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Bioturbation effects of *Corophium volutator*: Importance of density and behavioural activity

A. De Backer [a,*], F. Van Coillie [a], F. Montserrat [b], P. Provoost [b], C. Van Colen [a], M. Vincx [a], S. Degraer [a,c]

[a] Ghent University, Biology Department, Marine Biology, Krijgslaan 281-S8, 9000 Gent, Belgium
[b] NIOO-KNAW, Centre for Estuarine and Marine Ecology, Department of Spatial Ecology, Korringaweg 7, PO Box 140, NL-4400 AC Yerseke, The Netherlands
[c] Royal Belgian Institute of Natural Sciences, Management Unit of the North Sea Mathematical Model, Marine Ecosystem Management Section, Gulledelle 100, 1200 Brussel, Belgium

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ABSTRACT

Bioturbation is one of the major processes influencing ecosystem functioning. Population parameters such as species density, burrow density and species-specific life modes, determine the impact of bioturbation on the ecosystem. A laboratory experiment was developed, using microcosms mimicking a marine intertidal sediment–water interface which allowed for quantification of different population parameters. The vertical redistribution, bioturbation rate and maximum penetration depth of two sizes (41 and 129 μm) of luminophores were measured in five treatments (control, low density of burrows with and without *Corophium* (1989 ind./m^2), and high density of burrows with and without *Corophium* (14,921 ind./m^2)) after 1, 7 and 14 days. Results indicate that the behavioural activities of *Corophium* are of the utmost importance in sediment reworking, since they contributed to a five-fold increase in bioturbation rate compared to the passive transport induced by the static structure of the burrows. Furthermore, density is an important parameter because only high densities play a prominent role in particle transport and hence in organic matter processing, while the role of low *Corophium* densities is limited in sediment reworking. No evidence for differentiation in sediment size fractions was observed. Finally, bioturbation rates in this study were low compared to other studies, and these results suggest an influence of the tidal rhythmicity in the behavioural activity of *Corophium* on the bioturbation rate.

1. Introduction

Bioturbation, i.e. sediment reworking and bioirrigation by benthic fauna is recognised as one of the major processes that influence the structure and function of aquatic sedimentary environments (Lohrer et al., 2004; Meysman et al., 2006). Sediment particle reworking results from various activities (i.e. burrowing, feeding and locomotion), and strongly affects the physical, chemical and biological characteristics of marine sediments (Rhoads, 1974; Aller, 1982; Hall, 1994; Rowden et al., 1998; Solan et al., 2008). Hence, macrobenthos-mediated effects on sediment processes are strongly influenced by species-specific life modes (Mermillod-Blondin et al., 2005; Norling et al., 2007). The intensity of sediment reworking can vary according to population characteristics such as species density, animal size, biovolume, burrowing depth, density of and spacing between animal burrows (e.g. Rhoads, 1974; Sandnes et al., 2000; Duport et al., 2006, 2007; Gilbert et al., 2007), and environmental factors such as temperature and the availability of food (Ouellette et al., 2004; Lecroart et al., 2005; Maire et al., 2007; Nogaro et al., 2008; Braeckman et al., 2010).

Bioturbating benthic organisms have been classified in five types of functional groups according to their mode of particle mixing, and their main effects on sediment geochemistry and the benthic microbial community. Biodiffusers, upward conveyors, downward conveyors, regenerators and gallery-diffusers can be distinguished (François et al., 2002; Gérino et al., 2003). However, for a lot of bioturbators and bio-irrigators, no matter which functional group they belong to, population density is an important parameter determining the impact on ecosystem functioning, such as nutrient cycling and benthic mineralisation (Ieno et al., 2006; Bulling et al., 2008; Rossi et al., 2008; Braeckman et al., 2010). Furthermore, dominant species often contribute most to sediment reworking and ecological function (Mugnai et al., 2003; Maire et al., 2007), and the loss or density decline of dominant species might have serious repercussions for ecosystem functioning (Solan et al., 2004a). *Corophium volutator* is an abundant species in intertidal ecosystems along the North-Atlantic, and population densities are frequently recorded at >20,000 ind./m^2, while in summer, densities can locally

* Corresponding author. Present address: Institute for Agricultural and Fisheries Research, Animal Sciences, Fisheries, Ankerstraat 1, 8400 Oostende, Belgium.
E-mail address: annelies.debacker@ilvo.vlaanderen.be (A. De Backer).

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increase to 100,000–140,000 ind./m^2 (Möller and Rosenberg, 1982; Jensen and Kristensen, 1990; Gerdol and Hughes, 1994). Given the densities it can attain, its trophic position in the ecosystem (Murdoch et al., 1986; Boates et al., 1995), as well as the ecosystem engineering effect on the abiotic environment (Grant and Daborn, 1994), this amphipod can be considered a critical species in intertidal ecosystems. To our knowledge, there has been no attempt to assess the density effect of *C. volutator* on sediment reworking, and therefore, quantifying the density effect on sediment reworking is one of the objectives of the present study. In order to quantify sediment reworking, numerous tracer techniques have been developed over the last three decades (Maire et al., 2008), and especially the luminophore technique (Mahaut and Graf, 1987) is frequently used in bioturbation studies. In most studies, one size class of luminophores is used to track vertical redistribution of sediment particles and/or to calculate bioturbation rate (Mermillod-Blondin et al., 2004; Solan et al., 2004b; Duport et al., 2006; Gilbert et al., 2007; Maire et al., 2007). In this study, we used two different size classes of luminophores (median grain size 41 μm and 129 μm) to determine whether bioturbation by *Corophium* differentiates between the mud (<63 μm) and the sand sediment fraction.

Bioturbation is regarded as a dynamic process caused by the behavioural activities of bioturbating species. However, burrowing species often construct an entire network of (semi) permanent burrows or tubes, which alter the 'static' sediment structure, and which could be equally important in (passive) downward sediment transport and particle burial (passive bioturbation). Therefore, we aimed at assessing the importance of the active contribution of species to bioturbation as compared with the potential passive bioturbation caused by changes in the physical sediment structure.

To achieve our different objectives, a microcosm experiment was set up in the laboratory with different density treatments, both 'passive' (burrows only) and active (burrows with animals), and with two size fractions of inert fluorescent sediment tracers (luminophores), to be able to assess density effects, to distinguish between passive and active bioturbation and to determine potential size differentiation of the bioturbation by *Corophium*.

2. Material and methods

2.1. Collection of sediment and animals and experimental design

Sediment and *Corophium volutator* were collected in September 2006 in the Flemish nature reserve "IJzermonding", a mudflat-salt marsh area in Nieuwpoort (Belgium, 51°08′N, 2°44′E). The mudflat had a sediment consisting of muddy sand: 28% of mud (= silt + clay; <63 μm), 6% of very fine sand (63–125 μm), 50% of fine sand (125–250 μm) and 16% of medium sand (250–500 μm). The collected sediment was defaunated by three cycles 24 h freezing–24 h thawing. Freezing–thawing did not alter the sediment grain size characteristics (*t*-test, $p > 0.05$). To reduce natural heterogeneity and to obtain equal starting conditions, the sediment was homogenised before use. Sediment microcosms were established by transferring the homogenised sediment into PVC cores (15 cm long and 8 cm internal diameter) to a depth of 10 cm. The PVC cores had four holes (8 mm Ø), covered with a 250 μm mesh, at the same level as the sediment surface (≈10 cm) to allow gentle inflow of seawater and to avoid escape of the test animals. Sixty cores were placed in a large aquarium in a temperature controlled climate room (16 ± 1 °C) with a 12:12 h light:dark regime, and under a simulated tidal regime, resembling the natural tidal conditions of the collected *C. volutator* (i.e. 3 h of submersion and 9 h of emersion, salinity = 32 psu). *Corophium volutator* was added to the microcosms one day after sediment installation. In all treatments, adult individuals of *C. volutator* were used with similar sizes (≥7 mm). Movement of test animals between microcosms was prevented by the edges of the tubes, which protruded 5 cm above the sediment surface.

Five treatments were performed ($n = 4$ replicates per treatment per time interval): (1) without *Corophium* and without burrows, i.e. control (C); (2) with 10 individuals of *C. volutator*, i.e. low density (LD; 1989 ind./m^2); (3) burrows of 10 individuals of *C. volutator*, but without the animals (BLD); (4) with 75 individuals of *C. volutator*, i.e. high density (HD; 14,921 ind./m^2); and (5) burrows of 75 individuals of *C. volutator*, but without the animals (BHD). All treatments were randomised within the aquarium. No biofilm was present or no diatoms were added during the course of the experiment, but nevertheless the animals could be seen scraping, crawling or swimming.

To establish the treatments with burrows but without animals, *Corophium* was introduced as for the other treatments, but prior to the start of the experiment (after three days burrowing), these treatments were taken out of the aquarium and put carefully (without disturbing the sediment surface) in a 1% formaldehyde solution, chasing the animals out of their burrows immediately, but leaving the burrows intact. After all animals were removed, the core was placed in seawater to dilute the formaldehyde. After 10 min the cores were placed back in the aquarium. We should mention that in these formaldehyde treated cores, an increased oxygen penetration was observed after one day, most probably caused due to a change in bacterial community. However, this was restored quickly and oxygen penetration was relatively similar to the other treatments after seven days, and we can assume that this had no effect on luminophore redistribution.

2.2. Quantifying bioturbation

Bioturbation in the cores was quantified using the luminophore tracer technique (Mahaut and Graf, 1987). Two size types of luminophores were used (Environmental Tracing Systems, UK), corresponding to the two main sediment fractions: "UV Blue Mostyn" luminophores with 129 μm median grain size (i.e. fine sand, coarse tracer) and "Magenta" luminophores with 41 μm median grain size (i.e. mud, fine tracer). Two g of 129 μm and 1 g of 41 μm luminophores were added to 19 g of natural dried sediment and mixed homogeneously. Subsequently, seawater was gently added until a homogeneous mix was formed. The mix was poured in moulds of 8 cm diameter (= internal diameter of the experimental cores) and 4 mm deep and frozen at −20 °C. On day 0 of the experiment, just after removal of *Corophium* from the 'only burrow' treatments and just before the start of submersion, the frozen luminophore slices were placed on the sediment surface of the experimental cores to equally distribute the luminophores over the sediment surface.

Sampling of the cores was done at three sampling occasions: one day, seven days and 14 days after the start of the experiment. At each sampling occasion, 20 cores (5 treatments × 4 replicates) were taken out of the aquarium and put immediately in the freezer (−20 °C) to stop macrofaunal reworking. Frozen cores were subsequently sliced in layers of 2 mm down to 3 cm depth, then in 5 mm slices to 7 cm depth. However, the first two slices were combined, because the thickness of the initial luminophore slice was 4 mm. The sediment collected within each layer was homogenised thoroughly in a Petri dish, and pictures of a fixed surface area were taken under UV light under standardised conditions. Petri dish, camera and UV lamp (365 nm) were placed in a fixed setup. Pictures were taken with a digital mirror-reflex camera, Canon EOS 350D; aperture 1/8, shutter time 1s, ISO 400, manual focus and 46 mm focal length. Images were digitally processed in Matlab to count luminophore pixels. Using quadratic discriminant function analysis, pixels were classified into three classes (coarse tracer [129 μm; blue], fine tracer [41 μm; red] and background)

based on their brightness value in the red, green and blue bands. The use of the quadratic discrimant analysis prevented overlap between the three colour bands. Luminophore pixel counts of both size types were then converted to percentage of tracer in each sediment slice based on the total depth-integrated pixel counts for each size type. The image analysis revealed that no buried luminophores were present in the layers deeper than 3 cm (except for the artifactual one or two), for that reason, these data were not used for further analysis.

2.3. Bioturbation rate

Bioturbation rate was quantified by using a non-local model of bioturbation, the Continuous Time Random Walk model, based on Meysman et al. (2008). This model was preferred to the classical biodiffusion model, as the assumptions of the latter are usually not fulfilled in short-term bioturbation experiments (Meysman et al., 2008). Particle displacement is assumed to be a Poisson process, as the probability distribution of the waiting time until the next displacement is an exponential distribution, with a Gaussian step-length distribution (see Maire et al., 2007 for mathematical background on this model). Values for the two parameters σ (characteristic step-length) and τ (average waiting time) were determined by fitting the model to the respective log-transformed luminophore profiles using the R package FME (Soetaert and Petzoldt, 2009). Finally, a single quantity D_b^{NL}, representing the bioturbation rate, was calculated as follows:

$$D_b^{NL} = \frac{\sigma^2}{2\tau}$$

2.4. Data analysis

As it is of interest to determine differences in the shape or depth of tracer profiles between treatments, a split-plot ANOVA was performed. This allows for comparison of vertical tracer profiles between treatments (depth × treatment interaction) and between or within treatments over time (depth × treatment × time interaction). Tracer percentages were used as response variable, while time, treatment and tracer size were the 'between effect' explanatory variables and depth the 'within effect' explanatory variable, since depth intervals of the luminophores within cores are not independent. To enable ANOVA analysis, core identity was introduced as a new parameter treated as a random factor, nested within the time × treatment × tracer size interaction. Significance of the between effects (time, treatment and tracer size) and their interactions were tested over the mean square between cores within treatment × time × tracer size. Significance of depth (within effect) and all interaction terms involving depth were tested over the error mean square (Quinn and Keough, 2002). To fulfill homogeneity of variances, tracer percentages were arcsine-square root transformed. Because the sphericity assumption was violated, adjusted F tests using the Greenhouse–Geisser correction were performed, resulting in more conservative p levels (Quinn and Keough, 2002).

Furthermore, differences in maximum penetration depth (MPD, depth integrating 99.5% of the tracer) and bioturbation rates between treatments, sampling times and tracer size were tested using a three-way ANOVA. Whenever the homogeneity assumption was not met (Bartlett's test), data (multiplied by a power of 10) were log transformed. Whenever appropriate, a Tukey's *post hoc* test was used to assess differences between treatments and experimental duration. When interactions, e.g. time × treatment, were significant for any of the above analyses, interpretation of the main effects was done by splitting the original data per treatment and/or sampling occasion to allow for interpretation of the main treatment or time effect (Quinn and Keough, 2002). All analyses were performed using Statistica 7.

3. Results

3.1. Size selectivity

Corophium reworked the sediment particles irrespective of particle size. No significant differences in vertical distribution or maximum penetration depth (MPD, depth integrating 99.5% of the tracer) were found between fine (41 μm) and coarse (129 μm) tracers for none of the treatments (Tables 1 and 2). Furthermore, bioturbation rates for both size fraction were not significantly different between similar treatments and sampling occasions (Table 2), with for instance for the high-density treatment (HD) after 14 days, a bioturbation rate of 0.0035 ± 0.0007 cm^2/d for the fine tracer and 0.0029 ± 0.0003 cm^2/d for the coarse tracer.

3.2. Effects of density and biological activity over time

Bioturbation effects changed significantly between the treatments over time (time × treatment × depth, $p < 0.001$; Table 1). As time progressed, differences between treatments became more pronounced, and the percentage of luminophores worked down with time was higher for most treatments (Fig. 1). The empty burrows from the BLD and BHD treatments persisted during the experiment (personal observation during slicing), and they slightly influenced vertical tracer distribution in the sense that significantly more luminophores were buried over time for both, when analysing treatments separately (Fig. 1, Table 3). For the 'active' density treatments (LD and HD), more luminophores were buried over time, while for the C treatment, no significant vertical displacement of the luminophores was measured (Table 3). On each sampling occasion, a significantly higher percentage of luminophores was transported deeper for the HD treatment (14,921 ind./m^2) in comparison with all the other treatments, except on day 7 no difference was measured with LD (Table 4, Fig. 1). However, the LD treatment (1989 ind./m^2) only had a higher amount of buried tracer

Table 1
Time, treatment and tracer size differences for vertical tracer distribution patterns in five treatments at three sampling times (Split-plot ANOVA table). Adjusted p-levels were calculated for depth effects based on the Greenhouse–Geisser correction. Tracer % was arcsine-square root transformed. Significant p-values are bold.

Model term	df	Tracer % MS	Tracer % F	p	Adjusted p level
Between effects					
Cte	1	28.11007	72351.01	**<0.001**	
Time	2	0.01123	28.91	**<0.001**	
Treatment	4	0.02222	57.20	**<0.001**	
Size	1	0.00027	0.68	0.411	
Time × treatment	8	0.00196	5.05	**<0.001**	
Time × size	2	0.00006	0.15	0.860	
Treatment × size	4	0.00021	0.53	0.716	
Time × treat × size	8	0.00015	0.38	0.928	
Repl (Ti, Tr, Si)	90	0.00039	1.03	0.402	
Within effects					
Depth	13	221.2888	45217.67	**<0.001**	**<0.001**
Depth × time	26	0.2282	23.31	**<0.001**	**<0.001**
Depth × treatment	52	0.7645	39.06	**<0.001**	**<0.001**
Depth × size	13	0.0033	0.68	0.781	0.479
Depth × Ti × Tr	104	0.1927	4.92	**<0.001**	**0.013**
Depth × Ti × Si	26	0.0019	0.19	0.999	0.915
Depth × Tr × Si	52	0.0026	0.13	1000	0.994
Depth × Ti × Tr × Si	104	0.0114	0.29	1000	0.993
Depth × Repl (Ti, Tr, Si)	1170	0.4404	–	–	–

Table 2
Factorial ANOVA table for the effect of time, treatment and tracer size on the maximum penetration depth (MPD) and the natural logarithmic transformed bioturbation rate (ln(BR)). Significant *p*-values are bold.

	df	MPD MS	MPD F	*p*	Ln(BR) MS	Ln(BR) F	*p*
Cte	1	12040.03	864.8069	<0.001	5708.18	6022.98	<0.001
Time	2	162.13	11.6457	**<0.001**	22.11	23.33	**<0.001**
Treatment	4	419.87	30.1580	**<0.001**	26.31	27.77	**<0.001**
Size	1	32.03	2.3009	0.133	0.39	0.45	0.521
Time × treatment	8	32.97	2.3679	**0.023**	2.08	2.20	**0.036**
Time × size	2	5.73	0.4118	0.664	0.32	0.34	0.714
Treatment × size	4	9.20	0.6608	0.621	0.63	0.66	0.619
Ti × Tr × Si	8	6.40	0.4597	0.881	0.25	0.26	0.976
Error	90	13.92			0.95		

compared to the C treatment on day 14 (Tukey's *post hoc*; Table 4), and no differences in tracer percentage at depth were observed with the BLD treatment (Tukey's *post hoc*, Table 4).

Maximum penetration depth (MPD) was less subject to time (Fig. 2). MPD changed with time but this differed between treatments (time × treatment, $p = 0.023$; Table 2). MPD was only significantly different between sampling occasions for the LD and BHD treatment (Tukey's *post hoc*). MPD was deepest for the HD treatment with 18.5 ± 2.7 mm (mean ± SE) for the coarse (129 μm) tracer and 20 ± 2.8 mm for the fine (41 μm) tracer after 14 days (Fig. 2). We observed the same pattern in MPD as for the tracer profiles, where the HD treatment differed significantly from C and LD treatments on each sampling occasion, except not from the LD treatment on day 7 ($p = 0.31$, Tukey's *post hoc*; Fig. 2). Furthermore, the HD treatment also differed significantly from the BHD treatment at each sampling occasion. The LD treatment (13 ± 4 mm for fine and 11 ± 1 mm for coarse) showed only significant differences in MPD with the C treatment (6 ± 1 mm for both fraction sizes) on day 7, no significant differences with BLD treatment were observed (Tukey's *post hoc*; Fig. 2).

The bioturbation rate was significantly influenced by time (Table 2). For each treatment, except for the BLD treatment, the bioturbation rate at day 1 was significantly higher than the rates at day 7 and day 14 (Tukey's *post hoc*; Fig. 3). Bioturbation rate was

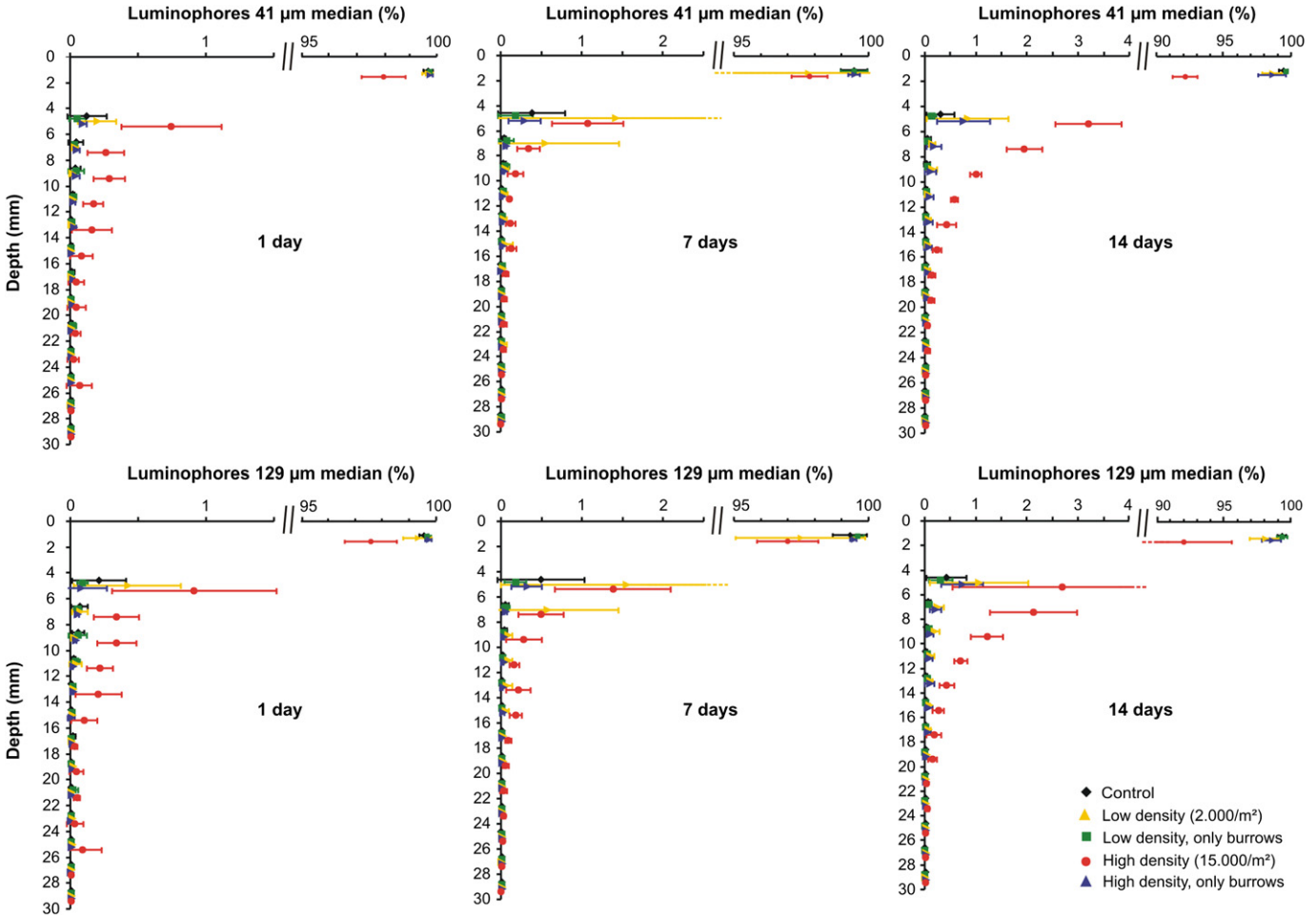

Fig. 1. Depth profiles (mean ± SD, $n = 4$) from 41 μm (upper graphs) and 129 μm (lower graphs) luminophores for the different treatments at the three sampling occasions. No significant differences between luminophore sizes were observed.

Table 3
Tukey's *post hoc* results for differences in vertical tracer distribution per treatment between different sampling occasions. For the C treatment, no overall significant effect of time was found, so no test was performed. Significant *p*-values are bold.

Treatment	Sampling occasion	Day 1	Day 7
BLD	Day 7	**0.002**	
	Day 14	**<0.001**	0.980
LD	Day 7	**0.039**	
	Day 14	**0.029**	0.994
BHD	Day 7	0.550	
	Day 14	**<0.001**	**<0.001**
HD	Day 7	0.998	
	Day 14	**<0.001**	**<0.001**

usually one order of magnitude higher in the HD treatment compared to the other treatments. The HD treatment showed significantly higher values on days 1 and 14 (resp. 0.02 cm^2/d and 0.003 cm^2/d for the fine tracer) than the LD treatment (resp. 0.001 cm^2/d and 0.0007 cm^2/d) and it differed significantly from the C treatment and BHD treatment on all sampling occasions (Table 2, Fig. 3). Again, the BLD and LD treatment did not show any significant differences in bioturbation rate.

4. Discussion

4.1. Size selectivity

No evidence for differentiation in sediment size fractions during sediment reworking by *Corophium* was observed, meaning that both the mud fraction and fine sediment fraction were transported at equal rates and in a similar way. In contrast with our results, high *Corophium* densities (20,000 ind./m^2) have been observed to stimulate loss of fine sediment (<4 μm) from the surface layer in the laboratory (De Backer et al., 2009), and to induce a coarsening of the sediment in the field (De Backer et al., 2010b). These contrasting results can probably be attributed to the fact that in the laboratory a loss of clay particles (particle diameter < 4 μm) was observed, while the fine tracer fraction used in this experiment had a median grain size of 41 μm. The difference with the field data can probably be explained by a large difference in external hydrodynamical forcing between laboratory and natural conditions. Furthermore, as *Corophium*, because of its bio-irrigating activities in a U-shaped burrow, was expected to induce non-local transport (i.e. transport of material from the surface directly to the deep part of the gallery) in addition to diffusive mixing, accumulations of (preferably coarse) particles were expected at the bottom of the burrow. Our vertical distribution profiles, however, showed no evidence of non-local transport, i.e. a peak of tracers at depth, which is consistent with the findings of Mermillod-Blondin et al. (2004). Mermillod-Blondin et al. (2004) attributed their results to an insufficient spatial resolution (slices of 5 mm), missing a possible luminophore peak at the bottom of the burrow. Our spatial resolution was higher (2 mm), but again no tracer peak at depth was observed. Possibly, *Corophium* removes the accumulation of sediment particles at the bottom of the burrow, whilst flushing during submersion (De Backer et al., 2010a). Further experiments on a very high spatial and temporal scale with the use of thin wall aquaria and time lapse camera could offer an outcome to actually see what is happening at the bottom of the burrow and to see if different sediment fractions are indeed transported in the same way (Solan et al., 2004b).

Table 4
Tukey's *post hoc* results for differences in vertical tracer distribution between the different treatments per sampling occasion. Significant *p*-values are bold.

Sampling occasion	Treatment	C	BLD	LD	BHD
Day 1	BLD	0.357			
	LD	0.811	0.948		
	BHD	0.965	0.770	0.993	
	HD	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Day 7	BLD	0.951			
	LD	0.117	0.454		
	BHD	0.999	0.980	0.168	
	BD	**<0.001**	**0.003**	0.290	**<0.001**
Day 14	BLD	0.929			
	LD	**0.015**	0.139		
	BHD	**0.013**	0.121	0.999	
	HD	**<0.001**	**<0.001**	**<0.001**	**<0.001**

4.2. Density effects and passive versus active bioturbation

Quantification of bioturbation by tracking the vertical distribution of luminophore tracers in different density treatments revealed that density is an important parameter determining sediment reworking by *Corophium*. High *Corophium* densities (±15,000 ind./m^2) reworked a significantly higher amount of tracer at each sampling occasion, while low densities of *Corophium* (±2000 ind./m^2) had only a slight, and mostly insignificant, influence on sediment reworking. Furthermore, differences between control and density treatments became more pronounced with time. Bioturbation rate and maximum penetration as well were positively influenced by density. *Corophium volutator* is an important deposit feeder, at least in the absence of phytoplankton (Riisgard and Schotge, 2007), and while foraging as deposit feeder, *Corophium* partly leaves the burrow to scrape surface sediment in the burrow (Meadows and Reid, 1966; Riisgard and Schotge, 2007; De Backer et al., 2010a). This feeding behaviour induces a displacement of surface particles down the burrow. If *Corophium* density increases, a larger surface area is occupied with burrows, and consequently the total scraping area, which surrounds the burrows, increases. Hence, a higher quantity of sediment tracers is buried at high densities. A similar particle displacement through feeding behaviour was described for *Hediste diversicolor* by Duport et al. (2006). It is important to mention that no food was added to this experiment, so feeding activity might have been reduced and might have resulted in less intensive sediment reworking (cf. Nogaro et al., 2008). This density-dependency of sediment reworking was as well observed for other taxa and other functional traits, and our results add to the recognition that density is an important parameter in sediment mixing (Sun et al., 1999; Sandnes et al., 2000; Duport et al., 2006; Braeckman et al., 2010).

The limited importance of low densities of *Corophium* is supported by the close relation in both depth profile and bioturbation rate between the low density treatment and the treatment with only burrows at low densities, indicating that the influence of the behavioural activities at low densities of *Corophium* was of minor importance. There is a small environmental, abiotic driven flux of passive particle transport down into the empty burrows, which does not differ significantly from the net animal activity at low densities. However, for high densities, significant differences were found between the 'passive' burrow treatment and the 'active' treatment with animals. Furthermore, the bioturbation rate was one order of magnitude higher in the animal treatment compared to the 'burrow only' treatment, indicating that bioturbation is actively driven by the burrow-flushing and particle-burial activities of *Corophium*. The behavioural activities of *Corophium* per se contribute to a downward sediment mixing of approximately 1 cm^2/y (for 15,000 ind./m^2), which is a five-fold increase compared to the passive transport induced by the static structure of burrows. These results indicate that in ecosystems where *C. volutator* is abundantly present, it may play a prominent role in downward particle transport and organic matter transformation, while a decline in *Corophium* densities due

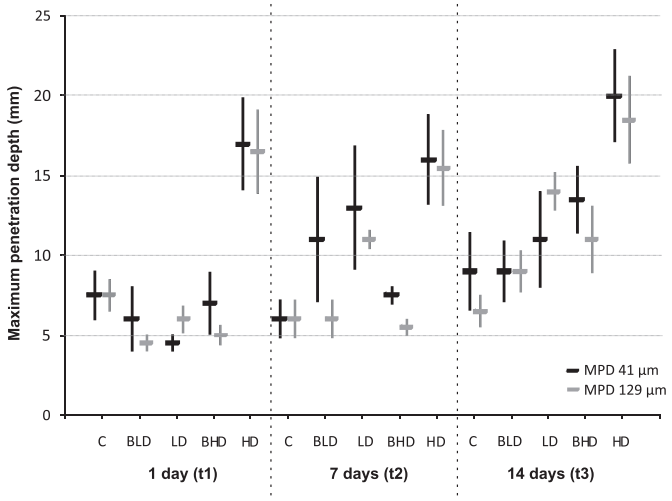

Fig. 2. Maximum penetration depth (mean ± SE, $n = 4$) for the different treatments (C = Control, BLD = Burrows Low Density, LD = Low Density, BHD = Burrows High Density, HD = High Density) from 41 µm (black) and 129 µm (grey) luminophores at the different sampling occasions. No significant difference between luminophore sizes were observed.

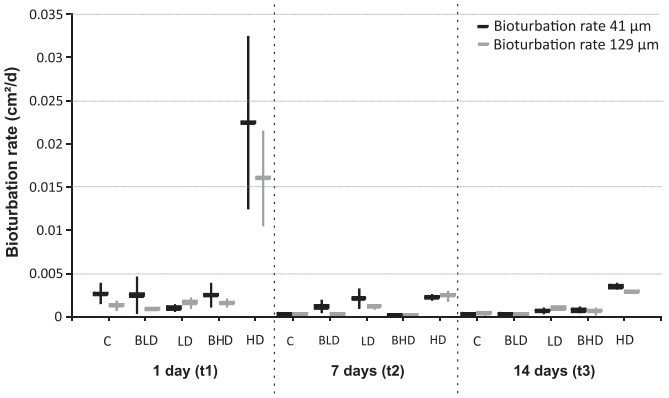

Fig. 3. Bioturbation rate (mean ± SE, $n = 4$) for the different treatments (C = Control, BLD = Burrows Low Density, LD = Low Density, BHD = Burrows High Density, HD = High Density) from 41 µm (black) and 129 µm (grey) at the different sampling occasions. No significant differences between luminophore sizes were observed.

to natural or anthropogenic disturbances might have negative effects on downward transport of organic matter. Moreover, the density of *Corophium* also proved to be an important parameter in biogeochemical processes, where ventilation activity increased oxygen consumption, nitrification and denitrification, and the release of nutrients from the sediment (Pelegri et al., 1994a; Pelegri and Blackburn, 1994b; Emmerson et al., 2001; Mermillod-Blondin et al., 2004) and furthermore, *Corophium* is known to stimulate microbial activity in the burrow (Mermillod-Blondin et al., 2004). These biogeochemical results, together with our quantification of sediment reworking imply that *Corophium*, at least at densities of 15,000 ind./m^2, is important in the functioning of intertidal mudflats. This density of 15,000 ind./m^2, and even much higher densities, are frequently observed in mudflats. For instance in the IJzermonding tidal flat, where the experimental animals were collected, average densities in summer easily reach 50,000 ind./m^2 with peaks towards 100,000 ind./m^2 (De Backer et al., 2010b). Further evidence pointing at the importance of *Corophium* in ecosystem functioning of mudflats was provided by Gerdol and Hughes (1993), who concluded that *Corophium* (12,500 ind./m^2) prevented the establishment of *Salicornia europaea* partly by burial of seeds, but mostly by preventing the establishment of the seedlings, which inhibits the expansion of salt marsh vegetation.

4.3. Bioturbation rate

For each of the cores, the bioturbation rate measured after 24 h was about one order of magnitude higher as compared to those measured on the later sampling occasions. Interpretation of these 24 h values should be done with care, because after one day only very small amounts of tracer particles have been reworked. At such short timescale, even minute percentages of tracer at depth (e.g. as a result of the slicing process, clearing the burrow opening after deposition of the luminophore slices, falling pieces of luminophore slice in the burrows after melting…) will result in an overestimation of the bioturbation rate by the model after 1 day. However, the effect of these experimental artifacts at the first day will subside over time to result in 'true' bioturbation rates, and when we compare our bioturbation rates measured at the later sampling occasions with other studies, lower values were observed. Mermillod-Blondin et al. (2004) for instance, measured a diffusion rate of 0.006 cm^2/d for *Corophium* at a density of 5000 ind./m^2 after 20 days, while in this study for both densities much lower values were observed, although control values in both studies are similar (Fig. 4). Other studies on intertidal animals, but using fully submersed experimental mesocosms, showed values ranging from 0.003 to 0.009 cm^2/d (François et al., 2002; Duport et al., 2006).

We hypothesise that this difference in bioturbation rates could be caused by the imposed tidal regime (3 h submersion versus 9 h emersion) in this study. A previous study (De Backer et al., 2010a) showed that *Corophium* is completely inactive for on average 70% of the time during emersion, meaning that sediment reworking is mainly restricted to submersion. Furthermore, comparison with the study of Mermillod-Blondin et al. (2004), which was done under similar temperature conditions (14 °C versus 16 ± 1 °C in this experiment) and also without the addition of food, but in total darkness and with 100% submersion, indicates that shifts in activity periods caused by the tidal regime in combination with the light regime, may be responsible for the different values in bioturbation rates, with a possible overestimation of bioturbation rates in the absence of a tidal regime in the dark. It is known that swimming activity by *Corophium* displays cyclic variation partially related to time of the day (Drolet and Barbeau, 2009 and references therein), but the effect of the light regime on the other activities has not yet been studied. Although diurnal periodicity, as a consequence of light conditions, is known to influence macrobenthic behaviour (Drolet et al., 2004), this is often subordinate to the tidal regime, but it can

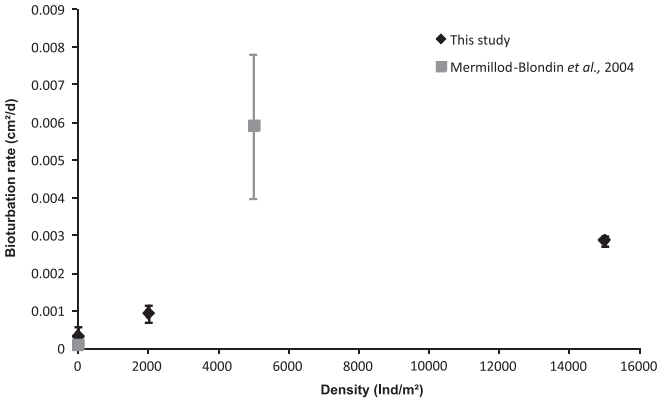

Fig. 4. Comparison of bioturbation rates measured in Mermillod-Blondin et al. (2004) at densities of 0 and 5000 ind./m^2 with rates measured in the current study at densities of 0, 2000 and 15,000 ind./m^2.

either hamper or enhance the patterns caused by the tidal regime (Orvain and Sauriau, 2002; Hampel et al., 2003). To our knowledge, no supporting literature exists linking tidal rhythmicity in behaviour to bioturbation rates. Therefore, it would be very interesting to test this hypothesis under experimental conditions with different tidal regimes. However, studies on seasonal variation in bioturbation rates measured lower sediment reworking rates in winter due to reduced feeding, burrowing and/or ventilation activities (Maire et al., 2007; Braeckman et al., 2010). Similarly, reduced bioturbation activity was also measured for *Hediste* due to decreased feeding behaviour without a food supply (Nogaro et al., 2008). Hence, the observed decrease in activity of *Corophium* during emersion (De Backer et al., 2010a) could similarly result in lower reworking rates.

5. Conclusion

The population density of *Corophium volutator* is a key parameter determining the impact of its bioturbation. Only when abundantly present in the mudflat ecosystem, *Corophium* will play an important role in reworking of the sediment surface. Density declines of *Corophium*, be it natural or anthropogenic, can thus have negative effects on downward particle and organic matter transport. We found, however, no evidence for size selectivity during bioturbation of *Corophium*. Furthermore, our results indicated that the bioturbation measured at high densities was actively driven by the burrow-flushing and particle-burial activities of *Corophium*, which contributed to a five-fold increase in sediment transport compared to the small abiotic driven flux of passive particle transport induced by the static burrow structures. Consequently, this suggests that all factors causing a decrease in behavioural activity (e.g. tidal regime, temperature, and food supply), cause a decrease in bioturbation activity.

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