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Functional response of an adapted subtidal macrobenthic community to an oil spill: macrobenthic structure and bioturbation activity over time throughout an 18-month field experiment

Franck Gilbert · Georges Stora · Philippe Cuny

Abstract An experimental oil spill was carried out in order to assess in situ responses of a macrobenthic community of shallow subtidal sediments historically exposed to petroleum contamination. Both structural and functional (bioturbation activity) parameters of the community, subjected or not to a pulse acute contamination (25,000 ppm), were studied for 18 months. No difference in the community structure was detected between contaminated and control sediments, from 6 to 18 months of experimentation. Vertical distributions of organisms, however, were affected by the presence of oil contamination leading to a deeper burial of some polychaete species. In the same time, changes in sediment-reworking activity and more especially a deeper particle burying in sediments subjected to acute oil contamination were shown. These results highlight the need to complete the analysis of community structure by assessing functional aspects, such as bioturbation activity, a process integrating various aspects of benthic behaviour (e.g. feeding, locomotion, burrow building) in order to estimate real (structural and functional) and long-term effects of oil contamination on benthic communities.

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Keywords Subtidal shallow sediments · Field experiment · Acute oil contamination · Macrobenthic community · Bioturbation · Sediment reworking

Introduction

Changes in the structure and composition of macrobenthic communities driven by pollution may have marked effects on biogeochemical cycles and other important benthic ecosystem processes and functions (Hale et al. 2014). As such, crude and refined petroleum hydrocarbon pollution of coastal sediments has generally been shown to induce pronounced community structural changes. These changes, which may appear at hydrocarbon concentrations above 50 ppm (Kingston 1992), are generally associated to an impoverishment of the diversity concomitant with the selection of opportunistic and tolerant species (e.g. Gómez Gesteira and Dauvin 2000; Nikitik and Robinson 2003; Gómez Gesteira and Dauvin 2005). Several studies also reported a decrease in species abundances with increasing or elevated hydrocarbon contamination levels such as those observed after an oil spill (e.g. Carman et al. 2000; Je et al. 2003; Venturini et al. 2008; Seo et al. 2014; Zabbey and Uyi 2014; Ferrando et al. 2015). Apart from the concentration, many other factors can, however, modulate the response of the community like the composition and toxicity of crude oils, the characteristics of the sedimentary matrix (e.g. grain size range, organic matter content; Je et al. 2003; Venturini and Tommasi 2004) and the bioavailability of hydrophobic contaminants (Di Toro et al. 1991). Initial benthic community structure and variable tolerances within and among species can also strongly affect the resistance capacities of the benthic system (Bickman and Smolen 1994; Depledge 1996; Hawkins 1998; Carman et al. 2000). This variability may be in part explained by differences in the life

style and feeding strategies between deposit feeders and carnivores (Venturini and Tommasi 2004). Historical or chronic exposures to hydrocarbon contamination of benthos lead to an adapted community with higher proportion of more tolerant species and/or increased tolerance among individual species (Carman et al. 2000). Instead, communities from pristine ecosystems seem to be particularly sensitive to oil spills (Ferrando et al. 2015). Whatever the previous history of contamination, the community shifts and the removal of organisms with specific biological traits induced by the contamination may have important consequences on the functioning of the whole ecosystem. For instance, the experimental removal from marine soft sediments of large suspension feeders or deposit feeders was shown to influence the flux of nitrogen and oxygen, surficial sediment characteristics, and also community composition (Thrush et al. 2006). Kristensen et al. (2014) also demonstrated that shifts in benthic fauna composition and species functional traits from a marine fjord could affect biogeochemical cycling resulting in modulation of primary productivity in the overlying water column with a feedback effect on the benthic system. Indeed, macrobenthic organisms have a pivotal role in the functioning of benthic ecosystems notably through their bioturbation activities (sediment reworking and ventilation, i.e. transport and mixing of particles and solutes, respectively; Kristensen et al. 2012). Due to their reworking activity, dwelling macroorganisms introduce temporal and spatial heterogeneity in the sediment system resulting in the modification of the expression of the main microbial activities, which in turn influences the degradation of organic matter (organic contaminants) (for a review, see Cuny et al. 2011). They notably ensure the oxygenation of the superficial sediments by the construction and ventilation of burrows and tubes (Aller 1994; Pischedda et al. 2008; Pischedda et al. 2012).

To date, most of the studies dealing with the effects of hydrocarbon contamination on macrobenthic communities were focused on the changes in species densities and faunal composition. In this study, time changes of macrobenthic community as well as of reworking activity (functional descriptor of macroorganism activity) induced by a field experimental oil spill in subtidal chronically contaminated sediments was assessed during 18 months.

Materials and methods

Experimental site

The experimental was carried out in the Gulf of Fos (Mediterranean Sea), a highly urbanized and industrialized site, notably surrounded by chemical and petroleum plants, characterized by heavy ships and tanker traffic (Fig. 1). In the Gulf of Fos, sediments are recognized having a long-time, from

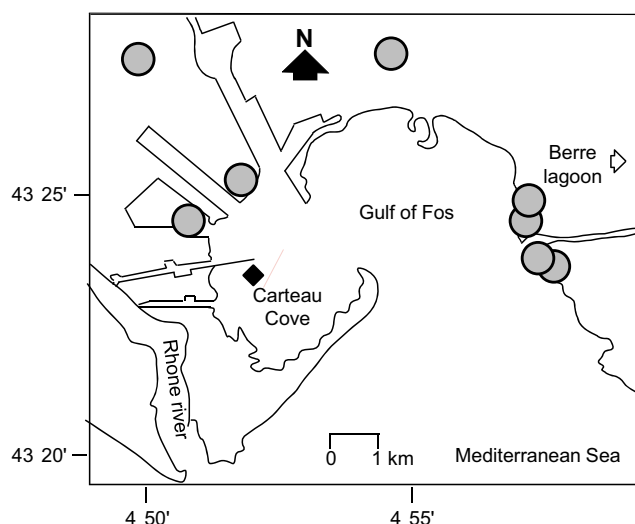


Fig. 1 Experimental site. Schematic map of the Gulf of Fos (Mediterranean Sea) indicating the experimental site (black diamond) and nearby oil and gas refining and chemicals main facilities (grey spots)

moderate to high level, contamination history by pyrogenic and petrogenic hydrocarbons (Mille et al. 2007). More especially, in the experimental subtidal site (northern Carteau Cove; 43° 23' 40" N, 4° 51' 35" E) organic rich muddy sand sediments contain $\sim 1240 \text{ mg kg}^{-1}$ sed. and $\sim 70 \text{ mg kg}^{-1}$ of extractable organic matter (EOM) and total hydrocarbon content (THC), respectively (Mille et al. 2007).

Experimental and analytical procedures

At the beginning of experiment (December 1994), 18 PVC cores (diameter 10 cm, height 25 cm) were separately embedded into sediments by divers, at 5-m water depth. Then, a frozen "cake" (diameter 10 cm; 1 cm thick) was deposited at the surface of each portion of sediment ecosystem delimited by a core. For half of the cores (OIL cores), the deposited cakes were a mixing of sediment and Brut Arabian Light topped at 250 °C petroleum (25.2 g oil kg^{-1} wet sediment). The rest of cores received a clean sediment cake and served as uncontaminated controls (UNC-Cores). In order to quantify the sediment reworking, particulate inert tracers (luminophores; 63–125 μm ; 8 g) were added to the different cakes.

Then, after 6, 12, and 18 months, three cores from each sediment type (oil-contaminated, control) were sampled by divers, transported to the laboratory, and sliced in 2-cm-thick segments. Then, each sediment slice was divided in eight different equal parts.

For macrofauna species identification, four parts of each slice were randomly selected and pooled while sieved through a 500- μm mesh. Retained organisms were then stored into a plastic bottle with fixating solution (4 % buffered formaldehyde with Rose Bengal) and further sorted and counted under optic microscopes.

For luminophore quantification, two other parts of each slice were randomly selected, pooled, freeze-dried, and homogeneously mixed. Luminophores' counting in each layer was then realized under UV light using an epifluorescence microscope. The quantification of sediment reworking by the gallery-diffusor model (François et al. 2002) was based on each vertical luminophore profile measured in each core (Duport et al. 2007). This model allows describing both the biodiffusion-like transport (D_b coefficient) due to the continuous displacement of the tracers and the nonlocal bioadvective displacement of the tracers (r coefficient). The best fit between observed and modelled tracer distribution with depth (i.e. producing the best D_b and r coefficient couple) was estimated by the least squares method.

For hydrocarbon quantification, the two remaining parts of each slice were pooled. The extraction of petroleum hydrocarbons from the sediment was achieved by alkaline digestion as described by Mille et al. (1988). The fractionation and capillary gas chromatography analyses of the saturated fraction of hydrocarbons (SF), used as a proxy to calculate oil budget and vertical repartition with time, were those used by Gilbert et al. (1997).

Similarity between macrobenthos samples was estimated using the Bray–Curtis index, a measure of the Bray–Curtiss dissimilarity (Bray and Curtis 1957).

Differences between biodiffusion coefficients (D_b) and bioadvection coefficients (r) were each analyzed by two-way ANOVA with time and conditions (contaminated or control) as factors, after assessing homogeneity of variance and normally distributed residuals using Barlett's and Shapiro–Wilk's tests, respectively.

Results

Contamination state of sediments

As indicated earlier, in the frame of our work, the saturated fraction of hydrocarbons (SF) was used as a proxy to calculate oil budget and vertical repartition with time. This was based on the assumption that all components of the Arabian Light crude oil (i.e. saturates, aromatics, resins, asphaltenes and waxes) were submitted to the same transport processes within sediments. To be consistent with this simple model, and despite previous studies carried out in the same area have shown that biodegradation processes were active in the sediments (Grossi et al. 2002; Miralles et al. 2007), we also considered the biodegradation as negligible compared to abiotic losses. Therefore, by now, we will only refer to oil in the text.

In order to allow long-time lasting contamination at the experimental site (Plante-Cuny et al. 1993), but without

Table 1 Sediment contamination

Period of experiment	Estimated oil content (g oil kg ⁻¹ sed)
Start to 6 months	25.2 to 4.8
6 to 12 months	4.8 to 2.3
12 to 18 months	2.3 to 1.0

Oil content, within contaminated (OIL) sediments, after 6, 12 and 18 months. Oil content was estimated from the saturated hydrocarbon fraction budget for an initial oil content of 25.2 g kg⁻¹ sed

confining sediments (Gilbert et al. 1996), an initial 25.2 g oil kg⁻¹ wet sediment oil contamination was applied. Indeed, results showed an important loss (i.e. resuspension and release into the water column) of added oil in the experimental oiled sediments of 81, 91 and 96 %, after 6, 12 and 18 months. This indicated, however, that highly polluted conditions (i.e. from 25.2 to 1 g oil kg⁻¹) were present throughout the experiment within oil-contaminated cores (Table 1). Remaining oil was found down to 10 cm deep but with a vertical of remaining hydrocarbons different with time (Fig. 2). After 6 months, oil presented a regular decrease with depth down to 6-cm depth (99 % of remaining oil). Then, after 12 and 18 months, oil repartition highlighted a subsurface peak, layer 2–4 cm containing 63 % of remaining oil.

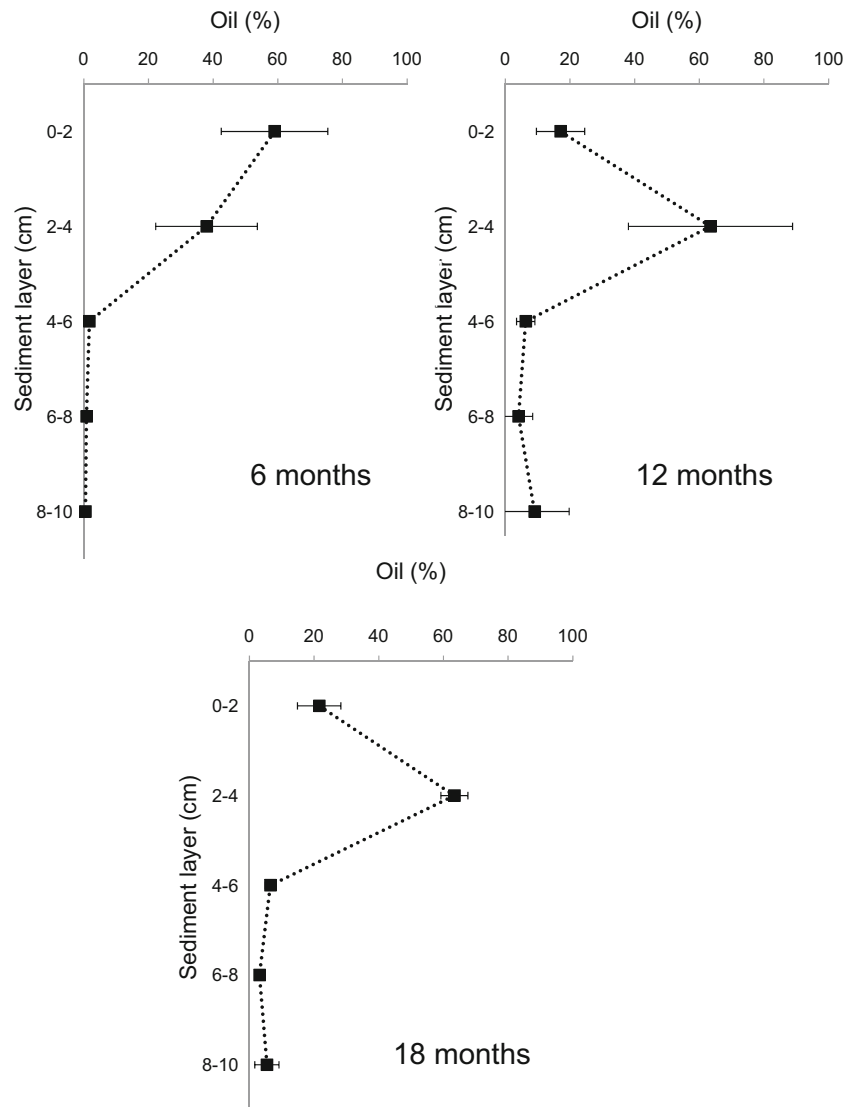
Macrobenthic community structure

Within the experimental sediments, the polychaetes (56.4 to 89.9 % of the total organisms) and the crustaceans (4.7 to 35.6 %) represented the two dominant macrobenthic groups. Molluscs and echinoderms were also present (see Online Resource 1). Throughout the experiment, global macrofauna density and specific richness in experimental sediments varied from 1336 to 2413 ind. m⁻² and from 10 to 38, respectively. None of these parameters of the community structure did show any significant difference with both time and type of sediments (Figs. 3 and 4).

Cluster analysis based on Bray–Curtis similarity distances indicated communities with affinities close or higher than 50 %, however, suggesting that the communities grouped with sampling time (Fig. 5).

Vertical repartitions of the organisms indicated that they were mainly (more than 75 %) located in the first 8 cm of sediment but with some individuals found down to 22-cm depth (Fig. 3). On a general way, in both contaminated or control sediment, the presence of organisms decreased regularly with depth. Contaminated sediments, however, showed a reduced presence of organisms in the surface layer, but a subsurface density peak centred on the 4–6-cm depth layer, from the 6th to the 18th month of experimentation.

Fig. 2 Sediment contamination. Vertical repartition of remaining oil within contaminated (OIL) sediments, after 6, 12 and 18 months. Values are mean $\pm$ SD for triplicates



Macrobenthic community sediment reworking

Luminophores deposited at the sediments' surface were tracked within the sedimentary columns, with time. Whatever the absence or presence of oil contamination or the time of experiment, the tracers were never found deeper than 16 cm deep and the majority of them (more than 95 %) in the first 8 cm (Fig. 6). In contaminated sediments, a subsurface repartition peak (layer 2–4 cm) was present throughout the experiment. On the other hand, after 6 months, the distribution of tracers initially deposited at the sediment surface decreased exponentially with depth in control sediments. After 12 months, a subsurface peak was detected in the 2–4 layer but was finally less marked at the end of experiment (18 months).

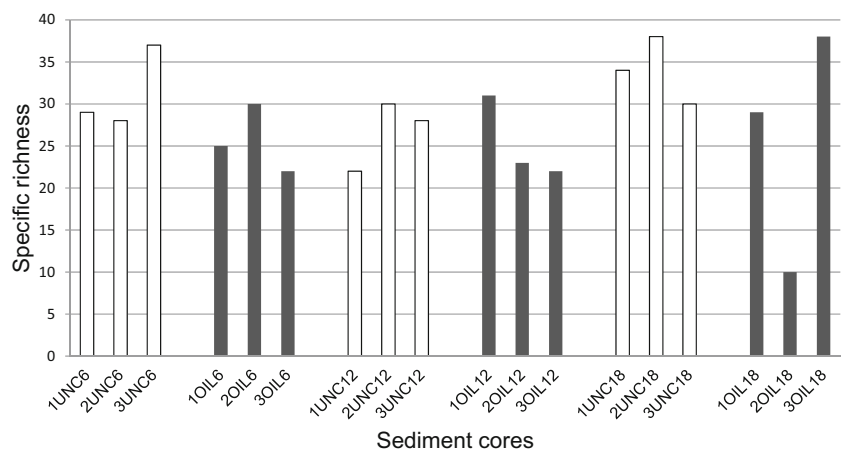
Quantification of sediment reworking showed mean Db and r coefficients, respectively, ranging from 2.1 to 10.1 $\text{cm}^2 \text{year}^{-1}$ and 1.1 to 3.7 year^{-1} , respectively (Table 2). Biodiffusive coefficient Db , but not the bioadvective

coefficient r , was found to significantly decrease with time ($P > 0.001$; $F 22.451$; $df 15$). No significant difference in both coefficients was found between control (UNC) and contaminated (OIL) sediments. The mean bioadvective coefficient, however, was higher in presence of oil contamination (2.98 year^{-1}) than for the control sediments (1.98 year^{-1}).

Discussion

Our results indicated very similar macrobenthic communities, from both qualitative and quantitative points of view, in controls and oiled contaminated sediments 6, 12 and 18 months following the initial experimental contamination. Both Gandra et al. (2006) and Egres et al. (2012) in situ experimental contamination works have previously shown equivalent communities in oil impacted and no impacted

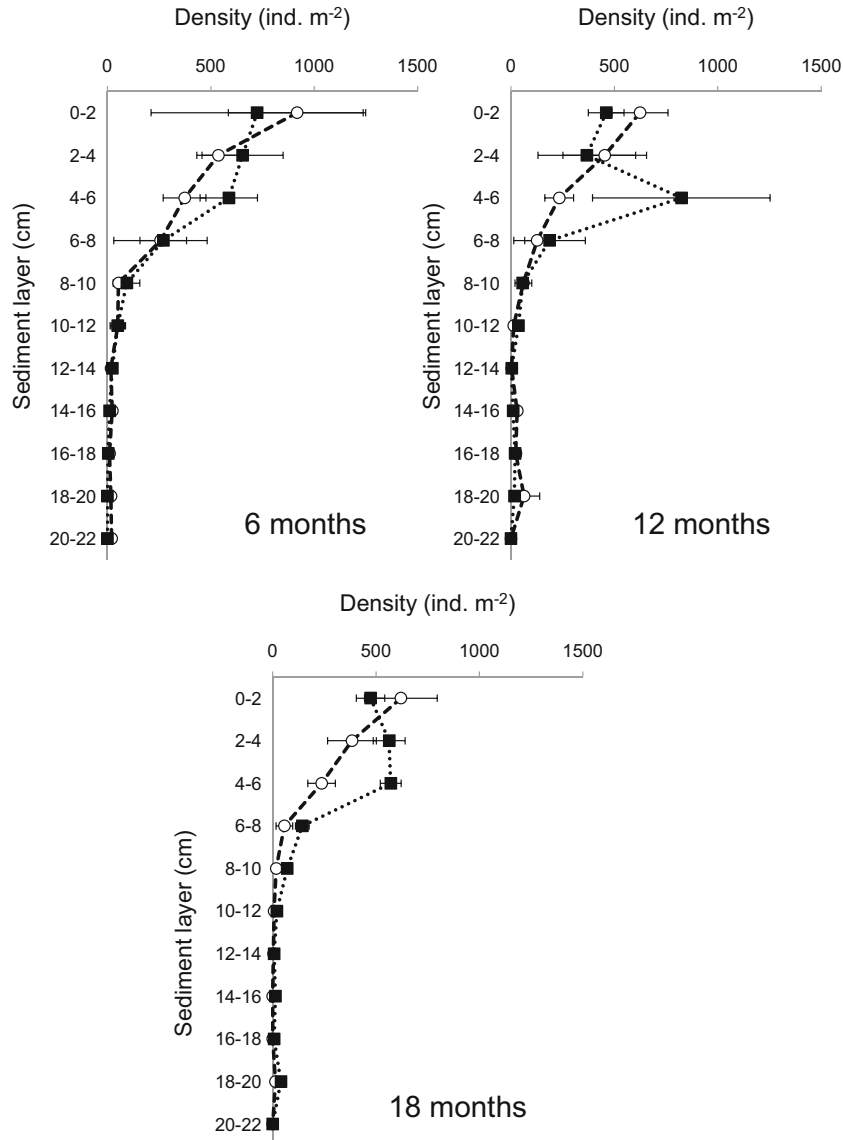
Fig. 3 Macrobenthic community. Specific richness for control (UNC, *white column*) and contaminated (OIL, *closed column*) sediments, after 6, 12 and 18 months



sediments. These works have also demonstrated quasi instantaneous (i.e. day-scale) acute effects on macrobenthic

community, more or less restricted to specific phyla such as crustaceans (Gómez Gesteira and Dauvin 2005; Gandra et al.

Fig. 4 Macrobenthic community. Density of organisms with depth for control (UNC; *open circle*) and contaminated (OIL; *closed square*) sediments, after 6, 12 and 18 months. Values are mean±SD for triplicates



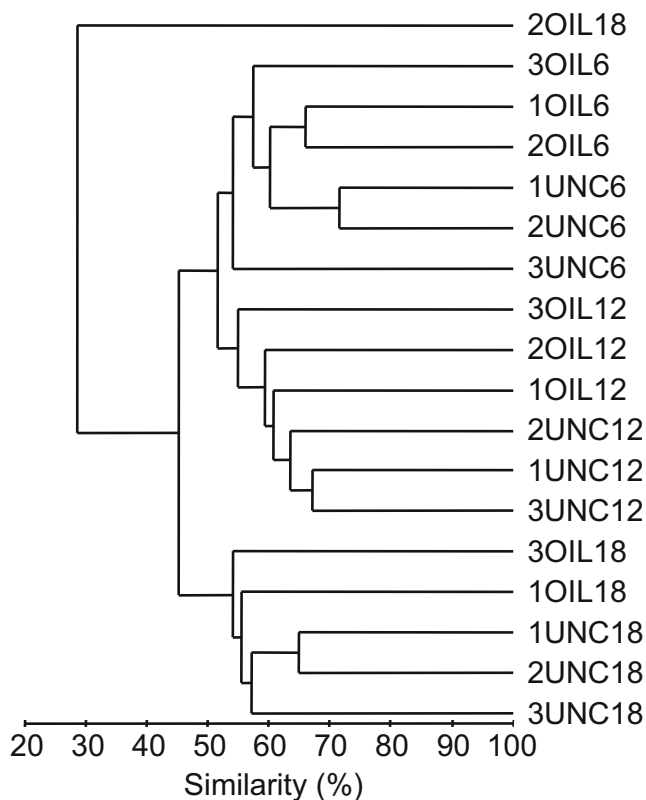


Fig. 5 Macrobenthic community. Cluster analysis based on Bray–Curtis similarity distances for control (UNC) and contaminated (OIL) sediments, after 6, 12 and 18 months

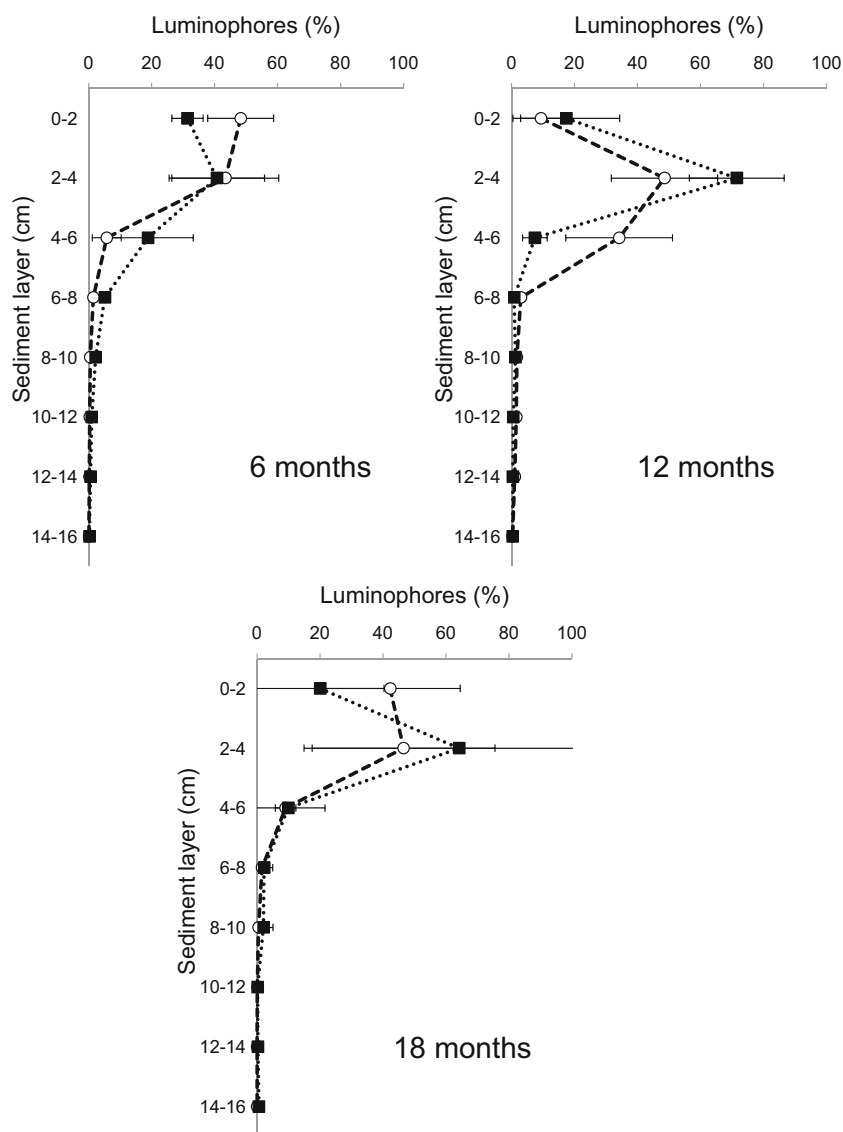
2006), followed by recoveries of population levels as soon as after 8 or 2 days, respectively. Such very fast recovery was suggested resulting from larval recruitment and surrounding migration of adults and juveniles (Carman et al. 2000; Negrello Filho et al. 2006). The contaminations applied were, however, far lower than our 25,000-ppm oil pulse. They resulted in pre-impact oil concentration reestablishment after only 73 days (Egres et al. 2012) while our experimental sediments were still highly contaminated 18 months after initial oil deposition. This ‘pulse’ disturbance (Glasby and Underwood 1996) allowing rapid recovery may have also occurred in our experimental conditions. Our 6-month sampling interval, however, was not appropriate to allow such detection. Furthermore, the investigated Carteau Cove community had a quite long history of contamination exposure and could have been potentially able to better endure contamination stress than communities with lower or almost absent previous contamination (Carman et al. 2000).

Community structures were similar between controls and contaminated sediments, but organisms were retrieved deeper in presence of oil. This macrofaunal vertical migration activity in presence of deposited material in sediments has been described as an efficient way for organisms to temporarily avoid unsuitable conditions (Bolam 2011). Within a community, this capacity is, however, not shared by all species (e.g. Miller et al.

2002; Hinchey et al. 2006). As a matter of fact, the presence of high amounts of oil in the surface leads to a reduction of the oxygen diffusion to the deeper layers of sediments that could be critical for some species. In the frame of our work, the polychaetes *Nereis caudata*, *Notomastus latericeus*, *Paradoneis lyra* and *Tharyx marioni* were the species that successfully vertically migrated in presence of oil.

Changes in organisms’ repartition into the sedimentary column were also accompanied by changes in global community mixing pattern: the control uncontaminated sediments presented a rather biodiffusive-like mixing pattern while the mixing of particulate tracers in oiled sediments tended to be non-local and more especially of conveying type (i.e. with production of a subsurface peak). It is possible that the deeper burial of organisms may have affected sediment reworking modes and rates (e.g. by a deeper extension of tracers spreading), as demonstrated in case of predation avoidance (Maire et al. 2010). Switches in feeding modes have been previously demonstrated for some infaunal species as a function of biological or environmental factors (Buchanan 1964; Lopez and Levinton 1987; Lindsay and Woodin 1995; Fodrie et al. 2007; Riisgård and Larsen 2010). Similarly, the general change in global community mixing in presence of surface oil contamination may have been linked to switches of individual modes of mixing by the totality or part of species. Actually, among the four species that were found buried deeper in oiled sediments, *Notomastus latericeus*, *Paradoneis lyra* and *Tharyx marioni* have the ability of both surface or subsurface feeding modes (Fauchald and Jumars 1979; Sánchez-Moyano and García-Asencio 2009). However, without any available supporting data on individual species reworking, caution must be applied. Indeed, as a function of the stressor applied, species which have the capacity to switch in feeding mode may not *ex abrupto*, or even never perform this ability. Moreover, a switch in feeding mode may not affect the sediment-reworking mode noticeably depending on factors such as mixing intensity or measurement time. For instance, *Hediste diversicolor*, described as a multiple feeding mode polychaete (e.g. Riisgård 1994; Scaps 2002; Riisgård and Larsen 2010), did not show any change in reworking mode when experimentally exposed to a surface oil contamination (Brut Arabian Light 250; from initial 4.5 g kg⁻¹ dry sed. to 1 g kg⁻¹ dry sed. after 45 days; Gilbert et al. 1994). On the other hand, according to biodiffusion and bioadvection mixing coefficients recently calculated from the Gilbert et al. (1994) data (mean control Db/r 0.8/7.6; mean contaminated Db/r 0.1/4.1), sediment-reworking intensity by this experimental *H. diversicolor* population was significantly lowered in presence of oil. In the frame of our experimental in situ work, no significant reduction of sediment mixing by the macrobenthic community was found under contaminated conditions. Nevertheless, results clearly suggested a deeper burying of tracers in presence of oil as indicated by higher bioadvection coefficients compared with uncontaminated controls.

Fig. 6 Sediment reworking. Vertical repartition of remaining luminophores within control (UNC; *open circle*) and contaminated (OIL; *closed square*) sediments, after 6, 12 and 18 months. Values are mean $\pm$ SD for triplicates



Conclusion

Despite high oil concentration still present, no difference in the macrobenthic community was detected between experimentally contaminated and natural control sediments historically exposed to petroleum contamination. Contrary to the structure of community, organisms' vertical distribution was

affected by the presence of oil contamination leading to a deeper burial (burial survival) of some polychaete species. This was accompanied by changes in sediment-reworking activity and more specifically by a deeper particle burying in sediments subjected to acute oil contamination. These results highlighted the need to complete the analysis of community structure by assessing functional aspects such as bioturbation activity, a process integrating various aspects of benthic behaviour (e.g. feeding, locomotion, burrow building), in order to estimate real (structural and functional) and long-term effects of oil contamination on benthic communities. Even if long-term toxicity effects could not be discarded, shallow subtidal macrobenthic communities adapted to chronic hydrocarbon inputs seemed to be particularly resistant to elevated hydrocarbon concentrations. In case of an oil spill, they would ensure the functioning of the benthic system by adapting their behaviour and maintaining a similar level of bioturbation

Table 2 Sediment reworking

		Db (cm ² year ⁻¹)	r (year ⁻¹)
Biodiffusion (Db) and bioadvection (r) mixing coefficients within control (UNC) and contaminated (OIL) sediments, after 6, 12 and 18 months. Values are mean $\pm$ SD for triplicates	UNC6	7.1 $\pm$ 0.8	1.4 $\pm$ 0.5
	OIL6	10.1 $\pm$ 0.4	3.1 $\pm$ 0.1
	UNC12	6.1 $\pm$ 0.8	3.4 $\pm$ 0.9
	OIL12	4.4 $\pm$ 0.5	2.1 $\pm$ 0.8
	UNC18	2.1 $\pm$ 0.8	1.1 $\pm$ 0.8
	OIL18	2.4 $\pm$ 2.0	3.7 $\pm$ 3.1

activity. Thus, the oxygenation of superficial sediments and the stimulation of aerobic oil-degrading bacteria would notably favour the natural attenuation of the sediments (Cuny et al. 2007).

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