

Effects of *Arenicola marina* on polychaete functional diversity revealed by large-scale experimental lugworm exclusion

N. Volkenborn*, K. Reise

Alfred Wegener Institute for Polar and Marine Research, Wadden Sea Station Sylt, Hafenstrasse 43, D-25992 List, Germany

Received 5 April 2006; accepted 8 August 2006

Available online 10 August 2006

Abstract

The lugworm *Arenicola marina* was excluded from sandy sediment areas in the mid and low intertidal zone of the Wadden Sea. Exclusion, control and ambient plots were 400 m² each, replicated six times and sampled in August of three consecutive years. Responses were analysed with respect to functional trait groups in the associated polychaete assemblage using uni- and multivariate statistical techniques. Tube-building worms and predacious worms were most abundant in exclusion plots, while subsurface deposit feeders tended to dominate in the presence of lugworms. Lugworm effects were stronger in low intertidal fine sand than in mid intertidal medium sand. In the third year, lugworm densities strongly decreased at the study site. Nevertheless the polychaete functional group composition in lugworm exclusion plots still significantly differed from that in control and ambient plots. We assume that the permanent exclusion of lugworms may have entailed a cumulative change in sediment properties in the exclusion plots. Overall, lugworm effects were highly dependent on space and time as well as on differential recruitment success in this intertidal polychaete assemblage. Sediment-mediated effects of an ecosystem engineer on associated species appear to be subtle and contingent in variable environments. © 2006 Elsevier B.V. All rights reserved.

Keywords: *Arenicola marina*; Benthic assemblage; Bioturbation; Functional groups; Intertidal; Polychaetes; Wadden Sea

1. Introduction

Substantial parts of the tidal flats in the Wadden Sea are populated and strongly bioturbated by the lugworm *Arenicola marina* (Beukema, 1976; Cadée, 1976; Reise, 1985). Its dominant appearance aroused interest in the effects of lugworms on other sediment-dwelling organisms and on implications of their bioturbating and bioirrigating activity for habitat properties (review by Riisgård and Banta, 1998). While lugworms were found to facilitate small zoobenthos in the immediate vicinity of ventilated

burrows by supplying oxygen to an otherwise anoxic subsurface sediment (Reise, 1981, 1983; Lackschewitz and Reise, 1998) disturbances at the sediment surface by their reworking activity was found to inhibit some other, primarily sedentary species (Brey, 1991; Flach, 1992a,b, 1993, 1996; Flach and De Bruin, 1993). These results were either based on sampling of burrow structures, on small scale field experiments (<2 m²) where lugworms were excluded or added, and on correlative studies on a km-scale. The assumed mechanisms are small-scale interactions directly related to burrow maintenance and feeding activity of the lugworms. Given the small size of most infauna and their limited motility, it is assumed that such biotic interactions are confined to small distances at the surface (Zajac et al., 1998; Zajac, 2004) as well as below

* Corresponding author.

E-mail address: nvolkenborn@awi-bremerhaven.de (N. Volkenborn).

(Lackschewitz and Reise, 1998). Much less is known about sediment-mediated biotic interactions on a meso-scale (Zajac et al., 1998) arising from ecosystem properties modified by populations of habitat engineering species (sensu Jones et al., 1994). This can be partly explained by the fact that most experimental studies of assemblage formation in soft sediment habitats have been conducted at small scales ($<1\text{ m}^2$, Hall et al., 1994). However, biogenic habitat transformations (sensu Reise, 2002) are well known to structure benthic assemblages and these may extend over wider spatial scales.

Indirect effects may be easily modified by changing boundary conditions and may require an extravagant approach to become visible. In fact, experimental exclusion of *A. marina* from replicated 400 m^2 plots induced significant changes in habitat properties, including an increase in microphytobenthic biomass, accumulation of sulphide, inorganic nutrients, fine particles and associated organic matter, and a decrease in sediment permeability (Volkenborn and Reise, 2006; Volkenborn et al., unpubl. ms). With this study we test whether ecosystem engineering by *A. marina* affects the benthic community on the scale of our experimental plots.

We here focus on the response of polychaete species to experimental lugworm exclusion since polychaetes are the most diverse group in the benthic macrofauna and comprise a variety of feeding and mobility modes (Fauchald and Jumars, 1979; Reise, 1985). The presence of different trophic and mobility traits at the study site allowed us to test predictions for functional modes. Furthermore, functional groups eased comparisons between years because inter-annual fluctuations of individual species are high in temperate intertidal sediments (Beukema, 1991). Other abundant macrofauna species on the experimental plots, the gastropod *Hydrobia ulvae* and the

juvenile bivalves *Macoma balthica* and *Mya arenaria*, were omitted to analyse their dynamics separately (Volkenborn et al., unpublished ms).

With univariate and multivariate statistical techniques we address the question: Does *A. marina* affect the polychaete community beyond the immediate vicinity of individual burrows? If so, (a) are there any differential inhibitive and promotive effects on polychaete functional groups?, and (b) are such effects consistent in space and time? Results of the present study may clarify the role of the most widespread ecosystem engineer of the Wadden Sea in structuring benthic assemblages relative to external drivers.

2. Materials and methods

2.1. Study area and experimental design

A large-scale lugworm exclusion experiment was conducted on an intertidal sandflat in Königshafen, a tidal embayment at the northern end of the island of Sylt in the North Sea ($55^{\circ}02'\text{ N}$; $8^{\circ}26'\text{ E}$, Fig. 1). *Arenicola* flats with consistently more than 20 worms m^{-2} comprise 76% of the intertidal area (Reise et al., 2001). Sediment at the experimental site is dominated by medium and fine sand and mean tidal range is 1.8 m. Due to aeolian sand input from surrounding dunes, medium sand dominates the edges of the embayment and grain size median decreases towards its centre (Austen, 1994). This is in contrast to most other tidal flats of the Wadden Sea where high intertidal zones are often muddy and grain size increases towards low tide line (Beukema, 1976). A detailed description of the tidal embayment is given by Reise (1985) and Reise et al. (1994). Exclusion of lugworms was achieved by inserting a 1-mm meshed

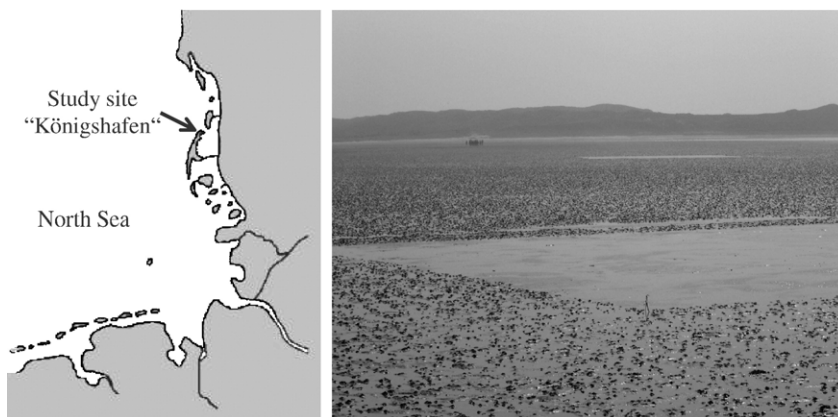


Fig. 1. Study site. The rectangular flat area lacking lugworm faeces mounds is one of the lugworm exclusion plots. Lugworms were permanently excluded from 400 m^2 areas by inserting a 1 mm meshed net to 10 cm depth. A second exclusion plot is visible in the background.

polyethylene net at 10 cm depth into the sediment in spring 2002. The horizontal net prevented lugworms maintaining their burrows and effectively kept them away. Surface sediment was excavated with a backhoe to bury the net and this was also done on control plots to account for the initial disturbance. Ambient plots were left untouched to represent natural conditions. The experiment was created in a 2-factorial nested block design (Fig. 2). One block consisted of three plots differing in treatment: Exclusion=buried net, Control= similarly dug up but left without a net, Ambient= untouched plot. Each plot was $20 \times 20 = 400 \text{ m}^2$ in area. The large size of the experimental plots was chosen to minimise effects of lateral sediment transport typical for sandy intertidal flats (Grant et al., 1997) and known from previous experiments at the study site (Flothmann and Werner, 1992; Zühlke and Reise, 1994). Six experimental blocks were nested with respect to the tidal zone: three blocks were conducted within a mid intertidal medium sand (emersion 6–7 h per tide; grain size median 330–340 μm) and three blocks within a low intertidal fine sand (emersion 3–4 h; grain size median 200–220 μm). Emersion time and sediment type were always interrelated in the area and thus could not be kept separate in our experimental design.

2.2. Macrofauna sampling

Analysis of macrofauna on experimental plots was done in August of three consecutive summers between 2002 and 2004. During this time of the year the polychaete community reaches its highest species richness at the study site and most species show highest abundances (Volkenborn, unpubl. data). Sampling was done by counting polychaetes retained on a 1-mm mesh sieve from 8 randomly chosen cores of 100 cm^2 taken within each experimental plot. Sampling depth was 20 cm and samples were divided into upper and lower 10 cm. At the exclusion site, only the upper layer could be sampled because of the net at 10 cm depth. Since polychaetes were encountered only occasionally below 10 cm (mainly adult *Nereis diversicolor*), results are presented as individuals m^{-2} with upper and lower core sections combined for the plots without a net. Sampling was generally completed within two weeks and was done block-wise, thus including possible effects of consecutive sampling into the block effect. Polychaete species were grouped according to their trophic and mobility guilds, based on published information (Fauchald and Jumars, 1979) and our own site-specific observations. Accounting for the plasticity of feeding and motility traits in single species (i.e. feeding guilds of tube

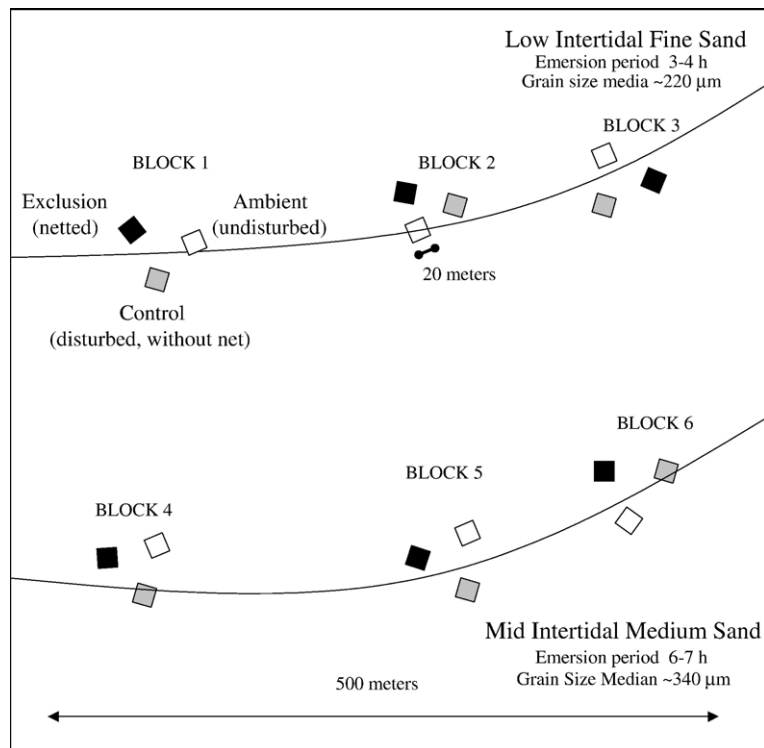


Fig. 2. Experimental set-up of the large-scale lugworm exclusion experiment in a 2-factorial nested block design. Six experimental blocks comprised three lugworm treatments and were nested within two tidal levels.

Table 1

Overview of uni- and multivariate statistical test to analyse factor effects on the polychaete community

Purpose	Univariate techniques	Multivariate techniques			
	Factor effects on abundance of single functional groups	Time consistent factor effects within each intertidal area	Spatially consistent factor effects within each year		Contribution of single species/functional groups to assemblage similarities/dissimilarities
Statistical test	multifactorial ANOVA	2-way crossed ANOSIM	2-way crossed ANOSIM 2	2-way nested ANOSIM	SIMPER
Investigated factors	year \times treatment \times tidal zone \times block	year \times treatment	treatment \times block	block \times tidal zone	year \times treatment \times tidal zone
Result shown in	Table 3	Table 5	Table 4	Table 4	Table 5, text

worms or motility of surface deposit feeders) we decided to use a bi-functional characterisation of species combining trophic and motility traits as these are the most fundamental attributes in functional group ecology (Pearson, 2001). This multifunctional approach (Bremner et al., 2003) allowed a clear classification without ambiguity and a comparison of years with different species composition. Within the

polychaete community four functional groups were classified: (a) mixed suspension and surface deposit feeding tube worms (*Pygospio elegans*, *Polydora cornuta*, *Spio martinensis*, *Lanice conchilega*), (b) surface deposit feeding discretely motile worms (*Nereis diversicolor*, *Nereis virens*, *Tharyx killariensis*, *Malacoceros fuliginosus*, juvenile *Arenicola marina*), (c) subsurface deposit feeding

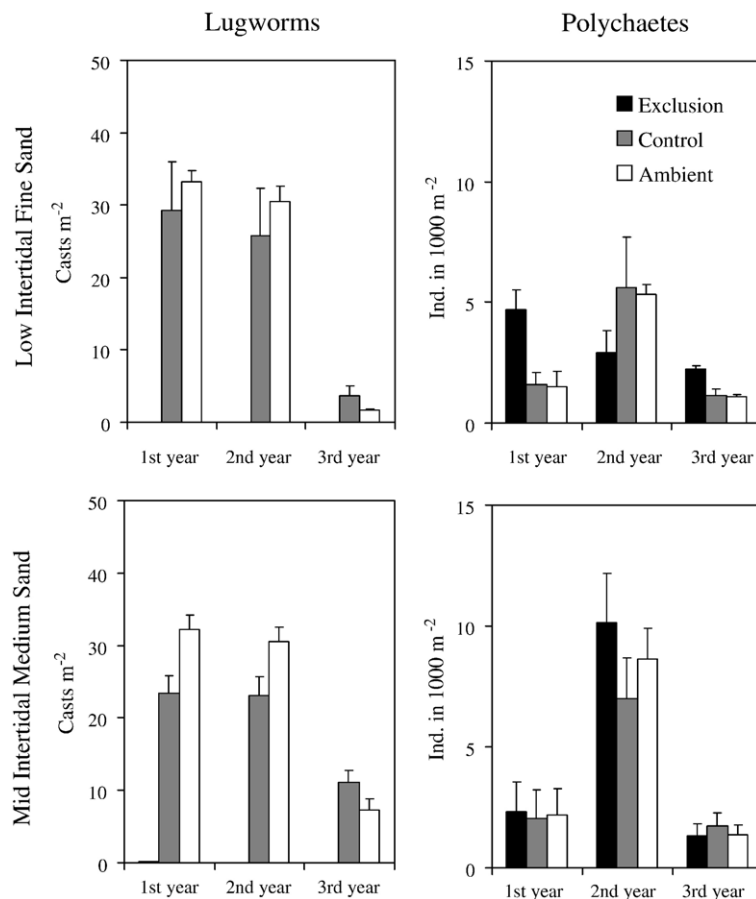


Fig. 3. Abundances of *Arenicola marina* estimated by cast counts and total polychaete abundances in experimental plots in the low intertidal fine sand and the mid intertidal medium sand area over the three year period. Shown are means ($n=3$) and SE.

discretely motile worms (*Scoloplos* cf. *armiger*, *Capitella capitata*, *Heteromastus filiformis*) and (d) carnivorous motile worms (*Eteone longa*, *Phyllodoce mucosa*, *Nephtys hombergii*). Adults of the lugworm *A. marina* were not included in the response analysis because they were part of the experimental treatment.

Densities of lugworm faecal mounds were estimated on calm days without rain within 10 randomly chosen quadrates of 0.25 m² on each experimental plot. Numbers of faecal castings vary with feeding activity but may serve as a proxy for abundances of *A. marina* at least in summer (Reise et al., 2001).

2.3. Statistical analysis

To test effects of lugworm presence/absence on the functional composition of the polychaete assemblage

with respect to time and space we used univariate and multivariate techniques (Table 1). Two-factorial Repeated Measures ANOVA (RM ANOVA) with experimental blocks nested in the tidal zones as additional factor was used to test the effects of

1. lugworm presence/absence and disturbance by initial dredging (exclusion, ambient and control plots),
2. tidal zone (low intertidal fine sand and mid intertidal medium sand),
3. experimental blocks (accounting for spatial heterogeneities on a scale of 10 s to 100 s of metres),
4. year-to-year variability (accounting for temporal heterogeneities),
5. and possible factor interactions on polychaete and functional group abundances. All ANOVA's were calculated with the General Linear Model (GLM)

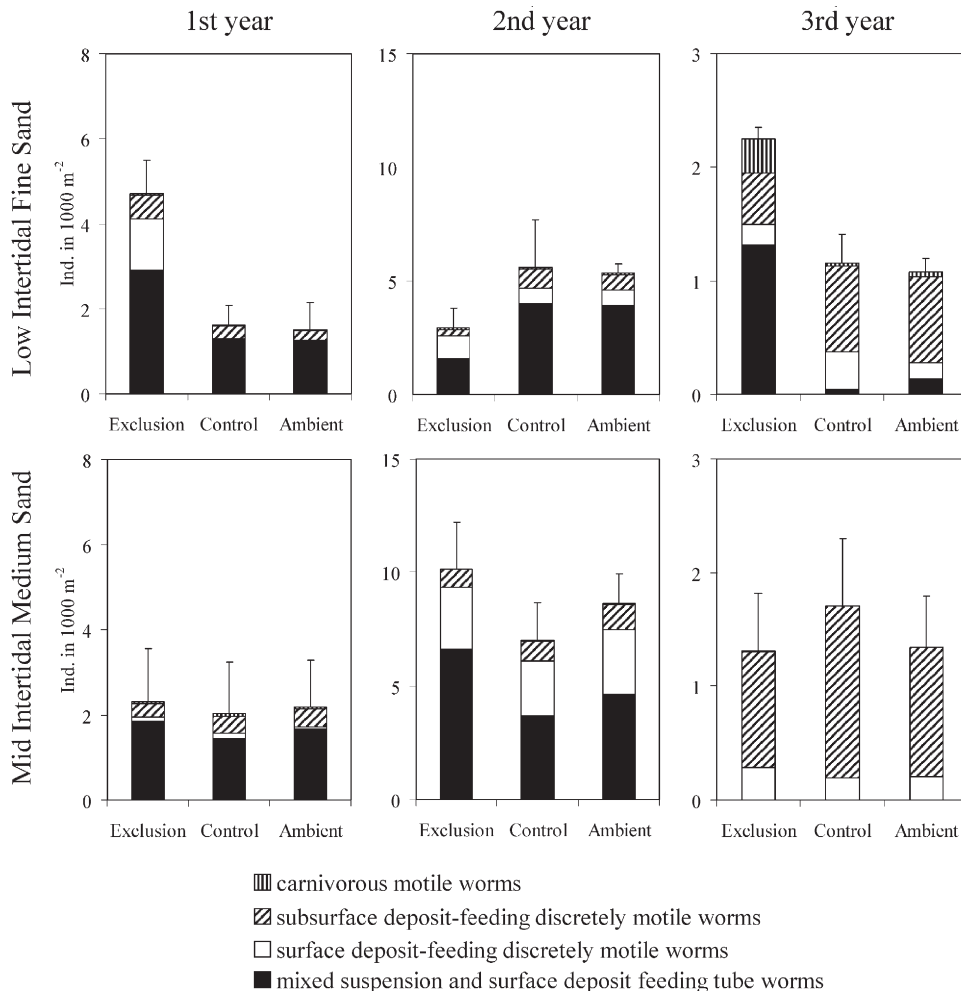


Fig. 4. Functional group composition of polychaete assemblages in relation to experimental treatments over the three years of observation. Shown are mean abundances (n=3) for each group. Error bars represent SE of total polychaete numbers (note different scale of y-axis).

procedure of STATISTICA (StatSoft, Inc.). Prior to analysis abundance data were log+1-transformed to achieve homogeneity of variances (Cochran's Test). Relative effect sizes were calculated as percent variance explained (Howell, 1992). Post-hoc multiple

means comparisons were performed using the Tukey-Kramer procedure at $\alpha=0.05$ significance level.

Table 2

Mean abundances of polychaetes (ind m⁻²) at mid and low intertidal zone on exclusion (E), control (C) and ambient plots (A) in August 2002, 2003 and 2004

	Mid Intertidal Medium Sand			Low intertidal Fine Sand		
	E	C	A	E	C	A
1st year						
<i>Arenicola marina</i> (juveniles)	0	0	0	4	0	0
<i>Capitella capitata</i>	123	132	102	208	102	38
<i>Eteone longa</i>	8	17	13	25	0	0
<i>Heteromastus filiformis</i>	4	8	21	72	30	0
<i>Nephtys hombergii</i>	0	0	0	17	21	18
<i>Nereis spec.</i>	106	115	21	1167	25	8
<i>Phyllodoce mucosa</i>	21	30	25	8	0	13
<i>Polydora cornuta</i>	1345	1205	1290	815	658	581
<i>Pygospio elegans</i>	382	140	238	1545	204	331
<i>Scoloplos cf. armiger</i>	195	263	301	263	153	204
<i>Spio martinensis</i>	119	102	153	552	407	314
<i>Tharyx killariensis</i>	0	13	9	34	4	8
Total individuals	2305	2024	2174	4711	1604	1516
2nd year						
<i>Arenicola marina</i> (juveniles)	13	4	21	0	0	0
<i>Capitella capitata</i>	296	58	17	21	33	38
<i>Eteone longa</i>	17	33	46	29	46	38
<i>Heteromastus filiformis</i>	4	13	46	50	25	17
<i>Lanice conchilega</i>	0	0	0	17	0	0
<i>Malacoceros fuliginosus</i>	4	4	0	0	0	0
<i>Nephtys hombergii</i>	0	0	0	8	17	21
<i>Nereis spec.</i>	2708	2383	2808	879	654	679
<i>Phyllodoce mucosa</i>	0	0	4	13	25	13
<i>Polydora cornuta</i>	658	154	96	567	242	54
<i>Pygospio elegans</i>	5958	3542	4542	958	3500	3458
<i>Scoloplos cf. armiger</i>	492	825	1063	225	767	596
<i>Spio martinensis</i>	0	4	0	42	250	417
<i>Tharyx killariensis</i>	0	0	4	117	46	8
Total individuals	10150	7021	8646	2925	5604	5338
3rd year						
<i>Arenicola marina</i> (juveniles)	42	13	8	13	4	4
<i>Capitella capitata</i>	0	0	0	4	8	8
<i>Eteone longa</i>	0	4	0	96	13	8
<i>Heteromastus filiformis</i>	8	4	13	29	50	13
<i>Lanice conchilega</i>	0	0	0	496	0	4
<i>Nephtys hombergii</i>	0	0	0	63	8	29
<i>Nereis spec.</i>	242	183	200	142	308	142
<i>Phyllodoce mucosa</i>	4	0	4	142	4	0
<i>Pygospio elegans</i>	0	0	0	817	46	129
<i>Scoloplos cf. armiger</i>	1013	1504	1121	413	704	738
<i>Tharyx killariensis</i>	0	0	0	33	13	0
Total individuals	1308	1708	1346	2246	1158	1075

Temporal, spatial and lugworm treatment effects on the functional assemblage composition were explored with non-parametric ANOSIM procedures (PRIMER software, Plymouth Marine Laboratory). Multivariate analysis and multi-dimensional scaling (MDS) were based on the Bray-Curtis similarity index of square-root transformed abundance data. To test for general effects consistent over all years, 2-way crossed ANOSIM was applied combining year as first factor and lugworm treatment, tidal zone or experimental block as second factor. To test for general effects of lugworm presence/absence on polychaete assemblages within both tidal zones we used 2-way crossed ANOSIM (Year \times Treatment) separated for both tidal zones.

To test for factor effects on the functional composition and to assess their relative importance within single years we used (a) 2-way crossed ANOSIM 2 (treatment \times experimental blocks) without replication within experimental blocks (Clarke and Warwick, 2001) for the effects of lugworm presence/absence and (b) nested ANOSIM (blocks nested in tidal zones) for the effects of spatial factors. With these statistical techniques we analysed (1) whether there were lugworm treatment effects while allowing experimental block effects, (2) whether there were assemblage differences between the low intertidal fine sand and the mid intertidal medium sand, and (3) whether there were assemblage differences among experimental blocks within both tidal zones.

To identify which species or functional group primarily account for assemblage differences SIMPER procedures were applied. With this exploratory technique we analysed the relative contribution of individual species or functional groups to within-treatment Bray-Curtis similarities and to factor-induced assemblage dissimilarities between treatments.

Prior to all uni- and multivariate statistical analysis we checked if there were significant differences between control and ambient plots. Since this was not the case, we lumped control and ambient plots as representatives for lugworm control plots.

3. Results

3.1. Control of experimental treatment

Lugworms were permanently excluded from all six plots by the inserted mesh (Fig. 3). Lugworm abundances were slightly reduced in control plots compared

Table 3

Repeated measures ANOVA of lugworm and tidal height effects on the abundances of polychaete functional groups

	df	mixed suspension and surface deposit feeding tube worms				surface deposit feeding discretely motile worms				subsurface deposits feeding discretely motile worms				carnivorous motile worms			
		MS	F	p	post-hoc	MS	F	p	post-hoc	MS	F	p	post-hoc	MS	F	p	post-hoc
Treatment	1	0.854	11.39	0.007	(E)	1.037	9.54	0.011		0.179	3.78	0.081		0.041	0.15	0.702	
Tidal Zone	1	7.445	99.27	0.000	(L)	0.006	0.06	0.817		0.117	2.47	0.147		5.702	21.67	0.001	
TR × TZ	1	0.307	4.09	0.071		0.880	8.10	0.017	(E, L)	0.009	0.19	0.672		2.247	8.54	0.015	(E, L)
Block	4	0.468	6.24	0.009		0.147	1.35	0.317		0.490	10.35	0.001		0.216	0.82	0.542	
Residual	10	0.075				0.109				0.047				0.263			
Year	2	22.59	388.8	0.000		6.838	64.91	0.000		0.343	14.02	0.000		0.587	2.47	0.110	
Year × TR	2	0.603	10.38	0.001		0.559	5.30	0.014		0.145	5.93	0.010		1.326	5.58	0.012	(E)
Year × TZ	2	9.173	157.9	0.000		1.120	10.63	0.001		0.076	3.11	0.066		2.501	10.52	0.001	(L)
Year × TR × TZ	2	0.907	15.62	0.000	(E, L)	0.951	9.03	0.002	(E, L)	0.136	5.56	0.012	(C, L)	0.072	0.30	0.741	
Year × Block	8	0.243	4.18	0.004		0.410	3.89	0.006		0.141	5.75	0.001		0.229	0.96	0.491	
Residual	20	0.058				0.105				0.024				0.238			

Since abundances of functional groups did not differ between control and ambient plots, both treatments were used as effective lugworm controls. Experimental treatments with significant higher abundances (as revealed by Turkey post-hoc test) are also given in parenthesis (E=exclusion, C=lugworm control, L=low intertidal, M=mid intertidal). Prior to analysis data were log-transformed. Bold p values indicate significant effects.

to ambient plots, presumably caused by increased mortality in conjunction with the dredging process. Lugworm abundances in ambient and control plots were high in the first two years but significantly lower in the third year (RM ANOVA; factor year: $F_{2,8}=438.2$; $p<0.001$; Tukey $p<0.001$).

Total polychaete abundances and abundances of single functional groups did not differ between control and ambient plots (Figs. 3 and 4), which suggests that the initial dredging of the sediment in early spring 2002 had no lasting effect on the polychaete assemblage. We therefore used control and ambient plots as equivalent lugworm control plots in the statistical analysis.

3.2. Polychaete total abundance and species composition

During the three years of this study, 15 polychaete species were encountered in the experimental plots (Table 2). Three species were abundant in all years (*Scoloplos cf. armiger*, *Nereis diversicolor*, *Pygospio elegans*), while others occurred in abundance only in one year (*Polydora cornuta*, *Spio martinensis*, *Lanice conchilega*). The assemblage was dominated by different species each year. In 2002, the three species *Polydora cornuta*, *Scoloplos cf. armiger* and *Pygospio elegans* together contributed 74.4% to an overall within-year assemblage similarity of 41.4%. In 2003 and 2004, the assemblage was characterised by single species: in 2003 by *Pygospio elegans* (57.0% contribution to 53.6% overall similarity) and in 2004 by *Scoloplos cf. armiger* (70.0% contribution to 55.4% overall similarity). Total polychaete abundance was highest in 2003 (Fig. 3) when *P. elegans* reached abundances of ~ 4000 ind m^{-2} and

contributed about 50% to total polychaete abundances. Overall, total polychaete abundances were significantly higher in the mid intertidal zone than in the low one ($F_{1,8}=12.3$; $p<0.01$) and spatially heterogeneous as indicated by a significant block effect ($F_{4,8}=7.4$; $p<0.01$).

3.3. Responses of functional groups: univariate approach

Treatment effects on functional groups were not consistent over space and time (Fig. 4). Two-factorial Repeated Measures ANOVA (RM ANOVA) revealed significant lugworm effects on all functional groups,

Table 4

ANOSIM analysis of the functional benthic community for each year of investigation

	1st year		2nd year		3rd year	
	R	p	R	p	R	p
Treatment groups	0.433	0.045	0.1	0.27	0.733	0.004
average across all Block groups						
Tidal zone groups	−0.111	0.7	0.37	0.2	0.963	0.1
using Block groups as samples						
Block groups	0.436	0.05	0.444	0.004	0.206	0.09
averaged across all tidal zone groups						

ANOSIM was based on Bray Curtis Similarities of square-root-transformed functional group abundance data.

Table 5

ANOSIM and SIMPER analysis of the functional benthic community on experimental plots in the mid and low intertidal between 2002 and 2004

	ANOSIM			SIMPER	
		R	p	similarity within treatment	dominating functional group (and contribution to similarities within treatments)
Mid Intertidal Medium Sand	Year	0.88	0.001		
	Treatment	−0.045	0.63		
Low Intertidal Fine Sand	Year	0.814	0.001	E 78.18%	mixed suspension and surface deposit feeding tube worms (44%)
	Treatment	0.537	0.001	C 66.20%	subsurface deposit feeding discretely motile worms (38%)

ANOSIM was based on Bay Curtis similarities of square-root-transformed data (E=exclusion, C=lugworm control).

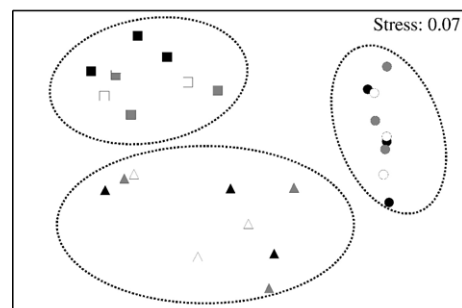
but these effects were variable in time and/or space as indicated by significant year \times treatment \times tidal zone interactions (Table 3). Temporarily and/or spatially confined inhibition by lugworms was found for mixed suspension and surface deposit feeding tube worms, surface deposit feeding discretely motile worms, and carnivorous motile worms (Tukey post-hoc tests, Table 3). Subsurface deposit feeding discretely motile worms were temporarily facilitated by the presence of lugworms in the low intertidal zone. Abundances of two functional groups, the tube worms and the discretely motile surface deposit feeding worms were extremely variable between the years and the factor time explained 70 and 65% of overall variance, while abundances of subsurface deposit feeders and carnivorous worms were intermittently less variable (variance explained 30 and 15%, respectively). Tube worms and discretely motile surface and subsurface deposit feeders were heterogeneously distributed over the tidal flat as indicated by significant time \times block interaction effects (Table 3).

3.4. Responses of functional groups: multivariate approach

Multivariate SIMPER analysis showed that different functional groups dominated the polychaete assemblage in each year (Fig. 4). Tube worms contributed most to the observed assemblage similarity in the first two years, while subsurface feeding polychaetes were dominant in the third year. The three investigated factors (lugworm exclusion, intertidal zone, experimental block) contributed in varying proportions to functional assemblage separation over the three years of investigation (Table 4). The effect of lugworm exclusion was moderate in the first year and highly significant in the third year, but played no role in the second year. The moderate effect of lugworm treatment in the first year was mainly based on lower abundances of tube worms in the presence of lugworms (51% contribution to average dissimilarities between treatment groups). In the third year, higher

abundances of tube and predacious worms and lower abundances of subsurface deposit feeders in the absence of lugworms accounted for more than 90% of overall dissimilarities between assemblages in the presence and absence of lugworms. In the first and second years, the functional composition was significantly separated regarding experimental blocks indicating a high spatial heterogeneity. The functional composition strongly

Mid Intertidal Medium Sand



Low Intertidal Fine Sand

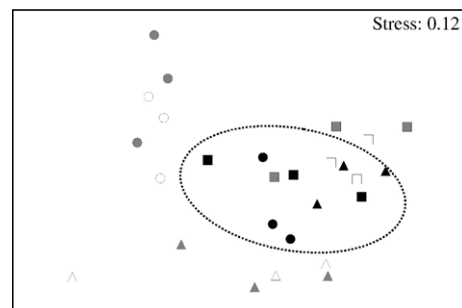


Fig. 5. MDS plots of functional polychaete composition in mid intertidal medium sand and the low intertidal fine sand. Shading indicates lugworm treatment (black=exclusion; grey=control; white=ambient) and symbols indicate years (triangle=1st year; square=2nd year; circle=3rd year). Plots are based on Bray Curtis Similarities of square-root-transformed functional group abundance data. Dotted line indicate the significantly different polychaete functional composition revealed by ANOSIM (Table 4).

differed between tidal zones in 2004. Mid range and even high values of R were not significant in some cases, which can be mainly attributed to low numbers of replicates and thus low numbers of possible permutations (i.e. only three block samples within both tidal zones).

Over all years and experimental blocks, lugworm treatment had no consistent effect on the functional composition of the polychaete assemblage (time \times treatment two-way crossed ANOSIM: $R=0.013$; $p=0.35$). Mid intertidal medium sand and low intertidal fine sand assemblages differed significantly, but separation was weak ($R=0.133$; $p<0.01$). Differentiation improved when experimental blocks were used as second factor ($R=0.378$; $p<0.001$). Pair-wise comparison of block assemblage dissimilarities showed that the assemblages of the three blocks in the low intertidal fine sand were not significantly different, while the functional compositions in the mid intertidal medium sand were highly heterogeneous and clearly separated ($R>0.512$ for all pair-wise tests). Multivariate statistical analysis was therefore applied separately for both tidal zones (Table 5). Significant treatment effects on the functional composition were restricted to the low intertidal fine sand. Pair-wise ANOSIM comparisons showed that the functional composition clearly differed between exclusion plots and control plots. In exclusion plots tube worms contributed 44% to within-treatment assemblage similarity, while subsurface deposit-feeding species were characteristic for control plots (38% contribution to overall similarity). Over the three years of observation, assemblage similarity in the low intertidal fine sand was higher in lugworm exclusion plots (78% similarity within treatment) than in control plots ($\sim 65\%$), indicating a decline in the year-to-year variation of the polychaete assemblage in the absence of *A. marina*. MDS plots also indicate a much higher variability of functional group composition in lugworms plots (Fig. 5). In the mid intertidal medium sand the functional composition clearly differed between individual years and was not consistently affected by lugworm treatment.

4. Discussion

Lugworms do affect the polychaete assemblage beyond the immediate vicinity of burrows in intertidal soft sediments, but effects vary in time and space. The impact of lugworms was found to be significant in single years and within one intertidal zone, while it was marginal in others. Time-consistent effects were restricted to the low intertidal fine sand, with strongest effects in the last year.

Small-scale experiments have shown that lugworms negatively affect juvenile densities of various worm and bivalve species with different trophic and motility traits

and that these effects increase with increasing lugworm abundance (Flach, 1992a,b, 1993; Flach and De Bruin, 1993). Negative response was found for tube worms (e.g. *Pygospio elegans*), surface deposit feeding worms (e.g. *Nereis diversicolor*) as well as for subsurface deposit feeding worms (e.g. *Scoloplos armiger*). Small-scale disturbance of infauna by lugworm bioturbating activity was suggested to be the main mechanism responsible for lower abundances of infaunal species. In our study polychaete functional groups responded differentially to lugworm exclusion. While abundances of mixed suspension and surface deposit feeding tube worms and surface deposit feeding discretely motile worms tended to increase in the absence of lugworms, subsurface deposit feeding worms were inhibited and became the characteristic functional group in the presence of lugworms. Even though our functional classification may be too strict for some species due to high plasticity of their feeding guilds, e.g. *N. diversicolor* (Riisgård and Kamermans, 2001), we observed a clear trend from rather sedentary species feeding at the sediment surface in lugworm exclusion plots to subsurface feeding motile species in the presence of lugworms. Lower abundances of sedentary species in the presence of lugworms (Brey, 1991; Flach, 1992b, this study) correspond to the mobility group hypothesis based on small-scale disturbance of infauna by a large bioturbator (Brenchley, 1981; Wilson, 1981). However, in the second year high lugworm abundances did not result in a changed functional composition of polychaetes, suggesting that the assemblage was highly adapted to lugworm bioturbating disturbance. Within-year assemblage dissimilarities between lugworm and exclusion plots were moderate in the first, low in the second and highly significant in the third year, when overall lugworm abundances happened to be low. Considering the relatively low abundances of *Arenicola marina* in the third year, small-scale disturbance alone is unlikely to cause the assemblage separation between lugworm exclusion and control/ambient plots and give rise to sediment-mediated effects of large bioturbators for benthic assemblage composition (Flint and Kalke, 1986; Posey, 1986; Posey et al., 1991; Botto and Iribarne, 1999). We do not have a cogent explanation for this decrease in lugworm abundance, which was also evident on the scale of the entire embayment (Reise, unpubl. data). However, the coinciding low lugworm abundances in control and ambient plots in 2004 and strongest separation in the polychaete assemblage in the presence/absence of lugworms in the same year suggest that changes in sediment properties cumulating in the course of three years due to lugworm exclusion might have been responsible for the conspicuous shift in the functional diversity of the polychaete assemblage. This

hypothesis is stressed by the time-consistent assemblage differentiation between exclusion and control plots in the low intertidal fine sand where changes in sediment properties were also most significant (Volkenborn and Reise, 2006). Surface deposit feeding worms may have been attracted by the higher availability of microphytobenthos and organic material in the sediment of lugworm exclusion plots (Volkenborn and Reise, 2006). Higher abundances of potential prey in the organic enriched sediment may have been responsible for abundant motile predacious worms in exclusion plots in 2004. Subsurface deposit feeding worms such as *Scoloplos cf. armiger* were found to take advantage from ameliorated sediment properties (e.g. low sulphide concentrations in the pore-water) in the presence of lugworms (Volkenborn and Reise, 2006). Indirect sediment-mediated effects may therefore be more important than direct small-scale disturbance by *A. marina* alone in influencing the functional composition of the assemblage on this larger scale.

The functional composition of the polychaete assemblage at our study site seems to be driven by the variable and contingent interplay of external factors, such as dispersal and environmental constraints (Belyea and Lancaster, 1999) and endogenous ecosystem engineering effects of *A. marina*, operating on different spatial scales. Moreover, the polychaete assemblage seems to be highly adapted to the dynamic and unstable conditions of intertidal sand. Dredging of experimental plots at the start of the experiment had no obvious effect on species and functional group composition and abundance. This reflects high resilience after disturbance (Boesch et al., 1976) and may apply to the small-scale disturbances caused by lugworm bioturbation as well. Our results clearly indicate that widely occurring lugworm densities in the Wadden Sea of 20–40 lugworms m⁻² (Beukema, 1976) do not necessarily result in a conspicuous change in the endobenthic assemblage. Direct disturbance of infauna may become important as lugworm abundances increase above these typical densities but a generally negative effect of lugworms on other benthic infauna, as assumed by Flach (1992b), may not exist.

Overall community dynamics may partly explain the observed inconsistencies of lugworm effects. The abundances of single species and functional groups were highly variable in time and space, and the assemblage was dominated by different species in each year. Especially in the mid intertidal medium sand in the third year species and functional diversity was very low. Even though we do not have environmental data to explain these patterns, this confounding variability caused by differential recruitment and propagule supply between years and tidal zones presumably overshadowed lug-

worm effects. The high temporal and spatial variability of macrobenthic dynamics may partly explain inconsistent lugworm effects and the absence of substantial lugworm effects in modelled community dynamics using long-term monitoring data (Williams et al., 2004). However, our experimental results imply that even contingent and subtle effects of lugworm presence may be important on a large scale in structuring the benthic community, particularly in fine-grained sediments of the low intertidal zone. Within this tidal zone, lugworms were found to modify the functional diversity in polychaetes and increase assemblage year-to-year variability, thus maintaining the functional diversity in polychaetes on a larger time scale within the spatio-temporal mosaic of soft-bottom habitats (Johnson, 1973).

The changing success of functional groups in time and space at our study site seems to be driven by a variable interplay of large-scale environmental variation, meso-scale ecosystem engineering by *A. marina* (this study) and small-scale disturbance caused by lugworm activity (Brey, 1991; Flach, 1992a,b; Lackschewitz and Reise, 1998). Their relative contributions to assemblage composition vary from time to time and site to site, which stresses the importance of combined biotic and abiotic variables for benthic community formation (Hagberg et al., 2003) and the importance of the spatial scale of controlling factors (Zajac et al., 1998). Our results have far-reaching implications for the explanatory power of small-scale and/or short-term experiments in the intertidal zone, in which biogenic effects of single species may easily be over- or underestimated depending on time and place of an experiment. We conclude that the spatial and temporal contingent interplay of (1) small-scale disturbance, (2) meso-scale sediment-mediated ecosystem engineering by the lugworm *A. marina*, and (3) external factors should be regarded as characteristic for assemblage formation in unvegetated intertidal sediments of the Wadden Sea.

Acknowledgements

We thank Elisabeth Herre for logistical support and Heike Lotze and Thorsten Reusch for their advice on statistical analysis. Els Flach and two anonymous reviewers gave valuable comments on the manuscript. This work was supported by the German Ministry for Education and Research and the State of Bremen through funding of the project NEBROC. We acknowledge the support by the MARBEF Network of Excellence 'Marine Biodiversity and Ecosystem Functioning', which is funded in the Community's Sixth Framework Programme (contract no. GOCE-CT-2003-505446).

References

- Austen, I., 1994. The surficial sediments of Königshafen – variations over the past 50 years. *Helgol. Meeresunters.* 48, 163–171.
- Belyea, L.R., Lancaster, J., 1999. Assembly rules within a contingent ecology. *Oikos* 86, 402–416.
- Beukema, J.J., 1976. Biomass and species richness of the macrobenthic animals living on the tidal flats of the Dutch Wadden Sea. *Neth. J. Sea Res.* 10, 236–261.
- Beukema, J.J., 1991. Changes in composition of bottom fauna of a tidal-flat area during a period of eutrophication. *Mar. Biol.* 111, 293–301.
- Boesch, D.F., Wass, M.L., Virnstein, R.W., 1976. The dynamics of estuarine benthic communities. In: Wiley, M. (Ed.), *Estuarine Processes*. Academic Press, New York, pp. 177–196.
- Botto, F., Iribarne, O., 1999. Effect of the burrowing crab *Chasmagnathus granulata* (Dana) on the benthic community of a SW Atlantic coastal lagoon. *J. Exp. Mar. Biol. Ecol.* 241, 263–284.
- Bremner, J., Rogers, S.I., Frid, C.L.J., 2003. Assessing functional diversity in marine benthic ecosystems: a comparison of approaches. *Mar. Ecol., Prog. Ser.* 254, 11–25.
- Brenchley, G.A., 1981. Disturbance and community structure: an experimental study of bioturbation in marine soft-bottom environments. *J. Mar. Res.* 39, 767–790.
- Brey, T., 1991. The relative significance of biological and physical disturbance: an example from intertidal and subtidal sandy bottom communities. *Estuar. Coast. Shelf Sci.* 33, 339–360.
- Cadée, G.C., 1976. Sediment reworking by *Arenicola marina* on tidal flats in the Dutch Wadden Sea. *Neth. J. Sea Res.* 10, 440–460.
- Clarke, K.R., Warwick, R.M., 2001. *Change in Marine Communities: An Approach to Statistical Analysis and Interpretation*, 2nd ed. PRIMER-E, Plymouth.
- Fauchald, K., Jumars, P.A., 1979. The diet of worms: a study of polychaete feeding guilds. *Oceanogr. Mar. Biol. Ann. Rev.* 17, 193–284.
- Flach, E.C., 1992a. The influence of four macrozoobenthic species on the abundance of the amphipod *Corophium volutator* on tidal flats of the Wadden Sea. *Neth. J. Sea Res.* 29, 379–394.
- Flach, E.C., 1992b. Disturbance of benthic infauna by sediment-reworking activities of the lugworm *Arenicola marina*. *Neth. J. Sea Res.* 30, 81–89.
- Flach, E.C., 1993. The distribution of the amphipod *Corophium arenarium* in the Dutch Wadden Sea: relationships with sediment composition and the presence of cockles and lugworms. *Neth. J. Sea Res.* 31, 281–290.
- Flach, E.C., 1996. Distribution of *Corophium* at different scales. *Senckenb. Marit.* 27, 119–127.
- Flach, E.C., De Bruin, W., 1993. Effects of *Arenicola marina* and *Cerastoderma edule* on distribution, abundance and population structure of *Corophium volutator* in Gullmarsfjorden western Sweden. *Sarsia* 78, 105–118.
- Flint, R.W., Kalke, R.D., 1986. Biological enhancement of estuarine benthic community structure. *Mar. Ecol., Prog. Ser.* 31, 23–33.
- Flothmann, S., Werner, I., 1992. Experimental eutrophication on an intertidal sandflat: effects on microphytobenthos, meio- and macrofauna. In: Colombo, G., et al. (Ed.), *Marine Eutrophication and Population Dynamics*. Proc. 25th EMBS. Olsen & Olsen, Fredensborg, Denmark, pp. 93–100.
- Grant, J., Turner, S.J., Legendre, P., Hume, T.M., Bell, R.G., 1997. Patterns of sediment reworking and transport over small spatial scales on an intertidal sandflat, Manukau Harbour, New Zealand. *J. Exp. Mar. Biol. Ecol.* 216, 33–50.
- Hagberg, J., Jonzén, N., Lundberg, P., Ripa, J., 2003. Uncertain biotic and abiotic interactions in benthic communities. *Oikos* 100, 353–361.
- Hall, S.J., Raffaelli, D., Thrush, S.F., 1994. Patchiness and disturbance in shallow water benthic assemblages. In: Giller, P.S., Hildrew, A.G., Raffaelli, D. (Eds.), *Aquatic Ecology*. Blackwell, Oxford, pp. 333–375.
- Howell, D.C., 1992. *Statistical Methods for Psychology*. Duxbury Press, Belmont, CA.
- Johnson, R.G., 1973. Conceptual models of benthic communities. In: Schopf, T.J.M. (Ed.), *Models in Paleobiology*. Freeman Cooper and Co., San Francisco, pp. 148–159.
- Jones, C.G., Lawton, J.H., Shachak, M., 1994. Organisms as ecosystem engineers. *Oikos* 69, 373–386.
- Lackschewitz, D., Reise, K., 1998. Macrofauna on flood delta shoals in the Wadden Sea with an underground association between the lugworm *Arenicola marina* and the amphipod *Urothoe poseidonis*. *Helgol. Mar. Res.* 52, 147–158.
- Pearson, T.H., 2001. Functional group ecology in soft-sediment marine benthos: the role of bioturbation. *Oceanogr. Mar. Biol. Annu. Rev.* 39, 233–267.
- Possey, M.H., 1986. Changes in a benthic community associated with dense beds of a burrowing deposit feeder, *Callianassa californiensis*. *Mar. Ecol., Prog. Ser.* 31, 15–22.
- Possey, M.H., Dumbauld, B.R., Armstrong, D.A., 1991. Effects of a burrowing mud shrimp, *Upogebia pugettensis* (Dana), on abundances of macro-infauna. *J. Exp. Mar. Biol. Ecol.* 148, 283–294.
- Reise, K., 1981. High abundances of small zoobenthos around biogenic structures in tidal sediments of the Wadden Sea. *Helgol. Meeresunters.* 34, 413–425.
- Reise, K., 1983. Experimental removal of lugworms from marine sand affects small zoobenthos. *Mar. Biol.* 74, 327–332.
- Reise, K., 1985. *Tidal Flat Ecology*. Springer, Berlin. 191 pp.
- Reise, K., 2002. Sediment mediated species interactions in coastal waters. *J. Sea Res.* 48, 127–141.
- Reise, K., Herre, E., Sturm, M., 1994. Biomass and abundance of macrofauna in intertidal sediments of Königshafen in the northern Wadden Sea. *Helgol. Meeresunters.* 48, 201–215.
- Reise, K., Simon, M., Herre, E., 2001. Density-dependent recruitment after winter disturbance on tidal flats by the lugworm *Arenicola marina*. *Helgol. Mar. Res.* 55, 161–165.
- Riisgård, H.U., Banta, G.T., 1998. Irrigation and deposit feeding by the lugworm *Arenicola marina*, characteristics and secondary effects on the environment. A review of our current knowledge. *Vie Milieu* 48, 243–257.
- Riisgård, H.U., Kamermans, P., 2001. Switching between deposit and suspension feeding in coastal zoobenthos. In: Reise, K. (Ed.), *Ecological Comparisons of Sedimentary Shores*. Springer, Berlin, pp. 73–101.
- Volkenborn, N., Reise, K., 2006. Lugworm exclusion experiment: responses by deposit feeding worms to biogenic habitat transformations. *J. Exp. Mar. Biol. Ecol.* 330, 169–179.
- Williams, I.D., Van der Meer, J., Dekker, R., Beukema, J.J., Homes, S.P., 2004. Exploring interactions among intertidal macrozoobenthos of Dutch Wadden Sea using population growth models. *J. Sea Res.* 52, 307–319.
- Wilson Jr., W.H., 1981. Sediment-mediated interactions in a densely populated infaunal assemblage: the effects of the polychaete *Abarenicola pacifica*. *J. Mar. Res.* 39, 735–748.
- Zajac, R.N., 2004. Macrofaunal responses to pit-mound patch dynamics in an intertidal mudflat: local versus patch-type effects. *J. Exp. Mar. Biol. Ecol.* 313, 297–315.
- Zajac, R.N., Whitlatch, R.B., Thrush, S.F., 1998. Recolonization and succession in soft-sediment infaunal communities: the spatial scale of controlling factors. *Hydrobiologia* 375–376, 227–240.
- Zühlke, R., Reise, K., 1994. Response of macrofauna to drifting tidal sediments. *Helgol. Meeresunters.* 48, 277–289.