ELSEVIER

Journal of Experimental Marine Biology and Ecology 330 (2006) 261 – 273

Journal of EXPERIMENTAL MARINE BIOLOGY AND ECOLOGY

www.elsevier.com/locate/jembe

Indirect effects of *Atrina zelandica* on water column nitrogen and oxygen fluxes: The role of benthic macrofauna and microphytes

Judi Hewitt [a,*], Simon Thrush [a], Max Gibbs [a], Drew Lohrer [a], Alf Norkko [b]

[a] *National Institute of Water and Atmospheric Research, P.O. Box 11-115, Hamilton, New Zealand*
[b] *Finnish Institute of Marine Research, PB 33, FI-00931 Helsinki, Finland*

Received 31 August 2005; received in revised form 13 December 2005; accepted 15 December 2005

Abstract

The effects of ecosystem engineers can be mediated by direct and indirect interactions. For suspension-feeding bivalves that contribute to benthic–pelagic coupling, indirect effects have been linked to hydrodynamics; however, the influence of these ecosystem engineers may also operate through interactions with surrounding sediments, microphytes and macrofauna that, in turn, affect nutrient and oxygen fluxes. This study investigated the indirect effects of an epibenthic suspension-feeding bivalve (*Atrina zelandica*) on ammonium and nitrate–nitrite effluxes from the sediment, and oxygen consumption in the overlying waters, under dark conditions, at two sites with different environmental characteristics. Location-dependent effects were observed in the relative strength of the effect of *Atrina* on microphyte and macrofaunal abundance. The difference between the strength of the effect of *Atrina* on macrofauna between sites was not driven by a single species or type of species; rather all the species decreasing in abundance away from *Atrina* were small species that utilised the sediment water interface. Location-dependent effects were also observed in the relative strength of the effect of microphyte and macrofaunal abundance on oxygen and nutrient fluxes. While microphytes were an important consumer of oxygen at one site, at the other site, small infaunal macrofauna were important. Similarly, the strength of the effect of surrounding macrofauna on ammonium efflux varied between sites. These findings demonstrate the importance of natural history and species functions for understanding complex responses. They suggest that indirect effects by key benthic macrofaunal species in marine systems can also be important to benthic–pelagic coupling. In particular, while key species are often large, excretion and respiration of smaller macrofauna can be important to the exchange of nutrients near the seafloor and oxygen consumption in the benthic boundary layer.
© 2006 Elsevier B.V. All rights reserved.

Keywords: Benthic–pelagic coupling; Macrofauna; Microphytes; Nutrient fluxes; Oxygen consumption; Suspension feeders

1. Introduction

Suspension-feeding bivalves can play important roles as ecosystem engineers through their influence on benthic–pelagic coupling and the use of their shells as attachment surfaces for encrusting organisms. When suspension-feeding bivalves protrude from the seafloor into the water column, the potential for both direct and indirect effects on surrounding macrofauna is frequently mediated by hydrodynamics affecting boundary flows, sedimentation and resuspension (Frechette et al., 1989; Green et al., 1998; Nikora et al., 2002). However, the potential for these ecosystem engineers to affect benthic–pelagic coupling via indirect effects on microphytes and surrounding macrofauna is relatively unexplored, even though direct effects due to respiration, excretion and increased fluxes from the water column of

* Corresponding author.
E-mail address: j.hewitt@niwa.cri.nz (J. Hewitt).

0022-0981/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jembe.2005.12.032

sediment and organic matter have been described (Loo and Rosenberg, 1989; Dame and Libes, 1993; Frechette and Bacher, 1998; Dame et al., 2001; Dowd, 2005; Gibbs et al., 2005 but see Newell et al., 2002).

Recent studies have demonstrated the potential for interactions and feedbacks between large bioturbators, microphytes and sediment nutrients (Hughes et al., 2000; Reise, 2002; Lohrer et al., 2004). Other studies suggest that not only bioturbators are important but that large, high density macrofauna may affect nutrient fluxes from the sediment into the water column and oxygen consumption in the overlying water (Welsh, 2003; Thrush et al., in press). A potential, usually not considered, also exists for smaller surrounding macrofauna to directly introduce nutrients into, and consume oxygen from, the water.

Atrina zelandica (Gray) is a large suspension-feeding pinnid bivalve that has been previously demonstrated to influence community structure and ecosystem function in coastal soft-sediment habitats (Warwick et al., 1997; Cummings et al., 1998; Norkko et al., 2001; Hewitt et al., 2002). *Atrina* are large (up to 30 cm long) and protrude above the sediment surface, and modify boundary flow conditions (Green et al., 1998; Nikora et al., 2002). *Atrina* can produce copious amounts of biodeposits (Hewitt and Pilditch, 2004) influencing nutrient and oxygen fluxes (Norkko et al., 2001; Gibbs et al., 2005), sediment fluxes and surrounding sediment characteristics (Norkko et al., 2001). Previous work suggests effects are location-dependent, with strongest effects on surrounding macrofauna and oxygen and nutrient fluxes at low turbidity (Gibbs et al., 2005; Norkko et al., in press). While Gibbs et al. (2005) provide an estimate of the separate effect of direct *Atrina* excretion and respiration on their measurements of nutrient and oxygen fluxes between the seafloor and the water column, they were not able to determine whether fluxes were affected by indirect interactions with microphytes or surrounding macrofauna. Some indications of a microphyte interaction were suggested by an observed increase in sediment chlorophyll a away from *Atrina*.

This study was designed to understand better the direct and indirect effects of *Atrina* highlighted by previous studies. Two key questions were posed. (1) Knowing that *Atrina* can indirectly affect nutrient and oxygen content of the water, is this indirect effect associated with effects on sediment characteristics, microphytes or surrounding macrofauna? And (2) if the strength of the effect of an ecosystem engineer on surrounding macrofauna is location-dependent, is the effect on microphytes and oxygen/nutrient fluxes also location-dependent? The answers to these questions were used to build a conceptual model of the direct and indirect linkages between *Atrina*, microphytes, surrounding macrofauna, and nitrogen and oxygen fluxes. This conceptual model was used to highlight the potential for, and importance of, location-specific indirect interactions.

2. Methods

2.1. Study location and sampling design

Based on information from previous studies (Norkko et al., 2001, in press), we chose two sites differing in environmental characteristics (e.g., turbidity), microphyte and macrofaunal communities (Table 1). The first site, MI, was located off Motoroa Island, at the mouth of Kawau Bay (Fig. 1). This site had sandy sediments, low suspended sediment loads, and weak currents. The second site, TK, was located in Mahurangi Harbour, near the mouth (Fig. 1). Sediments were fine sand, suspended sediment loads were high on the outgoing tide, and currents were strong at mid-ebb and flood.

At both sites, patches of various sizes and densities of *Atrina* were observed. As the effect of *Atrina* on macrofauna has been predicted to depend on the density of *Atrina* and patch structure (see, Hewitt et al., 2002), samples were distributed along gradients in these characteristics at both sites. Seven locations of differing densities/patch size of *Atrina* were selected at each site, varying from an area with one *Atrina* per square meter area to areas of 25 *Atrina* per square meter. A benthic chamber (13.9 cm diam.; volume 0.8 l) was placed next to an *Atrina* in each location (location A). Another chamber was placed adjacent to the first (location N), but as far away from other *Atrina* in the patch as possible, such that the distance between it and any *Atrina* was greater than the distance between the first chamber and an *Atrina*. Bare areas (>50 cm diam.) were selected as

Table 1
Environmental characteristics of the two sites, MI and TK, derived from unpublished data

Location	MI	TK
	Large coastal embayment	Small harbour
Mean peak tidal current	0.5 m s^{-1}	0.57 m s^{-1}
Mean peak bed orbital velocity	2.6 cm s^{-1}	1 cm s^{-1}
Baseline suspended sediment concentration	10–20 mg l^{-1}	20–60 mg l^{-1}
Predominant sediment type by weight	Coarse, medium, fine sands	Fine sands

Tidal current and bed orbital velocity were derived from hydrodynamic models of the area. Suspended sediment concentrations were measured on three occasions over a tidal cycle at nearby sites. Sediment type was measured once on a composite core of the surface 2 cm of sediment.

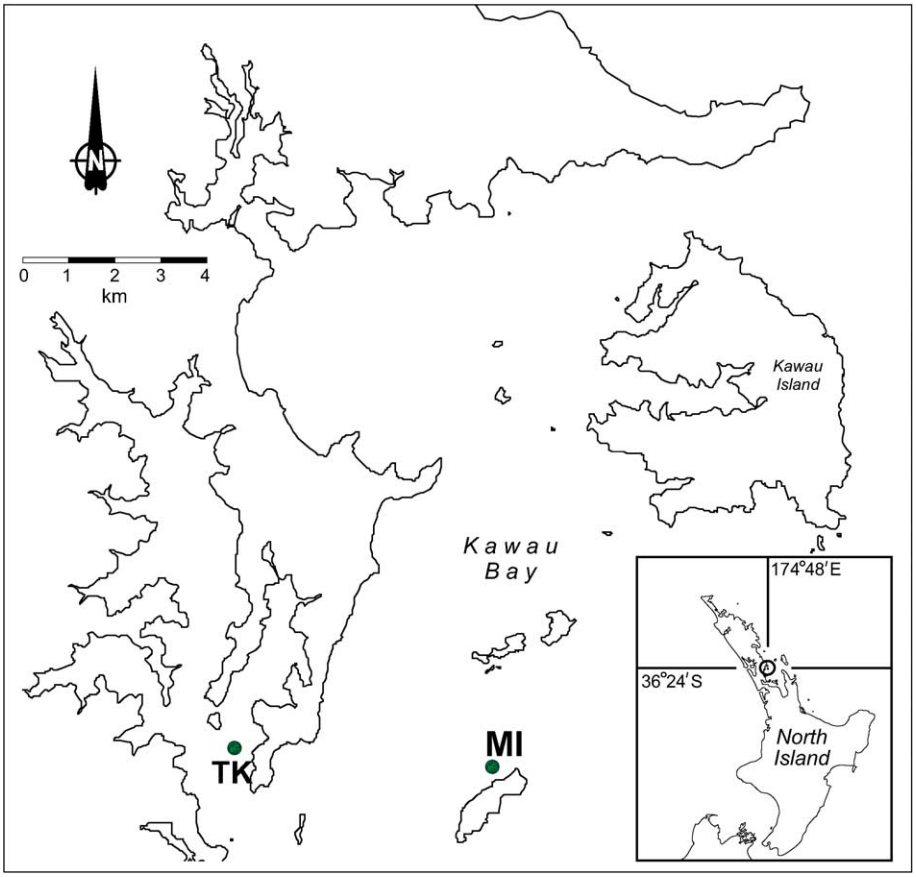

Fig. 1. Location of experimental study sites in Kawau Bay, New Zealand.

locations for another 5 chambers at each site (location B). Thus, 19 identical chambers were deployed at each site, at varying distances from varying densities of *Atrina*.

To provide data to statistically nest the deployed chambers within the *Atrina* landscape, an area 1 m in radius around each chamber was video taped and the distance between chambers and all *Atrina* surrounding them to a distance of 55 cm calculated. Four distance classes were calculated from these measurements; i.e., the number of *Atrina* within 10 cm, 10–30 cm, 30–45 cm and 45–55 cm of a chamber. An estimate of density weighted by distance to the chamber was also derived. These variables were tested for correlations with each other and for spread over the range of variables and three variables were selected for further use: number of *Atrina* within 10 cm (d10), number of *Atrina* within 45 cm (sum45); and density of *Atrina* within 55 cm weighted by distance (wtsum55). The last measure was included in case any response depended upon both density and distance and was calculated by taking the number of *Atrina* abutting the chamber and adding the number of *Atrina* <30 cm away (but not abutting the chamber) divided by 2, the number of *Atrina* between 45 and 30 cm away divided by 3 and the number of *Atrina* between 55 and 45 cm away and dividing by 4. For example, a chamber in a bare patch may have had an *Atrina* between 55 and 45 cm away; the chamber would have d10 = 0, sum45 = 1, wtsum55 = 0.25.

2.2. Chamber measurements of oxygen and nutrient fluxes

All nutrient and oxygen fluxes were measured in the dark, to overcome problems associated with varying light conditions (Gibbs et al., 2005). Water samples were taken from each chamber immediately after the chambers were installed and again at the end of the experiment (5 h later). Ambient water was also incubated in 1 l dark bottles at each site and incubated without sediment over the duration of the experiment.

Dissolved oxygen (DO) was measured immediately using a Yellow Springs Instrument (YSI) BOD self-stirring oxygen probe (model 5730). The water was then pressure-filtered through a 2.5 cm Whatman GF/C glass

fibre filter in a Swinnex® filter holder directly into a 100 ml polyethylene screw cap sample bottle. The bottles were sealed and placed on crushed ice in the dark for transport to the laboratory where they were frozen to −20 °C pending analysis. Ammoniacal and nitrate-plus-nitrite nitrogen were determined simultaneously on a Lachat flow injection analyser using standard Lachat colorimetric methods; NH_4–N was measured using the phenol-hypochlorite method (Liao, 2001), nitrate-plus-nitrite nitrogen (NO_x–N) was measured using a sulphanilamide diazotisation method following cadmium reduction of nitrate to nitrite (Diamond, 2001).

Fluxes were measured as difference between start and finish for each chamber, corrected for replacement water effects and any water-column-only effects, as determined from ambient water incubation.

2.3. Macrofaunal, microphyte and sediment sampling

Once the benthic chambers had been removed from the sediment, three sediment cores were taken from the position of the chamber, one each for macrofauna, microphytes and sediment characteristics. Macrofauna were sampled with a 10 cm diam. 13 cm deep core, sieved on a 0.5 mm mesh sieve, preserved in 70% isopropyl alcohol, and stained with 0.2% Rose Bengal. Microphytes were sampled with a 2 cm diam., 2 cm deep core, preserved with 1% Lugol's acidified iodine solution and stored in the dark. Sediment characteristics were sampled with a 2 cm diam., 2 cm deep core and frozen.

In the laboratory, macrofauna were sorted and identified to the lowest taxonomic level practicable (usually genus or species). For the macrofauna, a number of aggregate groups were defined: deposit feeders, suspension feeders, large organisms (>20 mm longest dimension) and organisms that protruded or migrated into the water column (e.g., ostracods, cumaceans, tube-dwelling suspension or surface deposit feeders). The number of individuals in each of these groups was determined, as well as the total number of individuals and number of taxa. Microphytes were identified to the lowest possible taxonomic level, usually species or genera and their abundance ranked on a scale from 0 to 10. Chlorophyll a was measured on a freeze-dried subsample of the sample collected to assess sediment characteristics. Chlorophyll a was extracted by boiling the subsample in 90% ethanol, and the extract processed using a spectrophotometer. An acidification step was used to separate degradation products from chlorophyll a (Sartory, 1982). The sediment particle size distribution for each site was calculated on a composite sample, consisting of the remaining sediment, by wet sieving. Sieving was done on 2, 0.5, 0.25 and 0.063 mm mesh sieves and the sediment was dried to constant weight at 60 °C to provide % composition of gravel, coarse sand, medium sand, fine sand and mud.

2.4. Statistical analysis

Initially, the effect of *Atrina* on sediment chlorophyll a, oxygen consumption, NH_4–N and NO_x–N effluxes, microphyte taxa richness, and macrofaunal taxa richness and number of individuals was determined using generalized linear models containing two fixed factors (site and location (A, N, B)) and their interaction term. Either raw data with normal errors, raw data with Poisson errors and a log-link function or rank transformed data was used in the analysis depending on the results of normality and homogeneity of variance tests. The effect of *Atrina* on the microphyte and macrofauna communities was similarly determined using NP-MANOVA (Anderson, 2001) on Bray–Curtis similarities calculated from raw data.

Secondly, the effect of *Atrina* on sediment chlorophyll a, fluxes (oxygen consumption, NH_4–N and NO_x–N effluxes), microphyte taxa richness and macrofaunal aggregate groups (richness, total number of individuals, number of deposit feeders, suspension feeders, surface dwellers and large organisms) was determined using the calculated measures of *Atrina* density at different spatial scales as continuous measures (D10, sum45, wtsum55). Due to the differences observed between sites in macrofaunal communities and the response of many of the variables to the *Atrina* (assessed graphically and suggested by other studies (e.g., Thrush et al., 2001; Gibbs et al., 2005; Norkko et al., in press)), this analysis was conducted on each site separately.

2.5. Model construction

A conceptual model was constructed, linking the effect of *Atrina* on macrofauna and microphytes to potential effects on oxygen and ammonium. In this conceptual model, indeed through the whole paper, the density and distance to *Atrina* were used as a surrogate for a number of unmeasured variables (small changes in particle size and organic content, provision of refugia and the effect of biodeposition on the microphyte and macrofauna etc.), but did not include (as *Atrina* were not within the chamber) direct excretion and respiration. The conceptual model allowed *Atrina* to directly affect chlorophyll a content of the sediment, macrofauna, oxygen consumption and NH_4–N efflux. Macrofauna were allowed to consume oxygen and chlorophyll a in the sediment and

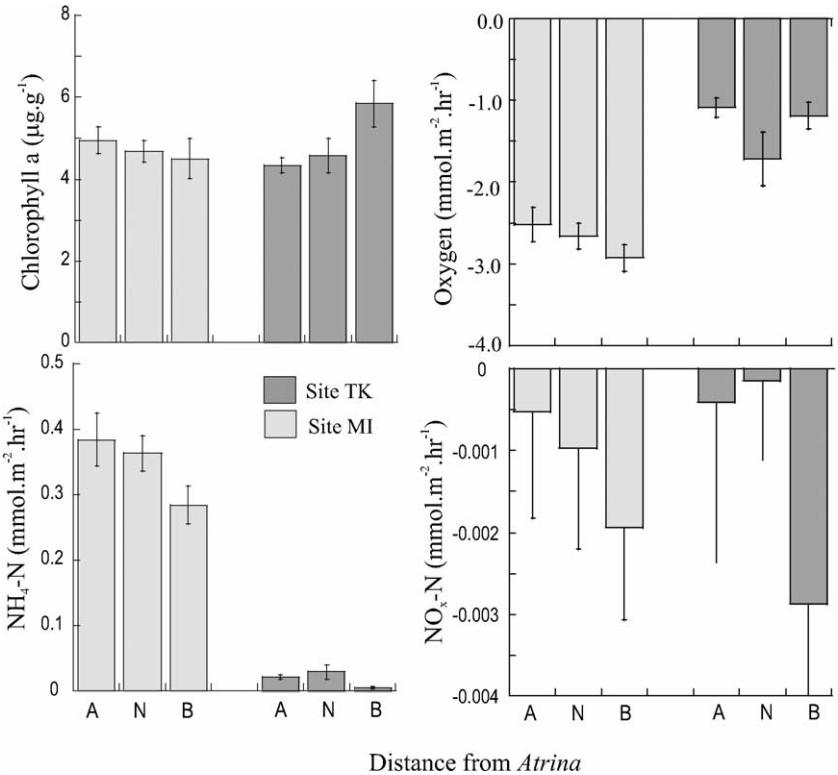

Fig. 2. Means and standard errors of sediment chlorophyll a content and oxygen and nutrient effluxes observed in the dark chambers over a 5 h period at each *Atrina* location for each site. A: chamber/core adjacent to *Atrina*, N: chamber/core immediately adjacent to A (not further than 30 cm from an *Atrina*), B: chamber/core further than 50 cm from an *Atrina*.

excrete NH_4–N. Chlorophyll a content of the sediment was allowed to affect macrofauna, oxygen consumption (through respiration in the dark), and NH_4–N efflux (through the slow growth which can occur in the dark). Oxygen consumption was allowed to affect NH_4–N efflux through the potential for conversion to NO_x–N in the presence of oxygen.

To simplify the model, a composite macrofaunal measure that reflected both abundance and body size was developed, based on three body-size classes (<3, 3–20 and >20 mm longest body dimension). The total abundance of each of these size classes was calculated; abundances in each size class were differentially weighted before adding to the model. Two different weighting systems were trialled as it was apparent that oxygen consumption and NH_4–N efflux were responding differently to the relative proportion of large to small individuals. The first equation weighted small individuals as 1, medium individuals by 2 and large individuals by 3 and was used in correlations with NH_4–N efflux, the second equation weighted small individuals as 1, medium individuals by 5 and large individuals by 25 and was used in correlations with oxygen consumption.

The relative strength of relationships between different variables was assessed at each site separately as our hypothesis was that the sites would behave differently. Relative strength was calculated using Pearson correlation coefficients, partial correlation coefficients from type 2 sum of squares, and standardized partial regression coefficients. As the three methods gave very similar results, only the standardised partial regression coefficients are used in this manuscript to demonstrate the model links.

3. Results

3.1. Differences between sites

Site MI sediments were dominated by coarse, fine and medium sands in decreasing order of percent by weight. Sediments at site TK were dominated by fine sands. However the chlorophyll a content at the two sites was similar (Fig. 2, Table 2), approximately 4.9 µg mg^{-1}.

Surprisingly, microphyte communities at the two sites were similar (64% Bray–Curtis similarity, Fig. 3), with both sites dominated by benthic diatoms (*Gyrosigma*, *Nitzschia* spp. and *Pleurosigma*) and the pelagic diatom

Table 2
Results of two-way analysis effect of site (MI, TK) and location (a: adjacent to *Atrina*, n: within 30 cm, f: greater than 50 cm away)

Variable	Error MS	Site * location		Site		Location		Multiple contrast
		MS	*p*-value	MS	*p*-value	MS	*p*-value	
Chlorophyll a	0.806	2.951	0.0386	0.28	0.5566	1.02	0.2977	TK f>n a
Oxygen consumption	0.210	0.196	0.4048	17.47	<0.0001	0.270	0.2922	
NH_4–N	0.197	0.768	0.0357	64.2	<0.0001	1.59	0.0023	TK n a>f
NO_x–N	0.041	0.001	0.9671	0.001	0.9995	0.009	0.8198	
Number of microphyte taxa	69.3	3.872	0.1936	11.8	0.0286	0.93	0.6633	
Number of macrofauna taxa	387	3.120	0.6619	62.9	0.0069	51.2	0.0036	a>n f
Number of macrofauna	583661	3705	0.0017	231710	<.0001	46232	0.0005	MI a n>f

MS = mean square. Most variables were analysed using normal errors and no transformation, numbers of taxa and individuals analyses used Poisson errors and a log-link, particulate organic nitrate had to be analysed using rank transformation. Where a significant interaction term is given ($p<0.05$), significant multiple contrast results for individual sites are given.

Thalassiosira. Slightly higher numbers of microphyte taxa were found at TK than MI (Fig. 4). However, macrofauna at the two sites were markedly different (93% Bray–Curtis dissimilarity, Fig. 3). MI was dominated by the suspension-feeding, tube-dwelling sabellid polychaete *Euchone*, with three species of ostracods and the small polychaete sub-family of Exogoninae also abundant. TK was dominated by the small deposit-feeding bivalves, *Theora lubrica* and *Nucula hartvigiana*, with the deposit-feeding, tube-dwelling polychaete *Boccardia syrtis*, two species of ostracods, and a small paraonid polychaete also abundant. The number of macrofaunal taxa was significantly higher at TK, and the reverse was true for total number of individuals (Table 2, Fig. 4).

3.2. Relationships between sediment characteristics, oxygen and nutrient fluxes and Atrina

ANOVA demonstrated no site * location interactions or effect of location (nearness to *Atrina*) on oxygen consumed in the dark or NO_x–N efflux (Table 2). For NH_4–N efflux, a significant interaction term between location and site was observed, with a lower NH_4–N efflux in bare areas (Table 2). The response of sediment chlorophyll a to *Atrina* differed significantly between the sites (site * location interaction term <0.05, Table 2), with sediment chlorophyll at TK significantly higher at 50 cm from *Atrina* than at locations closer. No significant effect of location on sediment chlorophyll was observed at site MI, although a general increase near *Atrina* was observed.

Regressing sediment chlorophyll concentrations against aspects of the *Atrina* landscape suggested that at MI chlorophyll was positively related to the weighted density of *Atrina* up to 55 cm away ($r^2=0.40$, Table 3), whereas at TK sediment chlorophyll was negatively related to the log of the density of *Atrina* within 10 cm ($r^2=0.39$) or the weighted density of *Atrina* up to 55 cm away ($r^2=0.42$). Oxygen consumption at MI was negatively related to the density of *Atrina* up to 45 cm away ($r^2=0.30$, Table 3), while there was no significant relationship at TK. NH_4–N and NO_x–N effluxes were

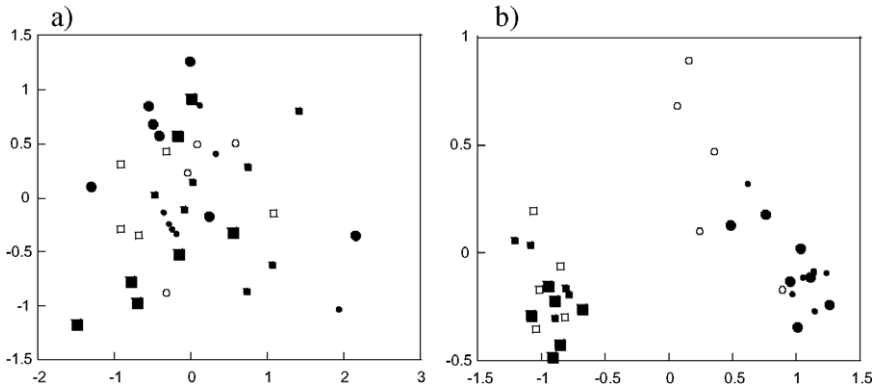

Fig. 3. Non-metric multi-dimensional scaling plot of (a) microphyte rank abundances and (b) macrofauna raw abundances based on Bray–Curtis similarities. Squares are samples from site TK, circles are samples from site MI. Large filled symbols are samples within 10 cm of an *Atrina*, small filled symbols are samples with 30 cm of an *Atrina*, open symbols are 50 cm away from an *Atrina*.

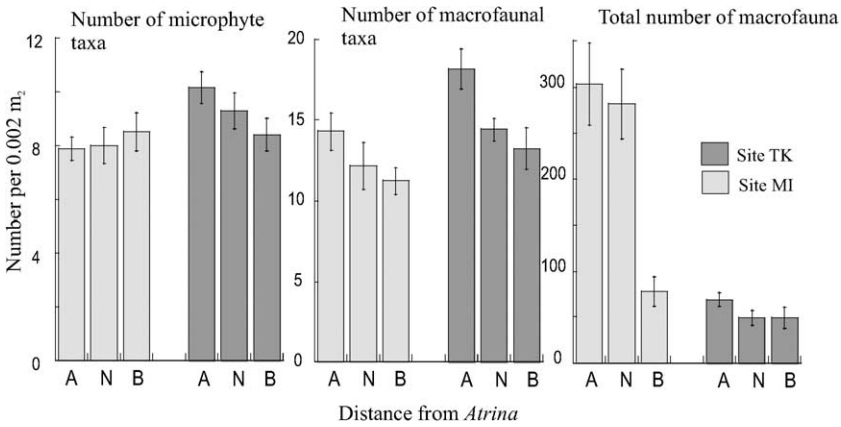

Fig. 4. Mean and standard errors of numbers of microphyte taxa, macrofaunal taxa and total number of macrofaunal individuals found in cores at each *Atrina* location for each site A: adjacent to *Atrina*, N: immediately adjacent to A (not further than 30 cm from an *Atrina*), B: further than 50 cm from an *Atrina*.

negatively related to the density of *Atrina* up to 45 cm away ($r^2 = 0.48$ and 0.39, respectively, Table 3), again no significant relationships were observed at TK.

3.3. Relationships between macrofauna and microphytes and Atrina

No site * location interactions or location (nearness to *Atrina*) effect was detected by ANOVA on the number of microphyte or macrofaunal taxa (Table 2). However, for total number of individuals of macrofauna, a site * location interaction was observed, with significantly more individuals found within 30 cm of *Atrina* at MI than further away (Fig. 4). No significant effect was observed at TK. Regression analysis detected a significant positive relationship between both the number of macrofaunal taxa and total number of individual macrofauna and the weighted density of *Atrina* within 55 cm ($r^2 = 0.28$ and 0.60, respectively) at MI and a positive relationship between the number of macrofaunal taxa and the density of *Atrina* 10 cm away at TK ($r^2 = 0.29$, Table 3).

Dissimilarity in microphyte communities adjacent to, and further away, from *Atrina* was not high (average 39% and 42% for MI and TK, respectively). Communities furthest away from *Atrina* were not necessarily most dissimilar to those adjacent to *Atrina*. However, there were some consistent patterns. At both sites the abundance of *Thalassiosira* increased away from *Atrina*, while *Nitzschia ventricosa* decreased. At TK, abundance of *Diplioneis* and *Gyrosigma* also increased away from *Atrina*. As *Gryosigma* and *Thalassiosira* were among the 5 most dominant genera at site TK, this probably accounted for the increased chlorophyll found away from *Atrina*.

Dissimilarity in macrofauna communities adjacent to, and further away, from *Atrina* was not high at TK (average 51%), and communities furthest away from

Table 3
Results from simple regression analysis to determine the effect of distance from, and density of *Atrina*, on the measured variables

Site	Variable	*Atrina* variable	R^2	*p*-value
MI	Chlorophyll a	Wtsum55	0.41	0.0044
	Oxygen consumed	Sum45	0.30	0.0208
	NH_4–N efflux	Sum45	0.48	0.0025
	NO_x–N efflux	Sum45	0.39	0.0092
	Number of microphyte taxa	Sum45	0.02	0.4970
	Number of macrofaunal taxa	Wtsum55	0.28	0.0490
	Number of macrofaunal individuals	Wtsum55	0.60	<0.0001
	Number of suspension feeders	Sum45	0.73	<0.0001
	Number of deposit feeders	D10	0.19	0.0724
	Number of surface utilisers	Wtsum55	0.71	<0.0001
	Number of large individuals	Sum45	0.37	0.0055
TK	Chlorophyll a	Wtsum55	0.42	0.0097
	Oxygen consumed	D10	0.10	0.2127
	NH_4–N efflux	Sum45	0.06	0.4045
	NO_x–N efflux	D10	0.22	0.0704
	Number of microphyte taxa	Wtsum55	0.01	0.899
	Number of macrofaunal taxa	D10	0.29	0.035
	Number of macrofaunal individuals	Sum45	0.03	0.527
	Number of suspension feeders	Sum45	0.11	0.4355
	Number of deposit feeders	Sum45	0.04	0.8667
	Number of surface utilisers	D10	0.12	0.1567
	Number of large individuals	Wtsum55	0.30	0.0309

Three different variables were used (d10: density of *Atrina* within 10 cm, sum45: density of *Atrina* within 45 cm, wtsum55: density of *Atrina* with 55 cm weighted by their distance) and results with the highest adjusted R^2 are shown. Non-linear relationships were incorporated by including logarithmic transformations and 2 degree polynomials.

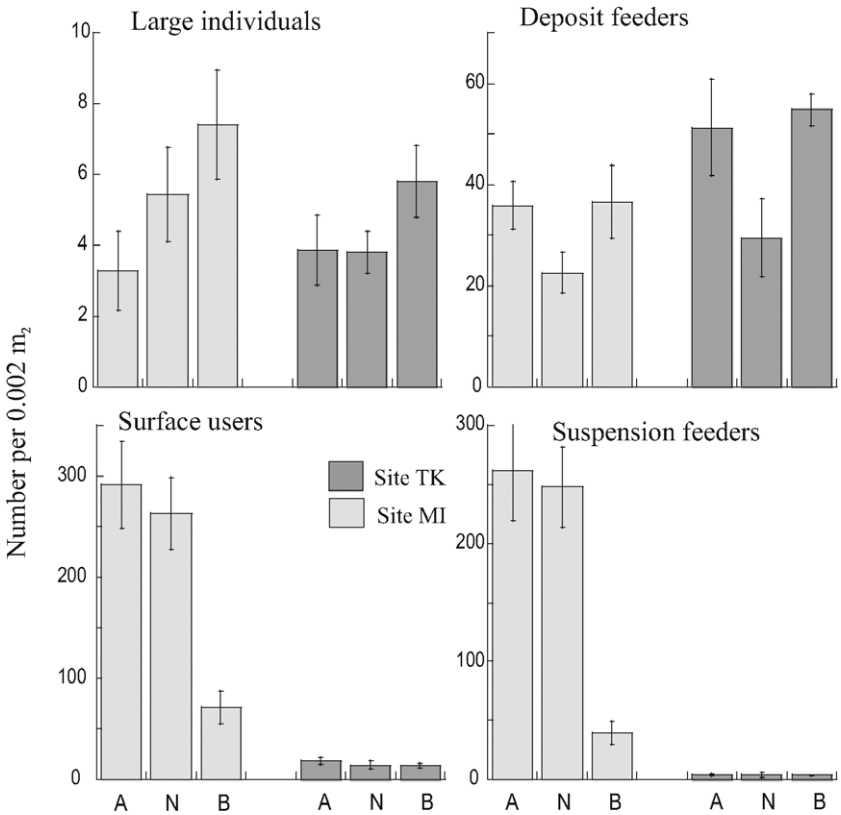

Fig. 5. Mean and standard errors of numbers of large individuals, surface utilising, deposit-feeding and suspension-feeding macrofauna found in cores at each *Atrina* location for each site A: adjacent to *Atrina*, N: immediately adjacent to A (not further than 30 cm from an *Atrina*), B: further than 50 cm from an *Atrina*.

Atrina were not necessarily most dissimilar to those adjacent to *Atrina*. At MI, in contrast, dissimilarity varied from 34% between 10 and 30 cm away from *Atrina* to 65% and 66% dissimilar in comparisons with 50 cm away from *Atrina*. At both sites there were consistent species changes moving away from *Atrina*, although only at MI did this include the dominant species present (*Euchone*). At TK, numbers of *Boccardia* and the dominant species of ostracod decreased away from *Atrina* while numbers of *Nucula* increased. At MI, numbers of *Euchone*, *Boccardia* and all species of ostracods decreased with decreasing distance from *Atrina*. All the species decreasing in abundance away from *Atrina* were small species that utilised the sediment water interface e.g., tube- or surface-dwelling, surface-deposit or suspension feeders. These findings suggested that division of the macrofauna into these type of species (hence called surface users) and large species (>25 mm longest dimension), rather than just total numbers of individuals may be worthwhile (Fig. 5).

Using regression analysis, at TK, no relationships were observed between numbers of deposit feeders or suspension feeders and any of the *Atrina* measures (Table 3). There was a suggestion of a relationship between numbers of surface users and the number of *Atrina* within 10 cm and a negative relationship between number of large individuals and both the log of the weighted density of *Atrina* within 55 cm and the number of *Atrina* in 45 cm ($r^2=0.30$ and 0.29, respectively). At MI, however, all aggregate variables were significantly related to at least one continuous measure of distance from *Atrina* (Table 4). Numbers of deposit feeders and suspension feeders were positively but weakly related to the number of *Atrina* within 10 cm ($r^2=0.19$ and 0.22, respectively, $p>0.1$). Numbers of suspension feeders were also positively related to both the number of *Atrina* within 45 cm of the core location ($r^2=0.73$) and the weighted density of *Atrina* within 55 cm ($r^2=0.50$). Numbers of surface users were positively related to both the weighted density of *Atrina* within 55 cm and the number of *Atrina* in 45 cm of the core location ($r^2=0.71$ and 0.65, respectively). Similar to TK, there was a negative relationship between numbers of large individuals and both the weighted density of *Atrina* within 55 cm

Table 4
Standarised partial regression estimates (SPRE) calculated from 4 multiple regressions conducted at each site using the connections specified in Fig. 6

Site	Independent variable	Dependent variable	SPRE
MI	Microphytes	Macrofauna	+0.170
		Atrina	+0.395
	Macrofauna	Microphytes	−0.301
		Atrina	+0.968
	Oxygen consumed	Macrofauna	+0.441
		Atrina	−0.408
		Microphytes	+0.074
	NH_4–N efflux	Macrofauna	+1.26
		Atrina	+1.109
		Microphytes	+0.095
		Oxygen	−0.247
TK	Microphytes	Macrofauna	+0.039
		Atrina	−0.670
	Macrofauna	Microphytes	−0.169
		Atrina	+0.843
	Oxygen consumed	Macrofauna	+0.239
		Atrina	−0.812
		Microphytes	+0.526
	NH_4–N efflux	Macrofauna	+0.365
		Atrina	+0.767
		Microphytes	−0.009
		Oxygen	−0.118

Microphytes = sediment chlorophyll a content, macrofauna = number of macrofaunal individuals weighted by size, *Atrina* effect = density of *Atrina* in 50 cm.

and the number of *Atrina* in 45 cm of the core location (r^2 = 0.35 and 0.37, respectively).

3.4. Modelling interactions between Atrina, macrofauna, microphytes and oxygen and nutrient fluxes

At both sites a negative relationship was observed between *Atrina* and oxygen consumption (Fig. 6). Sediment chlorophyll a and macrofauna were not strongly related at either site (i.e., p not <0.01). However, there were a number of differences in the strength of interactions defined by the conceptual model of the two sites. At MI, *Atrina* (as density within 50 cm) positively but weakly affected sediment chlorophyll a ($p>0.1$), while at TK there was a negative association. Although the relationship between *Atrina* and NH_4–N efflux was positive at both sites it was stronger at MI than TK. Similarly, the size-weighted estimate of macrofaunal abundance was positively related to NH_4–N efflux and oxygen consumption at both sites, but more strongly at MI than TK. Conversely the relationship between microphytes and oxygen consumption was stronger at TK than MI. Finally, while there was a positive effect of *Atrina* on macrofauna at both sites, the relationship was stronger at MI than TK.

4. Discussion

This study demonstrates the importance of indirect effects by *Atrina*, an ecosystem engineer that locally influence oxygen and nutrient fluxes. Effects of *Atrina* are not simply a direct result of *Atrina* respiration and excretion of dissolved nutrients. Instead, *Atrina* can also have strong indirect effects on ecosystem functioning by changing sediment characteristics, microphyte composition and biomass, and functional type and abundance of macrofauna. Such indirect effects are location-dependent and related to the complex interchange of location-dependent strength of effects of *Atrina* on these various aspects (Fig. 5).

The variability of effects of *Atrina* on surrounding macrofauna has been documented in a number of studies and is consistent with variable effects documented for other suspension-feeding bivalves (Olafsson, 1989). Specifically Norkko et al. (in press) observed stronger effects along a gradient of suspended sediment concentrations and suggested that the stronger effects of *Atrina* on macrofauna were related to a change from facilitation to inhibition related to sediment stress. The sites used in this study are at the facilitation end of their stress gradient and the responses observed fit within this facilitator-inhibition pattern, with stronger effects on numbers of macrofauna taxa and total numbers of individuals observed at site MI in clearer water than in the more turbid waters of site TK.

This clear difference between the strength of the effect of *Atrina* on macrofauna between sites was not driven by a single species or type of species and demonstrates the importance of natural history and species functions in understanding complex responses (Dayton, 2003). At both sites, the species changing in response to *Atrina* seemed to fall into two main groups, based on body size and surface utilization. All the species decreasing in abundance away from *Atrina* were small species that utilised the sediment water interface, e.g., tube- or surface-dwelling, surface-deposit or suspension feeders, while large species appeared to increase in occurrence. At site MI, strong relationships between numbers of suspension feeders, surface users and large individuals were observed, while at TK a significant relationship was observed for large individuals only. The increased numbers of surface utilisers around *Atrina* seem a likely response to micro-scale hypoxia resulting from increased organic content associated with biodeposits. Species protruding above the surface such as sabellid and polydorid tube worms (whether they filter or surface deposit feed) may have an advantage in an oxygen-depleted situation (Pearson and Rosenberg, 1978; Rhoads et al., 1978).

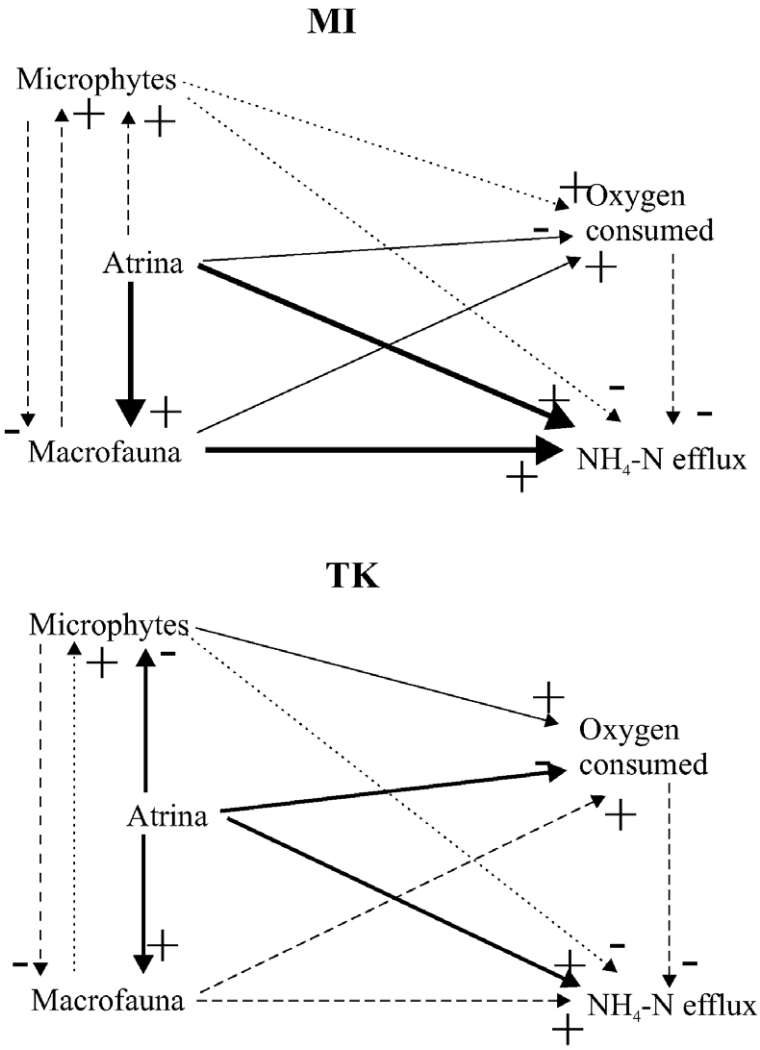

Fig. 6. Conceptual models indicating the relative strength and direction of relationships at the two sites, based on standardized partial regression estimate (SPRE) from Table 4. Dotted line (SPRE < 0.1), dashed line (SPRE 0.1–0.4), thin line (SPRE 0.4–0.6), thicker line (SPRE 0.6–0.9) and thickest line (SPRE > 0.9). These levels approximate to *p*-values for estimates of >0.2, 0.1–0.2, 0.05–0.1, 0.01–0.05 and <0.01. Microphytes = sediment chlorophyll a content, macrofauna = number of macrofaunal individuals weighted by size, *Atrina* effect = density of *Atrina* in 50 cm.

Mobile surface deposit/scavengers that spend much of their time in the water/surface interface (such as ostracods) may be responding to a resource (Van Blaricom, 1982; Oliver and Slattery, 1985).

While a strong response of *Atrina* on macrofauna was observed at MI, no strong effects were observed at either site on microphytes. Surprisingly the microphyte composition was very similar at the two sites with communities dominated by similar species of diatoms. Mainly these species were benthic-living motile diatoms, though at site TK a "suspensor" diatom species (i.e., a semi-pelagic species frequently resuspended off the seafloor) *Thalassiosira* was common. At both sites, this species increased away from *Atrina*, possibly in response to changes in hydrodynamics (Nikora et al., 2002). Dissimilarity in microphyte communities adjacent to and further away from *Atrina* was not high (average 39% and 42% for MI and TK, respectively) and at neither site were communities furthest away from *Atrina* necessarily most dissimilar to those adjacent to *Atrina*. Despite the lack of distinct changes in microphyte community structure, the response of chlorophyll a in the sediment to *Atrina* appeared to differ between sites. At MI, a small increase in chlorophyll a was observed near *Atrina*. Conversely at site TK, significantly higher chlorophyll a was found away from *Atrina*, possibly in response to increased abundances of two of the common species (*Gryosigma* and *Thalassiosira*). This increase in chlorophyll a away

from *Atrina* is consistent with that reported by Gibbs et al. (2005). These relatively weak responses for microphyte taxa suggest a reason for the lack of responses by meiofauna to *Atrina* noted at similar scales within *Atrina* patches (Austen and Thrush, 2001), despite the differences observed at larger scales between inside and outside patches (Warwick et al., 1997).

Changes to macrofauna, microphytes and sediment characteristics can be expected to have subsequent effects on ammonium effluxes from the sediment and oxygen consumption in the near surface waters (deDeckere et al., 2001; Bolam et al., 2002). Both of these would be expected to affect nitrification/denitrification rates (Bird et al., 1999; Reise, 2002). While nitrification/denitrification rates were not measured explicitly, negative nitrate–nitrite fluxes were observed at both sites, with these generally increasing away from *Atrina* (although the effect was only significant at site MI). Generally the trend of these fluxes reflected the trend of the ammonium efflux. Significant monotonic changes in oxygen consumption and ammonium efflux with increasing distance to *Atrina* were observed at site MI, but not at site TK. Changes at TK, for both these variables, were smaller and showed higher values at intermediate distances from *Atrina* (an inverse pattern to that of deposit feeders). At site MI, the correlation between oxygen consumption and ammonium efflux was negative, suggesting the use of oxygen to convert ammonium to nitrates. At site TK, the correlation was positive suggesting that the correlation between these two variables was actually a reflection of the two responding to another factor.

While microphytes were expected to provide the dominant link to oxygen consumption in the dark, this was not the case at either site. At site MI, a very weak positive correlation was observed between chlorophyll a content of the sediment and oxygen consumption in the dark, over shadowed by the stronger correlations with *Atrina* and macrofauna. At site TK, microphytes had a stronger correlation with oxygen consumption, although simple correlation, partial correlation and standardized regression coefficients all suggested that the effect of microphytes on oxygen consumption was not stronger than that of *Atrina*. The negative effect of *Atrina* on oxygen consumption initially appears counter-intuitive, but may be linked to decreased bioturbation near to *Atrina,* which would be expected to decrease oxygen consumption (deDeckere et al., 2001). The effect of macrofauna on oxygen consumption was positive at both sites and strongly affected by body size; the best relationship was obtained by a composite equation weighting individuals with body size >20 mm 25 times those of small individuals. However, the relationship was stronger at site MI, where a stronger gradient in macrofauna was observed.

Relationships with ammonium efflux were more consistent between sites. There was a weak negative relationship between microphytes and ammonium efflux at both sites, probably resulting from a small uptake of ammonium by dark growth of microphytes. This result is consistent with Gibbs et al. (2005) but does not provide support for the findings of Anderson et al. (2003), who suggest that dark uptake by microphytobenthos was likely to be an important sink for mineralized N. *Atrina* was a positive predictor of ammonium efflux at both sites, probably through the increased carbon content of the sediment (Caffrey et al., 1993; Strauss and Lamberti, 2000; Strauss et al., 2002) as observed by Norkko et al. (2001), although its effect was stronger at site MI than site TK. Macrofauna was a strong predictor of ammonium efflux at both sites, suggesting that excretion by even relatively small macrofauna (size difference between *Atrina* and the largest macrofauna found was 2 orders of magnitude) can strongly affect ammonium effluxes from the seafloor to the water column, increasing nitrogen inputs, as Gardner et al. (1993) suggested for the Gulf of Mexico. The potential for small macrofauna to be important in ammonium efflux was supported by the observation that the best relationship between ammonium efflux and macrofauna was obtained by a composite equation weighting individuals with body size >20 mm at only 5 times those of small individuals (cf. 25 for oxygen consumption).

5. Conclusions

Atrina have generally been demonstrated to have strong direct effects on benthic–pelagic coupling; increasing sediment fluxes to the seafloor, depletion of phytoplankton and changing ammonium and oxygen concentrations. This study suggests that indirect effects by key benthic macrofaunal species in marine systems can also be important to benthic–pelagic coupling. Indirect effects on benthic–pelagic exchanges of nutrients and oxygen were demonstrated to operate through changes in response of microphytes, sediment characteristics (organic content, particle size and porosity) and macrofauna. The relative strength of links between microphytes, sediment and macrofauna was generally location-dependent, however macrofauna were important, particularly for ammonium effluxes. This suggests that excretion and respiration of smaller macrofauna that directly respond to the presence of more dominant habitat modifiers can be important to the exchange of

nutrients near the seafloor and oxygen consumption in the benthic boundary layer.

Acknowledgements

We thank G. Funnell and Kay Vopel for assistance with field work, K. Safi for microphyte species identification and enumeration, and Luca Chiaroni and Jane Halliday for help with the macrofaunal species identification and enumeration and video analysis. This work was funded by the Foundation for Research, Science, and Technology, contract C01X0028. **[SS]**

References

Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. Aust. Ecol. 26, 32–46.

Anderson, I.C., McGlathery, K.J., Tyler, A.C., 2003. Microbial mediation of 'reactive' nitrogen transformations in a temperate lagoon. Mar. Ecol. Prog. Ser. 246, 73–84.

Austen, M.C., Thrush, S.F., 2001. Experimental evidence suggesting slow or weak response of nematode community structure to a large suspension-feeder. J. Sea Res. 46, 69–84.

Bird, F.L., Ford, P.W., Hancock, G.J., 1999. Effects of burrowing macrobenthos on the flux of dissolved substances across the water–sediment interface. Mar. Freshw. Res. 50, 523–532.

Bolam, S.G., Fernandes, T.F., Huxham, M., 2002. Diversity, biomass and ecosystem processes in the marine benthos. Ecol. Monogr. 72, 519–616.

Caffrey, J.M., Sloth, N.P., Kaspar, H.F., Blackburn, T.H., 1993. Effect of organic loading on nitrification and denitrification in a marine sediment microcosm. FEMS Microbiol. Ecol. 12, 159–167.

Cummings, V.J., Thrush, S.F., Hewitt, J.E., Turner, S.J., 1998. The influence of *Atrina zelandica* (Gray) on benthic marcoinvertebrate communities in soft-sediments habitats. J. Exp. Mar. Biol. Ecol. 228, 227–240.

Dame, R., Libes, S., 1993. Oyster reefs and nutrient retention in tidal creeks. J. Exp. Mar. Biol. Ecol. 171, 251–258.

Dame, R.F., Bushek, D., Prins, T.C., 2001. Benthic suspension feeders as determinants of ecosystem structure and function in shallow coastal waters. In: Reise, K. (Ed.), Ecological Comparisons of Sedimentary Shores. Springer, Berlin, pp. 11–38.

Dayton, P.K., 2003. The importance of the natural sciences to conservation. Am. Nat. 162, 1–13.

deDeckere, E.M.G.T., Tolhurst, T.J., deBrouwer, J.F.C., 2001. Destabilization of cohesive intertidal sediments by infauna. Estuar. Coast. Shelf Sci. 53, 665–669.

Diamond, D., 2001. Determination of nitrate/nitrite in brackish or seawater by flow injection analysis QuikChem method 31-107-04-1-A. LaChat instruments Milwaukee.

Dowd, M., 2005. A bio-physical coastal ecosystem model for assessing environmental effect of marine bivalve aquaculture. Ecol. Model. 183, 323–346.

Frechette, M., Bacher, C., 1998. A modelling study of optimal stocking density of mussel populations kept in experimental tanks. J. Exp. Mar. Biol. Ecol. 219, 241–255.

Frechette, M., Butman, C.A., Geyer, W.R., 1989. The importance of boundary-layer flows in supplying phytoplankton to the benthic suspension feeder, *Mytilus edulis* L. Limnol. Oceanogr. 34, 19–36.

Gardner, W.S., Escobar Briones, E., Cruz Kargi, E., Rowe, G.T., 1993. Ammonium excretion by benthic invertebrates and sediment–water nitrogen flux in the Gulf of Mexico near the Mississippi outflow. Estuaries 16, 799–808.

Gibbs, M., Funnell, G., Pickmere, S., Norkko, A., Hewitt, J.E., 2005. Benthic nutrient fluxes along an esturine gradient: influence of the pinnid bivalve, *Atrina zelandica*, in summer. Mar. Ecol. Prog. Ser. 288, 151–164.

Green, M.O., Hewitt, J.E., Thrush, S.F., 1998. Seabed drag coefficients over natural beds of horse mussels (*Atrina zelandica*). J. Mar. Res. 56, 613–637.

Hewitt, J.E., Pilditch, C.A., 2004. Short-term feeding responses of *Atrina zelandica* to suspended sediment concentrations: effects of environmental history and physiological state. J. Exp. Mar. Biol. Ecol. 306, 95–112.

Hewitt, J.E., Legendre, P., Thrush, S.F., Cummings, V.J., Norkko, A., 2002. Integrating results from different scales: a multi-resolution study of the relationships between *Atrina zelandica* and macrofauna along a physical gradient. Mar. Ecol. Prog. Ser. 239, 115–128.

Hughes, D.J., Atkinson, R.J.A., Ansell, A.D., 2000. A field test of the effects of megafaunal burrows on benthic chamber measurements of sediment-water solute fluxes. Mar. Ecol. Prog. Ser. 195, 189–199.

Liao, N., 2001. Determination of ammonia in brackish or seawater by flow injection analysis QuikChem method 31-107-06-1-B. LaChat instruments Milwaukee.

Lohrer, A.M., Thrush, S.F., Gibbs, M.M., 2004. Bioturbators enhance ecosystem performance via complex biogeochemical interactions. Nature 431, 1092–1095.

Loo, L.-O., Rosenberg, R., 1989. Bivalve suspension-feeding dynamics and benthic–pelagic coupling in an eutrophicated marine bay. J. Exp. Mar. Biol. Ecol. 130, 253–276.

Newell, R.I.E., Cornwell, J.C., Owens, M.S., 2002. Influence of simulated bivalve biodeposition and microphytobenthos on sediment nitrogen dynamics: a laboratory study. Limnol. Oceanogr. 47, 1367–1379.

Nikora, V., Green, M.O., Thrush, S.F., Hume, T.M., Goring, D., 2002. Structure of the internal boundary layer over a patch of horse mussels (*Atrina zelandica*) in an estuary. J. Mar. Res. 60, 121–150.

Norkko, A., Hewitt, J.E., Thrush, S.F., Funnell, G.A., 2001. Benthic–pelagic coupling and suspension feeding bivalves: linking site-specific sediment flux and biodeposition to benthic community structure. Limnol. Oceanogr. 46, 2067–2072.

Norkko, A., Hewitt, J.E., Thrush, S.F., Funnell, G.A., in press. Conditional outcomes of facilitation by a habitat-modifying subtidal bivalve. Ecology.

Olafsson, E.B., 1989. Contrasting influences of suspension-feeding and deposit feeding populations of *Macoma balthica* on infaunal recruitment. Mar. Ecol. Prog. Ser. 55, 171–179.

Oliver, J.S., Slattery, P.N., 1985. Destruction and opportunity on the sea floor: effects of gray whale feeding. Ecology 66, 1965–1967.

Pearson, T.H., Rosenberg, R., 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. Oceanogr. Mar. Biol. Ann. Rev. 16, 229–311.

Reise, K., 2002. Sediment mediated species interactions in coastal waters. J. Sea Res. 48, 127–141.

Rhoads, D.C., McCall, P.L., Yingst, J.Y., 1978. Disturbance and production on the estuarine seafloor. Am. Sci. 66, 577–586.

Sartory, D.P., 1982. Spectrophotometric analysis of chlorophyll a in freshwater phytoplankton. Hydrological Research Institute Technical Report TR 115. Pretoria, South Africa. 163 pp.

Strauss, E.A., Lamberti, G.A., 2000. Regulation of nitrification in aquatic sediments by organic carbon. Limnol. Oceanogr. 45, 1854–1859.

Strauss, E.A., Mitchell, N.L., Lamberti, G.A., 2002. Factors regulating nitrification in aquatic sediments: effects of organic carbon, nitrogen availability, and pH. Can. J. Fish Aquat. Sci. 59, 554–563.

Thrush, S.F., Cummings, V.J., Hewitt, J.E., Funnell, G.A., Green, M.O., 2001. The role of suspension feeding bivalves in influencing macrofauna: variations in response. In: Aller, J.Y., Woodin, S.A., Aller, R.C. (Eds.), Organism–Sediment Interactions. University of South Carolina Press, Columbia, SC, USA, pp. 87–101.

Thrush, S.F., Hewitt, J.E., Gibb, M., Lundquist, C., Norkko, A., in press. Functional role of large organisms in intertidal communities: Community effects and ecosystem function. Ecosystems.

Van Blaricom, G.R., 1982. Experimental analysis of structural regulation in a marine sand community exposed to oceanic swell. Ecol. Monogr. 52, 283–305.

Warwick, R.M., McEvoy, A.J., Thrush, S.F., 1997. The influence of *Atrina zelandica* Gray on nematode diversity and community structure. J. Exp. Mar. Biol. Ecol. 214, 231–247.

Welsh, D., 2003. Its a dirty job but someone has to do it: the role of marine benthic macrofauna in organic matter turnover and nutrient recycling to the water column. Chem. Ecol. 19, 321–342.