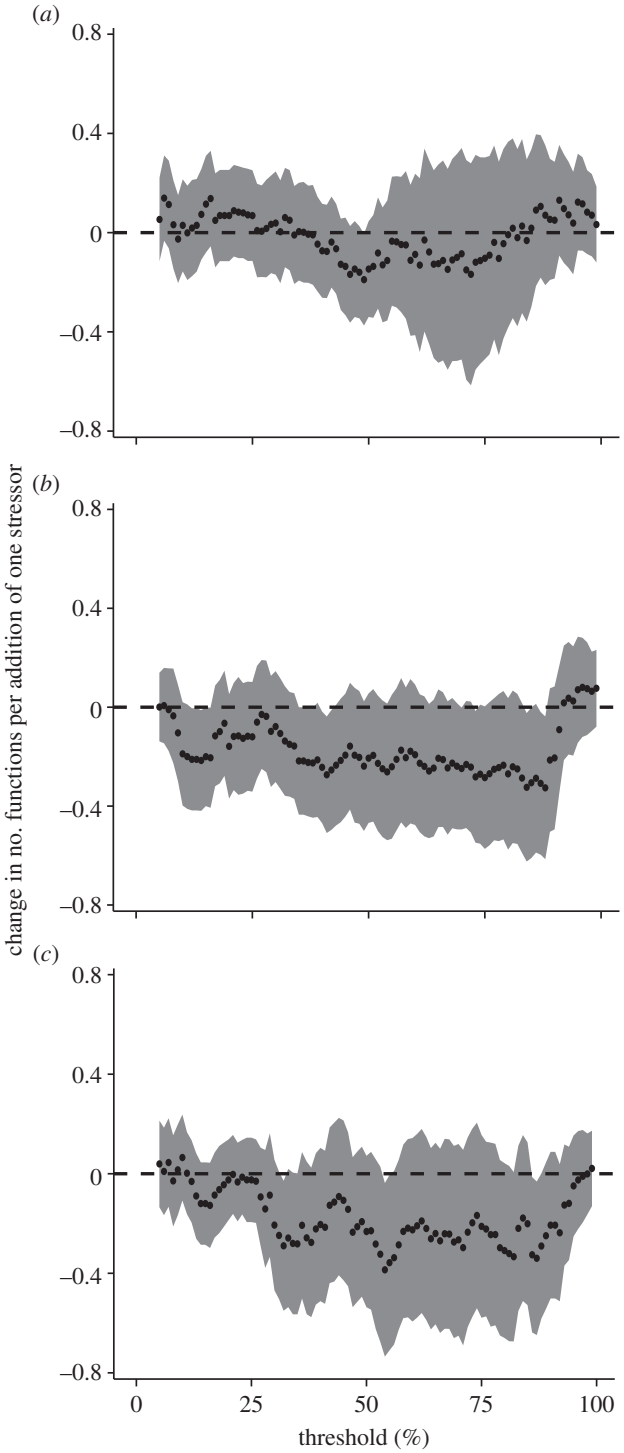

Figure 2. The slope of the relationship between number of stressors and the number of functions at or above a threshold of some proportion of the maximum observed function, at different threshold values during (*a*) start, (*b*) middle and (*c*) end for the two multiple stressor experiments. Points are the fitted values ± 1 confidence interval.

effect of the number of stressors on the number of functions performing above threshold levels (figure 2). However, there were a few exceptions at thresholds of around 40 and 85% in the middle of the experiments and 30, 55 and 85% at the end of the experiments (figure 2).

4. Discussion

We have shown that measuring multifunctionality can facilitate the understanding of how multiple stressors affect ecosystem functioning. While the relationship between multifunctionality and biodiversity has been previously studied [3], the link between the number of stressors and multifunctionality has not. We acknowledge the fact that exploring individual functions is important and can reveal whether specific processes or interactions are more or less sensitive to multiple stressors. However, measurements of many individual functions are still difficult to place in a general context when dealing with multiple stressor effects. This is because a negative effect on one particular function does not necessarily mean that the overall functionality of the ecosystem is significantly reduced.

In this study, stressors negatively affected three individual functions (net primary production, nitrate and phosphate fluxes), whereas others were unaffected (table 2). By contrast, multifunctionality, measured as (i) the average function or (ii) the change in the number of functions above multiple thresholds, was generally unaffected by multiple stressors. Thus, the previous suggestion that illuminated shallow-water sediment systems are resilient to stressors [4,23,24] was corroborated by our findings. Demonstrating resilience of an ecosystem greatly benefits from considering multifunctionality. For aquatic ecosystems, the trophic state has been suggested as a possible controller of resilience, so that net autotrophic ecosystems are better buffered against stressors than are net heterotrophic systems [11,13,25]. In illuminated benthic systems, a generally robust benthic diatom community quickly restores oxygenation after a disturbance, thereby contributing to the resilience and sustainability of the coastal filtering service [26].

We calculated the multifunctionality of shallow-water sediments using six benthic ecosystem functions. The results suggest that moderate levels of common stressors and their combinations may not significantly affect the filtering capacity of these systems. Although multifunctionality as such cannot be used as a direct proxy for the filtering service of coastal systems, it can be used as a tool for understanding the underpinning ecological functioning of the filter. Generally, there is still a lack of useable experimental data for examining the effects of multiple stressors on ecosystem functions and services. Future studies should thus aim to include a wider range of stressors and their interactions. Applying a multifunctionality approach has great potential to enhance our understanding of how stressors affect the overall functioning of ecosystems, thereby making these types of studies more relevant for management and conservation.

Data accessibility. Data available from figshare: http://dx.doi.org/10.6084/m9.figshare.1120485 and http://dx.doi.org/10.6084/m9.figshare.1181670.

Acknowledgements. We thank Fredrik Larson, Dorthe Groth Petersen and Ingela Dahllöf who have contributed to the stressor experiments.

Funding statement. Main funding was provided by the Research Council for Environment, Agricultural Sciences and Spatial Planning (Formas) to K.S. and from the Swedish Research Council (VR) to L.G. and K.S.

References

1. Darling ES, Cote IM. 2008 Quantifying the evidence for ecological synergies. *Ecol. Lett.* **11**, 1278–1286. (doi:10.1111/j.1461-0248.2008.01243.x)

2. Folt CL, Chen CY, Moore MV, Burnaford J. 1999 Synergism and antagonism among multiple

Downloaded from http://rsbl.royalsocietypublishing.org/ on January 26, 2017

stressors. *Limnol. Oceanogr.* **44**, 864–877. (doi:10.4319/lo.1999.44.3_part_2.0864)

3. Byrnes JEK *et al*. 2014 Investigating the relationship between biodiversity and ecosystem multifunctionality: challenges and solutions. *Methods Ecol. Evol.* **5**, 111–124. (doi:10.1111/2041-210X.12143)
4. McGlathery KJ, Sundbäck K, Anderson IC. 2007 Eutrophication in shallow coastal bays and lagoons: the role of plants in the coastal filter. *Mar. Ecol. Prog. Ser.* **348**, 1–18. (doi:10.3354/meps07132)
5. Cook PLM, Van Oevelen D, Soetaert K, Middelburg JJ. 2009 Carbon and nitrogen cycling on intertidal mudflats of a temperate Australian estuary. IV. Inverse model analysis and synthesis. *Mar. Ecol. Prog. Ser.* **394**, 35–48. (doi:10.3354/meps08270)
6. Underwood GJC, Kromkamp J. 1999 Primary production by phytoplankton and microphytobenthos in estuaries. *Adv. Ecol. Res.* **29**, 93–153. (doi:10.1016/S0065-2504(08)60192-0)
7. Halpern BS *et al*. 2008 A global map of human impact on marine ecosystems. *Science* **319**, 948–952. (doi:10.1126/science.1149345)
8. Defeo O, McLachlan A, Schoeman DS, Schlacher TA, Dugan J, Jones A, Lastra M, Scapini F. 2009 Threats to sandy beach ecosystems: a review. *Estuar. Coast. Shelf Sci.* **81**, 1–12. (doi:10.1016/j.ecss.2008.09.022)
9. Intergovernmental Panel on Climate Change. 2007 Intergovernmental panel on climate change. In *2007: synthesis report.* (eds RK Pachauri, A Risinger), pp. 45–47. Geneva, Switzerland: IPCC.
10. Crain CM, Kroeker K, Halpern BS. 2008 Interactive and cumulative effects of multiple human stressors in marine systems. *Ecol. Lett.* **11**, 1304–1315. (doi:10.1111/j.1461-0248.2008.01253.x)
11. Alsterberg C, Hulth S, Sundbäck K. 2011 Response of a shallow-water sediment system to warming. *Limnol. Oceanogr.* **56**, 2147–2160. (doi:10.4319/lo.2011.56.6.2147)
12. Alsterberg C, Sundbäck K. 2013 Experimental warming and toxicant exposure can result in antagonistic effects in a shallow-water sediment system. *Mar. Ecol. Prog. Ser.* **488**, 89–101. (doi:10.3354/meps10357)
13. Sundbäck K, Petersen DG, Dahllöf I, Larson F. 2007 Combined nutrient–toxicant effects on a shallow-water marine sediment system: sensitivity and resilience of ecosystem functions. *Mar. Ecol. Prog. Ser.* **330**, 13–30. (doi:10.3354/meps330013)
14. Alsterberg C, Sundbäck K, Hulth S. 2012 Functioning of a shallow-water sediment system during experimental warming and nutrient enrichment. *PLoS ONE* **7**, e51503. (doi:10.1371/journal.pone.0051503)
15. Petersen DG, Sundbäck K, Larson F, Dahllöf I. 2009 Pyrene toxicity is affected by the nutrient status of a marine sediment community: implications for risk assessment. *Aquat. Toxicol.* **95**, 37–43. (doi:10.1016/j.aquatox.2009.07.023)
16. Larson F, Sundbäck K. 2012 Recovery of microphytobenthos and benthic functions after sediment deposition. *Mar. Ecol. Prog. Ser.* **446**, 31–44. (doi:10.3354/meps09488)
17. Lorenzen CJ. 1967 Determination of chlorophyll and pheopigments: spectrophotometeric equations. *Limnol.* Oceanogr. **12**, 343–346. (doi:10.4319/lo.1967.12.2.0343)
18. Pasari JR, Levi T, Zavaleta ES, Tilman D. 2013 Several scales of biodiversity affect ecosystem multifunctionality. *Proc. Natl Acad. Sci. USA* **110**, 10 219–10 222. (doi:10.1073/pnas.1220333110)
19. Pinheiro J, Bates D, DebRoy S, Sarkar D, Team RC. 2014 nlme: linear and nonlinear mixed effects models. R package v. 3.1–117. http://CRAN.R-project.org/package=nlme.
20. Zavaleta ES, Pasari JR, Hulvey KB, Tilman GD. 2010 Sustaining multiple ecosystem functions in grassland communities requires higher biodiversity. *Proc. Natl Acad. Sci. USA* **107**, 1443–1446. (doi:10.1073/pnas.0906829107)
21. Wickham H. 2009 *ggplot2: elegant graphics for data analysis*. New York, NY: Springer.
22. R Development Core Team. 2010 *A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. http://www.r-project.org/.
23. Larson F, Petersen DG, Dahllöf I, Sundbäck K. 2007 Combined effects of an antifouling biocide and nutrient status on a shallow-water microbenthic community. *Aquat. Microb. Ecol.* **48**, 277–294. (doi:10.3354/ame048277)
24. Underwood GJC, Paterson DM. 1993 Seasonal-changes in diatom biomass, sediment stability and biogenic stabilization in the Severn estuary. *J. Mar. Biol. Assoc. UK* **73**, 871–887. (doi:10.1017/S0025315400034780)
25. de Wit R *et al*. 2001 ROBUST: the role of buffering capacities in stabilising coastal lagoon ecosystems. *Cont. Shelf Res.* **21**, 2021–2041. (doi:10.1016/S0278-4343(01)00040-1)
26. Larson F, Sundbäck K. 2008 Role of microphytobenthos in recovery of functions in a shallow-water sediment system after hypoxic events. *Mar. Ecol. Prog. Ser.* **357**, 1–16. (doi:10.3354/meps07426)