Relative importance of macrofaunal burrows for the microbial mineralization of pyrene in marine sediments: impact of macrofaunal species and organic matter quality

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ABSTRACT: The significance of macrofaunal burrows for microbial organic matter mineralization is well recognized. However, despite the importance of marine sediments as main sinks for organic particle-reactive contaminants such as polyaromatic hydrocarbons (PAHs), the significance of biogenic structures for microbial pollutant mineralization is largely unknown. We measured microbial mineralization of the PAH pyrene in different sediment compartments (surface, burrow and reduced bulk sediments) as a function of contamination history (uncontaminated and pyrene-exposed), macrofaunal species (*Amphiura filiformis* and *Nereis diversicolor*) and sediment organic matter quality (labile *Tetraselmis* sp. and refractory lignin). Sediments were exposed to combinations of the 3 factors for 43 d, after which sediment samples from the 3 compartments were incubated for radio-respirometric measurements in ^{14}C-pyrene-coated flasks to monitor $^{14}CO_2$ production (i.e. pyrene mineralization) for 128 d. Pyrene exposure enhanced microbial pyrene mineralization (MPM) rates by an order of magnitude in all compartments, signifying MPM as inducible. MPM rates increased successively from reduced bulk to burrow and surface sediments in uncontaminated treatments, while MPM rates were highest in burrows when exposed to pyrene. This emphasizes the oxygen dependence of MPM and pinpoints burrows as MPM hot spots. Enrichment with labile organic matter doubled MPM rates in pyrene-exposed surface sediment, likely fertilizing MPM where oxygen is readily available. Burrow sediment MPM rates were doubled with *A. filiformis*, suggesting species-specific stimulation of the microbial community within the burrows. In conclusion, burrow sediment appears to be at least as important as surface sediment for microbial PAH degradation in marine sediments. Furthermore, macrofaunal biodiversity, sedimentation events and the trophic state of the system should be taken into account when the fate and effects of organic contaminants are assessed in marine systems.

KEY WORDS: Polyaromatic hydrocarbon · PAH · Biogenic structures · Biodegradation · *Nereis diversicolor* · *Amphiura filiformis* · Organic carbon quality

INTRODUCTION

Marine sediments constitute important sinks in the cycling of particle-reactive, persistent organic contaminants released into our biosphere. While point sources are being regulated, marine sediments storing old discharges have become major sources, accounting for contaminant effects propagating along food chains (Landrum & Robbins 1990, Burgess & Scott 1992). A thorough understanding of ecological processes in marine sediments is, therefore, fundamental for the prediction of contaminant fate and effects on many trophic levels. Polyaromatic hydrocarbons (PAHs) are a group of contaminants which are continuously

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released into our environment. Pyrene is a 4-ringed high molecular weight PAH of pyrogenic origin and, together with fluoranthene, benzo(*b*)fluoranthene and benzo(*e*)pyrene, it is the most abundant of the PAHs found in estuarine and marine sediments (HELCOM 1996, OSPAR-Commission 2000). In contrast to many other organic contaminants, PAHs are more readily degraded by most organisms but to a greatly varying and fairly unexplored extent (Cerniglia & Heitkamp 1989, McElroy et al. 1989). Unfortunately, PAH metabolites may display higher toxicity than parent compounds, and due to their chronic release, acute toxicity, mutagenicity and carcinogenicity, PAHs are a contaminant group evoking high environmental concern (Jerina & Daly 1974, OSPAR-Commission 2000).

Microbial degradation is considered the primary process leading to general PAH decontamination in both sediment and soil (Cerniglia & Heitkamp 1989, Neff 2002). Degradation of PAHs has generally been proven more efficient under oxic conditions as the microbes involved are dependent on molecular oxygen for the initial enzymatic attack of the aromatic rings (Atlas 1981, Cerniglia 1992). Consequently, the highest sedimentary microbial PAH degradation rates have been detected in oxic surface layers (Gardner et al. 1979, Cerniglia & Heitkamp 1989, Kure & Forbes 1997). Repeated PAH exposure has also been proven to enhance microbial PAH degradation rates as well as the number of hydrocarbon-utilizing micro-organisms both in laboratory and field studies (Atlas 1981 and references therein, Bauer & Capone 1985, Cerniglia & Heitkamp 1989).

Sediment-dwelling macrofauna transform sediment into a structural and functional 3-dimensional medium. By constructing burrows and tubes, they extend the sediment water interface, thereby increasing the likely contact between sediment and oxygenated water. Around active biogenic structures (i.e. burrows and tubes), the sediment displays a significantly different biogeochemistry compared to the surrounding bulk sediment (Aller & Yingst 1978, Forster 1996). Rapidly changing fluid dynamics and oxygen conditions, caused by intermittent species-specific macrofaunal irrigation, create burrow linings with steep chemical gradients and short diffusive distances, strongly favoring a great diversity of microbes (Aller 1994, Hulthe et al. 1998). Inhabited macrofaunal tubes and burrows may, thus, enhance the microbial degradation capacity of the deeper sediments by displaying surface-like interface properties (Aller 1988, Fenchel 1996, Kristensen 2000). The general stimulating effect of bioturbation on the oxygenation of deeper anoxic or reduced sediment, microbial activity and mineralization of organic matter is well documented (Aller & Yingst 1985, Kristensen & Black-

burn 1987, Andersen & Kristensen 1991). However, bioturbation also affects the general whole sediment distribution, release and microbial degradation of sediment-associated organic contaminants (Lee II & Swartz 1980, Holmer et al. 1997, Kure & Forbes 1997, Gunnarsson et al. 1999a,b). The intensity of the macrofaunal impact on biogeochemical processes has, however, been shown to be highly species-specific and particularly attributed to differences in macrofaunal behavior (Aller & Yingst 1985, Andersen & Kristensen 1991). Since most studies apply whole sediment or 2-compartment models when studying contaminant fate, the relative importance of macrofaunal burrows, compared to surface and bulk sediments, for the microbial degradation of PAHs and other contaminants in marine sediment is yet highly unexplored with, to our knowledge, only 2 published studies (Chung & King 1999, 2001).

Estimating contaminant fate in marine sediments is further complicated when organic matter input and sedimentation events are considered. The supply of organic matter to the sediment fuels the benthos, and changes seasonally both in terms of quantity and quality (Smetacek 1980, Gremare et al. 1997). Consequently, properties of sedimenting organic matter directly affect overall microbial metabolic activity, but may also indirectly affect microbes through changes in macrofaunal activity (Graf et al. 1983, Christensen & Kanneworff 1985, 1986, Graf 1992), with anticipated consequences for the degradation of organic contaminants. Changes in sediment organic matter (SOM) quality may also influence microbial PAH degradation by affecting sedimentary oxygen conditions, and through altered bioavailability of sediment-associated contaminants (Xing 1997, Lueking et al. 2000).

In the present study, we experimentally investigated the relative capacity for microbial pyrene mineralization (MPM) in different compartments of the sediment, i.e. surface, burrow and reduced/anoxic bulk sediments. The aim was further to explore effects of macrofaunal species, SOM quality and pyrene-exposure history on the MPM rates. Macrofauna were represented by the semi-stationary suspension- and surface-deposit feeding brittle star *Amphiura filiformis* (Echinodermata:Ophiuroidea), and the mobile omnivorous and predacious common ragworm *Nereis diversicolor* (Annelida:Polychaeta). SOM quality was manipulated by enriching sediment with low quality (refractory) terrestrial lignin or high quality (labile) microalgae (*Tetraselmis* sp.). The choice of substrates was based on a definition of reactive, labile high quality organic matter which, in line with Tenore (1977) and Gunnarsson et al. (1999a), refers to a substrate high in nitrogen and available amino acids and low in condensed structural hydrocarbons.

MATERIALS AND METHODS

The study was conducted in 2 steps including an initial exposure phase followed by an incubation phase (Fig. 1). During the exposure phase, sediments were presented with specific treatments. The treatments were constructed as combinations of enrichments with either lignin or *Tetraselmis* sp. together with addition of *Amphiura filiformis*, *Nereis diversicolor* or no fauna. Double treatment sets were prepared in order to keep 1 set uncontaminated, while pyrene was added to the sediment in the other set. The resulting exposure treatment combinations are displayed in Table 1. At termination of the 43 d exposure phase, samples were collected from surface, burrow and bulk sediments. Sediment samples were transferred to individual radio-respirometric incubation flasks coated on the inside with radioactively-labelled pyrene (^{14}C-pyrene). During a 128 d incubation period, radioactive carbon dioxide ($^{14}CO_2$) produced during microbial mineralization of ^{14}C-pyrene was continuously collected in NaOH-filled CO_2 traps (Reid et al. 2001), with subsequent quantification using liquid scintillation counting. The system enabled us to compare the impact of the different exposure treatments on microbial pyrene-mineralization potential in the different sediment compartments.

Collection of sediment and organisms. Sediment and brittle stars *Amphiura filiformis* were collected in March 2000 from 40 m depth north-west of the Gullmarsfjord (58° 20′ 30″, 11° 21′ 60″) on the Swedish west coast using a 0.062 m^2 box corer (Jonasson & Olausson 1966). On deck, the upper oxidized sediment layer containing most of the macrofauna was removed from the lower reduced bulk sediment, separating the sediment from each core into 2 pools. In the lab, the bulk sediment was stored without sieving under reducing conditions (covered with a thin layer of N$_2$-saturated water and a thick plastic film) in the dark at 5°C until use. Brittle stars *A. filiformis* were carefully collected from the oxidized sediment and were kept on a 1 cm layer of sieved (1 mm mesh size) surface sediment in tanks with continuous water flow (32 psu, 5°C) until use. Polychaetes *Nereis diversicolor* were collected in April 2000 on a muddy sand flat on the shore of the Gullmarsfjord by digging. The worms were kept in flow-through aquaria (32 psu, 5°C) with a 5 cm layer of sieved surface sediment (1 mm mesh size) until use. Additional oxidized surface sediment was collected in April 2000 from the same location as previously (northwest of the Gullmarsfjord); it was sieved

(1 mm mesh size) and stored with continuous flow (32 psu, 5°C) until use. One month prior to the experiment, thirty 1 l wide-mouth glass jars were filled with ~600 g wet wt (WW) homogenized reduced bulk sediment. The jars were covered with parafilm, lidded and placed in the same water bath (5°C) in a dark constant temperature room (5°C), allowing for microbial community adaptation to prevailing oxygen conditions.

Sediment preparation. The 2 carbon sources used for enrichment of the surface sediment layer were terrestrial lignin (Curan 100, Holmen paper AB) and a microalgae concentrate consisting of intact non-living cells of the chlorophyte *Tetraselmis* sp. (Reed Mariculture). Lignin is considered a low-quality, refractory carbon source, microbially low reactive, unpalatable to marine detrivorous macrofauna (e.g. Tenore et al. 1982, Gunnarsson et al. 1999a). *Tetraselmis* sp. has a high lipid and amino acid content (http://www.instant-algae.com/microalgae/tetraselmis.htm), and compared to lignin, it is much higher in total nitrogen (TN), and enriches the sediment content of total amino acids (TAA) and enzymatically hydrolyzable amino acids (EHAA) to a greater extent (Table 2). Consequently, *Tetraselmis* sp. is considered a labile, high-quality carbon source, microbially reactive and highly palatable to marine macrofauna. Equal amounts of total organic carbon (TOC) of the 2 carbon sources were added to the sediment. The TOC content of the sediment was increased from ~2% dry wt (DW) sediment (Table 2) to 3% DW sediment by addition of 18 g WW of *Tetraselmis* sp. and 5.9 g DW of lignin per kg WW of sieved (1 mm mesh size) and homogenized oxidized surface sediment. This resulted in a TN content 24 times higher in the sediment enriched with *Tetraselmis*
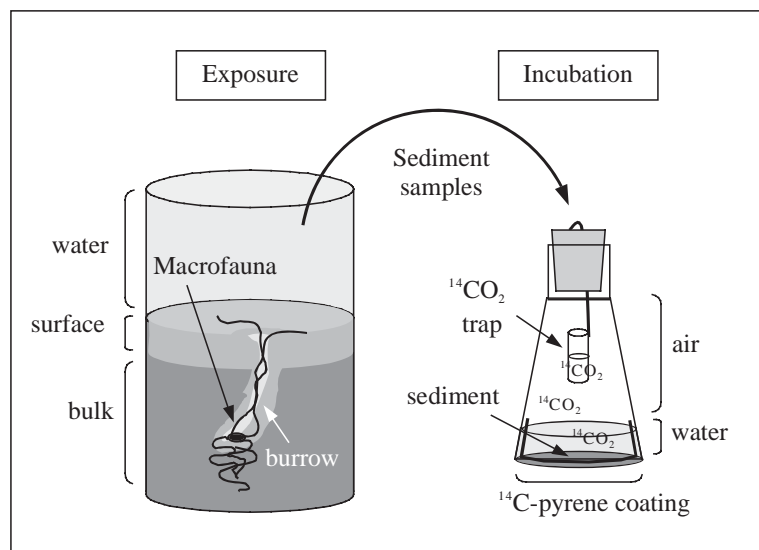

Fig. 1. Schematic diagram of the experimental design including exposure and incubation phases

Table 1. Exposure phase treatments. X: indicates an existing combination; –: indicates absence of the combination, n = 3

| | Uncontaminated | | Pyrene-exposed | |
	Tetraselmis sp.	Lignin	*Tetraselmis* sp.	Lignin
No fauna	–	–	X	X
Amphiura filiformis	X	X	X	X
Nereis diversicolor	X	X	X	X

sp. (156 mg kg WW^{-1} sediment) compared to the lignin-enriched sediment (6.5 mg kg WW^{-1} sediment). A small amount of seawater was added to the sediment in order to make it less cohesive for a more homogenous mixing of the organic matter; this was accounted for in the calculations. After enrichment, the sediments were agitated for 24 h on a shaking table at 5°C in darkness. Contents of total carbon (TC) and TN in sediment and organic substrates were measured on freeze-dried samples with a Carlo Erba elemental analyzer (NA 1500 NC, Fisons Instruments). TOC was determined accordingly after treating samples with HCl to remove carbonates (Hedges & Stern 1984). TAA and EHAA were also analyzed on freeze-dried sediment samples according to the method of Mayer et al. (1995). The sedimentary EHAA contents representing the amount of amino acids available to deposit-feeders, were analyzed by incubating 100 mg of dry sediment with proteolytic enzymes (Proteinase K, Sigma P-8044) in a shaking incubator in the dark at 37°C for 6 h, followed by precipitation of non-digested proteins with trichloro-acetic acid. The supernatant, containing digested peptides and amino acids, was then hydrolyzed in HCl (6 N) under N$_2$ for 24 h at 110°C. The samples were neutralized in a pH 8 boric acid buffer, stained with 0-phthaldialdehyde (OPA) (Sigma P-8044) and fluorescence was measured with a Hitachi F-4500 fluoresence spectrophotometer. Sedimentary TAA contents were analyzed by 24 h acid hydrolysis (6 N HCl) of 50 mg of dry sediment followed by neutralization, staining with OPA and fluorescence measurement. All samples were extracted in triplicates, and fluorescence

was counted using duplicate cuvettes (excitation: 340 nm; emission: 455 nm). EHAA samples were subtracted for protease blanks. Amino acid concentrations were determined by calibrating the fluorescence to a diluted solution of amino acid standard (Sigma AA-S-18) at 0.5, 1.0, 2.5 and 5.0 nmol ml^{-1}.

Contaminated and uncontaminated surface exposure sediments were prepared for both carbon sources in acetone-rinsed glass jars (3 l), by adding a known volume of pyrene (contaminated) (3.1 mg chrystalline pyrene ml^{-1} acetone, purity >98%, Sigma-Aldrich) or a corresponding volume of acetone (uncontaminated) to respective jars. The jars were agitated in darkness allowing for the solvent to evaporate (~4 h). A known amount of the organically-enriched wet sediment was added to the prepared glass jars, producing a pyrene/acetone and acetone-only spiked set for each carbon source. The jars were left for another 24 h on the shaking table in darkness allowing for the contaminant to bind and mix with the sediment reaching a final nominal concentration of 30 µg pyrene g DW^{-1} sediment.

Exposure phase. One hundred g WW of the prepared surface sediment was added to each of the 30 jars (1 l, diameter: 10 cm, height: 15 cm) on top of the bulk sediment. Each jar finally contained reduced bulk sediment (~6 cm deep) covered by a layer (~2 cm) of organically-enriched (with lignin or *Tetraselmis* sp.) and pyrene-contaminated (18 jars) or uncontaminated (12 jars) oxidized surface sediment with a surface area of 0.008 m^2. The jars were sealed with lids perforated for in- and out-flowing water and were then carefully filled with seawater (32 psu, 5°C) preventing resuspension. The jars were placed in random order in a large water bath (5°C) in a dark climate room (5°C), individually connected to a continuous flow of fresh seawater (32 psu, 5°C) of 40 ml min^{-1}, giving a water residence time of 13 min. After connection to the flow, the jars were left to stabilize for 24 h before the addition of ani-

Table 2. Initial specifications of sediment and organic carbon sources. TN: total nitrogen; TC: total carbon; TOC: total organic carbon. TAA (total amino acids) and EHAA (enzymatically hydrolyzable amino acids) both measured on initial sediment enriched with respective organic carbon source. DW: dry wt of organic carbon source or sediment; –: not analyzed. Means of 3 replicates (±SD) are shown

Source	TN (% DW)	TC (% DW)	TOC (% DW)	TAA (µmol g DW$_{sed}$$^{-1}$)	EHAA (µmol g DW$_{sed}$$^{-1}$)
Sediment	0.33 ± 0.03	4.25 ± 0.15	2.15 ± 0.41	–	–
Lignin	0.11 ± 0.004	61.1 ± 0.9	52.5 ± 2.9[a]	699 ± 51	101 ± 6
Tetraselmis sp.	1.88 ± 0.13[a]	37.7 ± 0.7[a]	37.5 ± 1.0[a]	799 ± 40	160 ± 2

[a]n = 2

mals and initiation of the experiment. Brittle stars and ragworms were carefully sieved out from storage tank sediments. Seven *Nereis diversicolor* (~7 cm in length) with a total biomass of ~3 g WW were added to each of 12 jars and 4 *Amphiura filiformis* (1 cm disk diameter) with a total biomass of ~1 g WW were added to each of another 12 jars. The total exposure period lasted for 43 d with continuous water flow at 5°C in darkness.

Incubation phase ($^{14}CO_2$ production). At the termination of the exposure phase, the jars were dismantled from the flow system, the water was carefully removed, and sediment samples were collected from the different compartments and transferred to separate polyethylene zip-lock bags. Surface and bulk sediment samples were collected with a stainless steel spoon rinsed between sampling in 96% EtOH. *Amphiura filiformis* burrows were sampled by extracting sediment with sterile disposable syringes and thick long needles (diameter: 2.1 mm, length: 80 mm). Only burrows visible through the glass walls were sampled and only sediment from the arm burrow linings, with a lighter color than the bulk sediment was collected. When collecting samples from *Nereis diversicolor* burrows, the entire sediment was forced out of the jar and carefully 'opened' in halves exposing the burrows. A stainless steel spoon with a very small bowl was used to scrape off the burrow linings. The spoon was rinsed in 96% EtOH between sampling. The sediment samples were carefully packed and shipped overnight in a refrigerated box (~6°C) to Roskilde University, Roskilde, Denmark. There, the samples were transferred to sterile 200 ml Erlenmeyer flasks coated on the inside with 0.15 µCi (4, 5, 9, 10-^{14}C) pyrene (58.7 mCi mmol^{-1}, purity >98%, Sigma-Aldrich). Coating was accomplished by adding 100 µl ^{14}C-pyrene dissolved in acetone (1.5 µCi ml^{-1}) to each flask, then allowing the acetone to evaporate. The sediment was covered with a thin layer of water (50 ml, 32 psu) and the flasks, containing a large headspace of air, were sealed with silicone stoppers. Replicated ^{14}C-pyrene-coated flasks filled with seawater only served as controls for the incubation-flask system (SW only controls). Traps for CO_2, consisting of 6 ml plastic scintillation vials, were fitted in the center of the flasks attached with steel wires to the silicone stoppers (Fig. 1). The vials were filled with 5 ml 0.5 M NaOH trapping the $^{14}CO_2$ produced by microbes mineralizing the ^{14}C-pyrene in the sediment. Incubations were run under oxic conditions at 10°C in darkness for a period of 128 d, during which the CO_2 traps were sampled on 12 occasions and flasks were simultaneously aerated. On each sampling occasion, 3 ml of NaOH was collected and transferred to scintillation vials, 15 ml scintillation liquid (Ecoscint A, National Diagnostics) was added and samples were left overnight, after which radioactivity was quantified

by liquid scintillation counting (LSC) on a Wallac 1409 liquid scintillation system, with corrections made for quenching by the external-standards ratio method after background subtraction. After sample collection, trap vials were emptied and refilled with fresh NaOH (5 ml, 0.5 M). At the termination of the incubations, samples were collected from the sediment and overlying water, and plastic traps and glass walls were extracted in order to measure the remaining amount of radioactivity and make a budget for the dynamics of ^{14}C-pyrene for the incubation systems.

Flask budget samples. Water samples from the incubation flasks were assayed for radioactivity in forms of $^{14}CO_2$, dissolved organic carbon (DO^{14}C) and particulate organic carbon (PO^{14}C). Three ml of the overlying water was collected with a syringe and needle, and dispensed into a glass scintillation vial through a glass fiber filter (GF75, pore size <0.7 µm, Advantec, Frisenette ApS). The GF filter collecting aqueous PO^{14}C was dissolved with 1 ml Soluene 350 (Packard) in a glass scintillation vial, 15 ml of scintillation cocktail (Ultima Gold XR, Packard) was added and radioactivity was quantified by LSC. The vials containing the filtered water were sealed with silicone stoppers, each fitted with a 0.5 M NaOH soaked GF filter hooked onto a steel wire connected to the stopper. Three drops of HCl (fuming) were added to each water sample with a syringe by pushing the needle through the silicone stopper and gently dispensing the acid. To allow for complete release from water and uptake of $^{14}CO_2$ on filters, vials were placed on gentle agitation overnight at 10°C in darkness. Following incubation, filters were transferred to new scintillation vials, dissolved as above and prepared for quantification of $^{14}CO_2$-derived radioactivity. Ultima Gold XR (15 ml) was added to the remaining water samples to quantify DO^{14}C-derived radioactivity. The sediment was removed from the flasks, weighed and oven-dried in semi-enclosed aluminium cups at 105°C for 24 h, left to cool and then reweighed. Samples of 0.1 g were collected from the dried sediment and transferred to scintillation vials. One ml of Soluene 350 was added, to each vial and the mixture was ultrasonicated for 10 min. Ten ml of Ultima gold XR was added, and samples were left overnight then analyzed by LSC. To extract ^{14}C-pyrene from the glass walls, the flasks were rinsed 3 times with 5 ml acetone. The acetone was transferred to glass scintillation vials then left to evaporate before addition of 10 ml Ultima gold XR followed by LSC. The plastic trap vials were placed in large scintillation vials, 18 ml Ultima Gold XR was added and radioactivity was quantified by LSC.

Trapping efficiency. A test was conducted in order to investigate the trapping efficiency of $^{14}CO_2$ in the incubation flask systems. Three different quantities of sed-

iment, corresponding to the sample weight variation in the main experiment, (0.5, 2 and 4 g DW sediment, n = 4), were tested in order to control for possible effects of the amount of sediment on CO_2 retention. Sediment was collected from the Gullmarsfjord as described previously; only the oxidized top sediment layer was sieved (1 mm mesh size), autoclaved in appropriate amounts in individual blue cap flasks and stored in darkness at 6°C until use. The sediment was mixed with 50 ml of 0.3 µm filtered and autoclaved seawater (32 psu, 5°C, pH 8) and decanted into 200 ml Erlenmeyer flasks. A total of 900 µl of radioactive sodium carbonate ($NaH^{14}CO_3$) dissolved in filtered and autoclaved seawater (0.38 µCi ml^{-1}) was added to the sediment slurries, and flasks were sealed and left in darkness at 6°C overnight for the sediment to settle. Four of the flasks containing 2 g DW sediment were sealed with silicone stoppers fitted with CO_2 traps (as described above) in order to control for possible early breakdown of $NaH^{14}CO_3$. The following day, glass vials containing 1 ml of concentrated HCl were carefully placed on the bottom of the remaining flasks. The flasks were resealed with silicone stoppers fitted with CO_2 traps filled with 5 ml 0.5 M NaOH as above. The HCl-vials were then knocked over and HCl was released into the sediment and water, lowering the pH sufficiently (pH < 2) for all of the $NaH^{14}CO_3$ to be converted into $^{14}CO_2$. The flasks were left in darkness at 10°C and sampled for $^{14}CO_2$ production after 2, 4, 8 and 16 d, when 3 ml of NaOH was removed from the trapping vials and transferred to scintillation vials. Ten ml of Ultima Gold XR was added and samples were counted on a Beckman LS5000TD liquid scintillation system (Beckman instruments) and corrected for quench by the external-standards ratio method after background subtraction.

Calculations and statistical analyses. Radioactivity values (DPM) derived from the amounts of $^{14}CO_2$ trapped during the incubation were corrected for the weight of incubation flask sediment using an empirically derived formula from the extraction efficiency test — f(x) = $-4.95x + 97.4$, r^2 = 0.866, x = g DW sediment and f(x) = $^{14}CO_2$-derived DPM — and were then normalized to dry weights of incubation flask sediments. Microbial pyrene-mineralization rates (MPM rates, g DW$_{sed}$$^{-1}$ d^{-1}) were calculated as DPM/(DPM$_{initial}$ × d × g DW$_{sed}$) from the slopes of regressions calculated from weight-specific relative $^{14}CO_2$ production versus time graphs, using the 3 initial data points. Calculated slope values were log-transformed (log$_{10}$) before being statistically tested. Levene Median and Kolmogorov-Smirnov tests were used for verification of homogeneity of variances and normality of data sets, respectively. Among compartments (surface, burrow and bulk sediments), log$_{10}$-transformed MPM rates were compared using 1-way ANOVAs, al-

though with separate testing of uncontaminated and pyrene-exposed data sets. The factors carbon source (lignin or *Tetraselmis* sp.) and fauna (*Amphiura filiformis*, *Nereis diversicolor* or no fauna) were tested independently within each compartment using orthogonal 2-way ANOVAs, where pyrene-exposed and uncontaminated sets were again treated separately. Ranking among levels within significant factors were resolved by Tukey post hoc tests. Budgets and percentages were calculated on the basis of empirical DPM values obtained during the initial additions of ^{14}C-pyrene.

RESULTS

Pyrene exposure induced the greatest overall treatment effect, resulting in increased microbial pyrene-mineralization (MPM) rates in all compartments by approximately an order of magnitude (differences between uncontaminated and contaminated sediments; surface $10^{0.91}$, burrow $10^{1.37}$, anoxic $10^{1.30}$) (Fig. 2). The highest MPM rates among sediment compartments within each treatment were generally observed in surface sediment, while the lowest rates were found in bulk sediment (Table 3). Generally, MPM rates in burrow sediment were almost equal to surface sediment MPM rates. However, the highest MPM rates detected in the entire experiment were measured in pyrene-exposed, lignin-enriched sediment from burrows made by *Amphiura filiformis* (Table 3). The MPM rate interrelationship among compartments depended on whether or not sediments were exposed to pyrene (Fig. 2). In uncontaminated treatments, MPM rates increased successively from bulk to burrow and surface sediment (ANOVA $F_{2,33}$ =
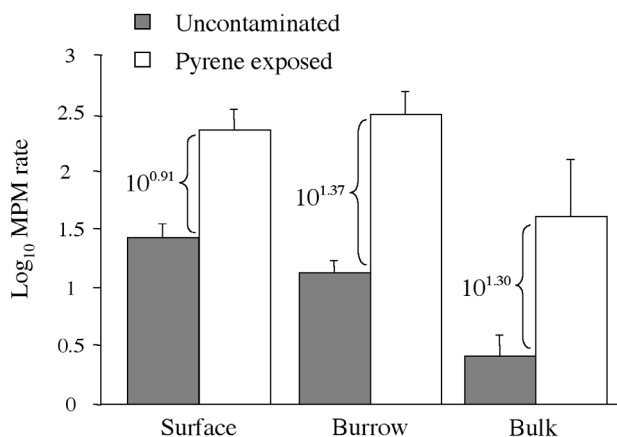

Fig. 2. Comparison of microbial pyrene-mineralization rates (MPM rates, g DW$_{sed}$$^{-1}$ d^{-1}) in different sediments compartments, i.e. surface, burrows, and bulk sediment either uncontaminated or exposed to pyrene. Faunal treatments are pooled. Error bars indicate + SD

Table 3. Microbial $^{14}CO_2$ production rates (10^{-3} g DW_{sed}^{-1} d^{-1}) per initially added ^{14}C-pyrene in sediment from the different treatments and sediment compartments. Means of 3 replicates ± SD are shown. –: no burrows present in treatments without fauna

| Carbon source | Fauna | Microbial $^{14}CO_2$ production rate (10^{-3} g DW_{sed}^{-1} d^{-1}) | | |
		Surface	Burrow	Bulk
Pyrene-exposed				
Tetraselmis sp.	No fauna	3.65 ± 1.20	–	0.23 ± 0.30[a]
Tetraselmis sp.	*Amphiura filiformis*	3.37 ± 0.23	3.27 ± 1.33[a]	0.41 ± 0.33
Tetraselmis sp.	*Nereis diversicolor*	2.38 ± 0.28	2.16 ± 0.14	0.83 ± 0.17
Lignin	No fauna	1.63 ± 0.50	–	0.36 ± 0.07[a]
Lignin	*A. filiformis*	1.51 ± 0.17	5.74 ± 2.45	0.23 ± 0.14
Lignin	*N. diversicolor*	1.72 ± 0.27	2.34 ± 0.24	1.50 ± 0.78
Uncontaminated				
Tetraselmis sp.	*A. filiformis*	0.30 ± 0.12	0.16 ± 0.03[a]	0.017 ± 0.001
Tetraselmis sp.	*N. diversicolor*	0.27 ± 0.02	0.15 ± 0.02	0.029 ± 0.002
Lignin	*A. filiformis*	0.24 ± 0.03	0.11 ± 0.03[a]	0.030 ± 0.002
Lignin	*N. diversicolor*	0.28 ± 0.08	0.12 ± 0.04	0.033 ± 0.02
[a]n = 2				

178, p < 0.001; Tukey p < 0.05 for all pairwise comparisons). In pyrene-exposed treatments, burrow and surface sediment MPM rates were equally high, while significantly lower rates were observed in the bulk sediment (ANOVA $F_{2,33}$ = 24.5, p < 0.001; Tukey p < 0.05). The strong effect of pyrene exposure called for a separate treatment of pyrene-exposed and uncontaminated data sets in subsequent statistical comparisons.

Furthermore, MPM rates in seawater-only controls nearly equaled zero (0.006×10^{-3} g DW_{sed}^{-1} d^{-1}) (Fig. 3A), indicating that the mineralization measured during the incubation phase almost exclusively originated from exposure sediments.

Visual observations confirmed an identical development of redox patterns (see Fig. 5) in pyrene-exposed and uncontaminated treatments. No difference was
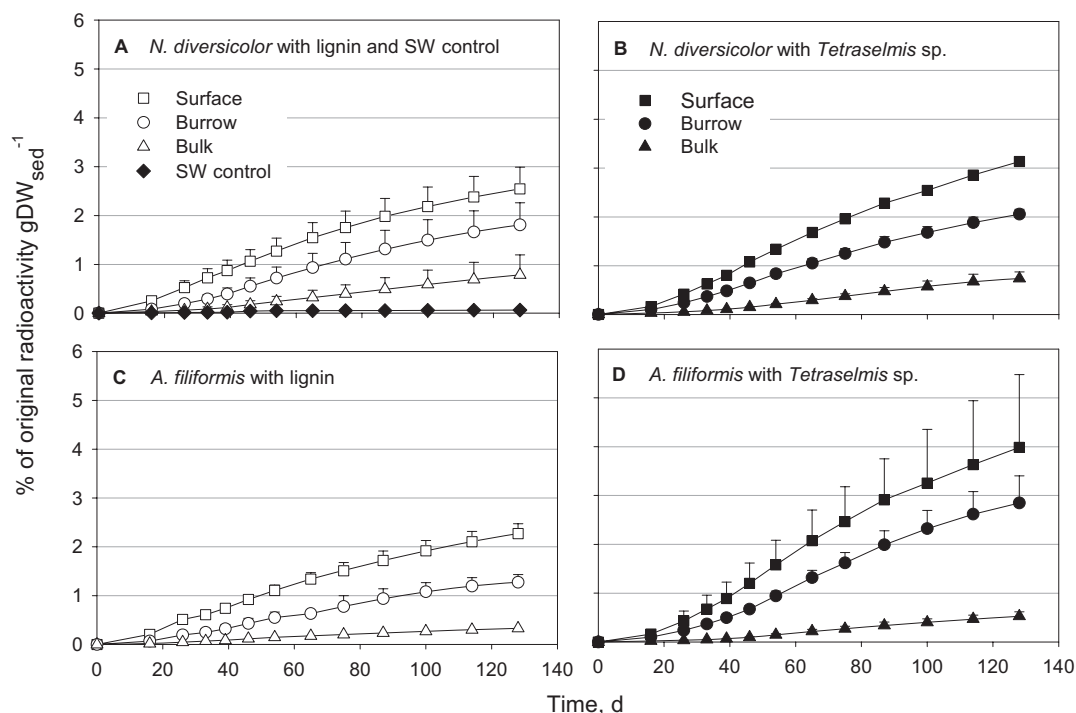

Fig. 3. Cumulative ^{14}C-pyrene-derived microbial $^{14}CO_2$ production from incubations of uncontaminated surface, burrow and bulk sediments. $^{14}CO_2$ production is expressed as % g DW_{sed}^{-1}, i.e. the isotopic activity of the produced ^{14}C-pyrene-derived $^{14}CO_2$ divided by the isotopic activity of the ^{14}C-pyrene originally added and the amount of sediment (g DW) in each incubation flask. Each point represents 3 replicates and error bars indicate +SD

observed in macrofaunal behavior or mortality between uncontaminated and pyrene-exposed treatment. All brittle stars originally added to experimental jars were recovered at the end of the exposure phase, while single polychaete worms were lost, i.e. escaped from or died in single exposure phase jars.

Surface sediment

Microbial pyrene-mineralization rates ranged from 0.24×10^{-3} to 0.30×10^{-3} g DW_{sed}^{-1} d^{-1} in uncontaminated surface sediment, with no effect of either fauna or organic matter quality (ANOVA $F_{1,8} = 0.07$ and 0.27, p >> 0.05) (Table 3). In pyrene-exposed sediment, organic matter quality had a significant effect on MPM rates, with higher mineralization rates measured in sediments enriched with *Tetraselmis* sp. than in sediment enriched with lignin (ANOVA $F_{1,12} = 42.05$, p < 0.001). The MPM rates ranged between 1.51×10^{-3} g DW_{sed}^{-1} d^{-1} in sediment exposed to pyrene/lignin/*Amphiura filiformis*, and 3.65×10^{-3} g DW_{sed}^{-1} d^{-1} in sediment exposed to pyrene/*Tetraselmis* sp./no fauna (Table 3), and were independent of the presence of fauna or faunal species (ANOVA $F_{2,12} = 0.804$, p >> 0.05).

Burrow sediment

Microbial pyrene-mineralization rates in uncontaminated burrow sediments were approximately half of the MPM rates measured in surface sediments within each treatment. There was a significant effect of organic matter quality, with higher MPM rates measured in sediments enriched with *Tetraselmis* sp. than in those enriched with lignin (ANOVA $F_{1,8} = 6.95$, p = 0.03) (Table 3). There was no effect of macrofaunal species on the MPM rates in uncontaminated sediments (ANOVA $F_{1,8} = 0.171$, p >> 0.05). When exposed to pyrene, however, burrow sediment MPM rates showed a significant effect of fauna (ANOVA $F_{1,8} = 12.24$, p = 0.008) with MPM rates 1.5 to 2.5 times higher in *Amphiura filiformis* burrow sediment than in sediment from *Nereis diversicolor* burrows (Table 3 & Fig. 4C–F). No effect was observed of organic matter quality (ANOVA $F_{1,8} = 2.92$, p >> 0.05) in pyrene-exposed burrow sediments. The highest MPM rates observed in the entire experiment were measured in pyrene-exposed sediment, enriched with lignin, from burrows made by *A. filiformis* (pyrene/lignin/*A. filiformis*) (Table 3, Fig. 4E). The average MPM rate in these burrows (5.74×10^{-3} g DW_{sed}^{-1} d^{-1}) was 3.8 times higher than the average rate measured in surface sediment (1.51×10^{-3} g DW_{sed}^{-1} d^{-1}) within the same treat-

ment. High MPM rates were further accompanied by greater variances (Table 3, Fig. 4E). The greater variance was a pattern more frequently observed in pyrene-exposed burrow sediment of *A. filiformis* than of *N. diversicolor*, and seemed independent of organic matter quality (Fig. 4C–F).

Bulk sediment

Microbial pyrene-mineralization rates in bulk sediment were generally an order of magnitude lower than MPM rates in surface and burrow sediments. Uncontaminated bulk sediment showed the lowest sedimentary MPM rates measured in the experiment, ranging between 0.017×10^{-3} and 0.033×10^{-3} g DW_{sed}^{-1} d^{-1}, with no significant effect of either fauna or organic matter quality (ANOVA $F_{1,8} = 1.3$ and 1.1, p >> 0.05) (Table 3, Fig. 3). Fauna was, however, the governing factor in pyrene-exposed sediment, with higher MPM rates measured in sediment with *Nereis* diversicolor compared to treatments with both *Amphiura filiformis* and no fauna (ANOVA $F_{2,12} = 5.8$, p = 0.017, Tukey p < 0.05) (Table 3, Fig. 4). In fact, the highest average bulk sediment MPM rate measured in the experiment (1.50×10^{-3} g DW_{sed}^{-1} d^{-1}) occurred with *N. diversicolor* in pyrene-exposed, lignin-enriched sediment, almost equalling the average surface sediment MPM rate (1.72×10^{-3} g DW_{sed}^{-1} d^{-1}) within the same treatment (Table 3, Fig. 4C).

Flask budget

The flask budget for added radioactivity was performed by extracting ^{14}C-pyrene-derived radioactivity from the sediment, the water (as $PO^{14}C$, $DO^{14}C$ and $^{14}CO_2$), from glass walls, plastic traps, and as total $^{14}CO_2$ collected during the incubation phase. On average, most of the recovered radioactivity (64%, SD ± 29) was found still remaining in the sediment, while 32% (SD ± 30) was recovered as $^{14}CO_2$ in the traps. A small radioactive fraction was found in the water phase, mainly associated with $DO^{14}C$ (2.3%, SD ± 1.7). On the inner glass walls of the flasks and on plastic trap vials, respectively, 0.14% (SD ± 0.08) and 0.42% (SD ± 0.88) of the total radioactivity was found. The average total amount of ^{14}C-pyrene-derived radioactivity recovered from all partial extractions together ranged from 30 to 86% with an average of 52% (SD ± 15) of the initially added radioactivity. One replicate from surface sediment exposed to pyrene enriched with *Tetraselmis* sp. and without fauna was rejected due to methodological and statistical classification as an outlier (Barnett & Lewis 1984).
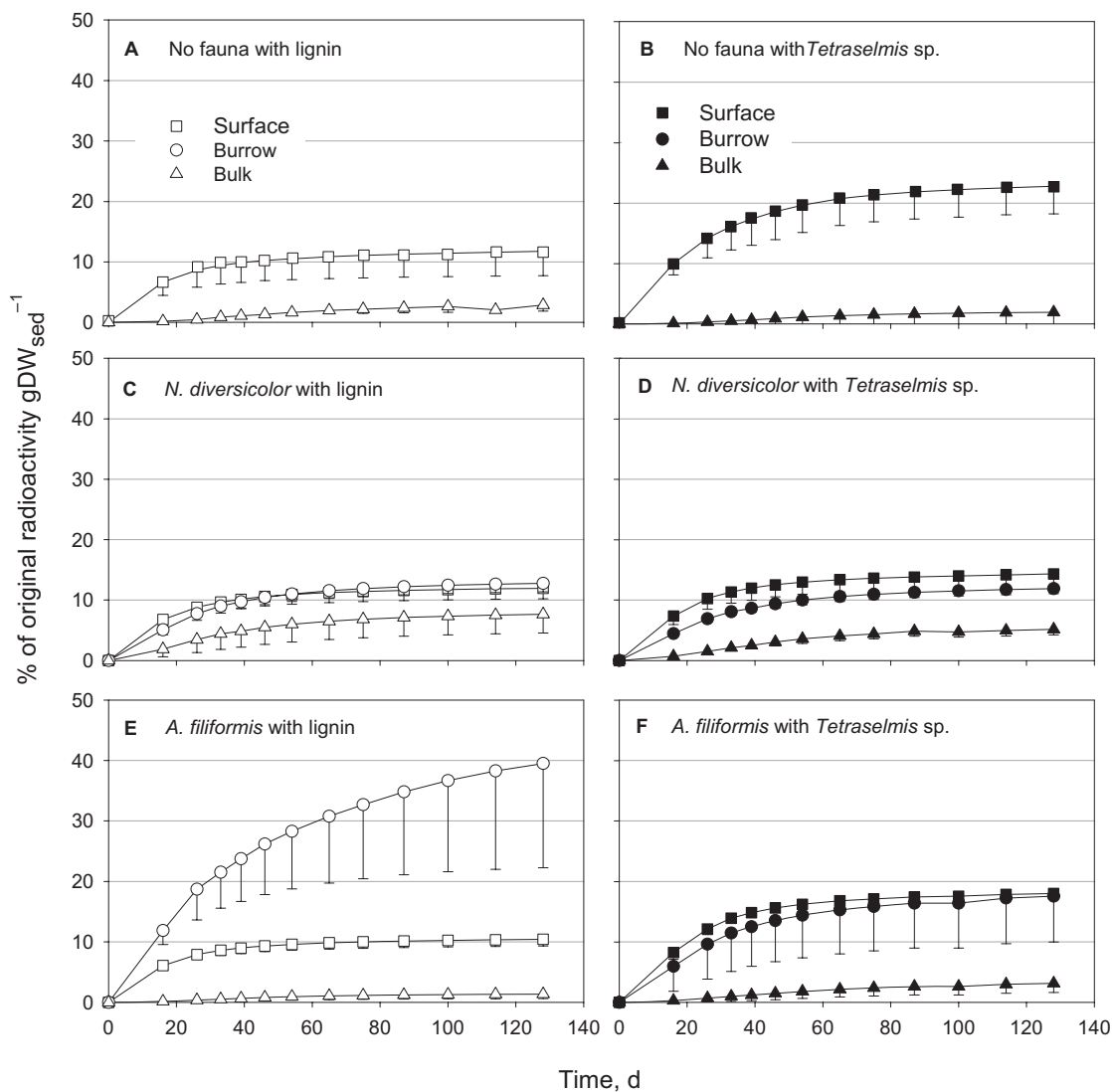
Fig. 4. Cumulative ^{14}C-pyrene derived microbial $^{14}CO_2$ production from incubations of pyrene-exposed surface, burrow and bulk sediments. $^{14}CO_2$ production is expressed as % g DW_{sed}^{-1}, i.e. the isotopic activity of the produced ^{14}C-pyrene-derived $^{14}CO_2$ divided by the isotopic activity of the ^{14}C-pyrene originally added and the amount of sediment (g DW) in each incubation flask. Each point represents 3 replicates and error bars indicate –SD

Trapping efficiency of $^{14}CO_2$

The retentive effect of sediment on the release of produced $^{14}CO_2$ into the air-filled headspace appeared to increase with the amount of sediment present in the incubation flasks. Extraction/release efficiencies decreased seemingly linearly from 95% (SD ± 2.5) for 0.5 g DW sediment to 78% (SD ± 2.4) for 4 g DW sediment. Linear regression was performed to produce the sediment weight correction formula for $^{14}CO_2$ trap data, $f(x) = -4.95x + 97.4$, $r^2 = 0.866$, in order to achieve proper comparisons among replicates and treatments. Furthermore, no early breakdown of $NaH^{14}CO_3$ due to insufficient sterilization procedures was detected.

DISCUSSION

Exposure to pyrene had a very strong effect, enhancing microbial pyrene-mineralization (MPM) rates in all compartments and treatments, a response which previously has been described for other PAHs in various systems (Bauer & Capone 1988, Cerniglia 1992, Coates et al. 1996). However, more intriguing was how pyrene exposure generated mineralization patterns, influenced by macrofauna and organic matter quality, which were absent in uncontaminated treatments (Table 4). The strong response in pyrene-exposed treatments likely resulted from a microbial community succession or from the induction of appropriate meta-

bolic pathways in microbes already present. MPM rates were also higher in sediments with readily available oxygen as illustrated by the successive decrease in mineralization rates from surface and burrow to bulk sediments (Fig. 2). These results are consistent with the established view of microbial PAH degradation being highly favored by the presence of molecular oxygen (Cerniglia & Heitkamp 1989), and also with results from Chung & King (1999) and Coates et al. (1996) who found no correlation between PAH mineralization and the availability of other electron acceptors such as nitrate, sulfate and ferric iron in moderately polluted field-collected sediment.

Microbial biodegraders and their requirements

Ring fission, i.e. the opening of aromatic molecular moieties, is required in order for pyrene and other PAHs to be mineralized, i.e. converted into CO_2. Various microbes are capable of performing ring fission, which involves initial incorporation of oxygen into the pollutant molecules facilitated by oxygenase or peroxidase enzymes. The main actors in environmental PAH-mineralization are considered to be certain bacteria which produce prokaryote-specific dioxygenases which efficiently catalyze ring fission, allowing the use of PAHs as their primary carbon source (Cerniglia 1993). Eukaryotes, in general, do not mineralize PAHs, but instead they metabolize these compounds to hydroxylated metabolites by using monooxygenase systems such as the detoxifying cytochrome P450 system (CYP), whereupon metabolites are excreted directly or as conjugates with more polar molecules (Hammel 1995). Nevertheless, some eukaryotes such as the microalgal species *Selenastrum capricornutum* and fungi belonging to the white-rot family (e.g.

Phanerochaete chrysosporium and *Pleurotus* sp.) are capable of complete PAH mineralization, as they produce prokaryote-like dioxygenases or other unique oxidative enzymes (Bumpus et al. 1985, Hammel 1989, Warshawsky et al. 1995, Wolter et al. 1997). The white-rot fungi are primarily specialized in the degradation of lignins, and ligninolysis involves extra-cellular release of a battery of highly nonspecific, oxidative enzymes such as ligninperoxidase (LIP), manganese-dependent peroxidase (MnP) and laccase (Tien & Kirk 1983). Due to the non-specificity of these enzymes, other lignin-like molecules, such as PAHs, can be attacked and degraded as well (Bumpus et al. 1985, Reddy 1995). Several marine fungi display ligninolytic activity and have been shown to possess lignin-modifying enzymes similar to those of terrestrial white-rot fungi (Raghukumar et al. 1994, Pointing et al. 1998).

Nutrient and substrate availability regulate microbial activity in very specific ways. Bacterial PAH mineralization depends on the relative lability of other carbon sources present, as well as on the versatility of the existing bacterial community. Keyser et al. (1978) reported that the presence of lignin itself does not induce fungal ligninolysis. It is, therefore, reasonable to assume that ligninolysis is not induced by the presence of PAHs either, which is contradictory to the inducible PAH mineralization observed in bacteria. Microbes require supplementary nutrients for the mineralization of carbon, and nitrogen has in particular been proven vital for efficient bacterial PAH mineralization (ZoBell 1946, Atlas 1981). Nitrogen also plays a pivotal role in the regulation of white-rot fungal metabolism, but in this case, the expression of ligninolytic enzymes is suppressed by nitrogen enrichment and stimulated by nitrogen deficiency (Kirk et al. 1978). By considering the specific nutritional constraints, oxygen requirements and metabolic abilities of different microbes in relation to our experimental exposure conditions, we may interpret the observed MPMs and suggest organisms likely to be responsible for them.

Surface sediment

Oxygen was always present in the uppermost surface sediment of all treatments (Fig. 5, light-colored surface layer) during the experimental exposure phase, as fresh seawater was continuously provided by the flow through system. In pyrene-exposed surface sediments, enrichment with *Tetraselmis* sp. resulted in almost a

Table 4. Summary of factors significantly affecting microbial pyrene-mineralization (MPM) rates in surface, burrow and bulk sediments derived from 2-factor ANOVAs with sediment organic matter (SOM) quality (Carbon source) and macrofauna (Fauna) as fixed factors ($^*p < 0.05$, $^{**}p < 0.001$). The organic enrichment type or macrofaunal species inducing the highest MPM rate is displayed beneath the significant factor. Indicated in parentheses is the level of the non-significant factor with which the significant factor induced the highest MPM rate

Sediment compartment	Uncontaminated	Pyrene-exposed
Surface	**No significant effect**	**Carbon source**** *Tetraselmis* sp. (no fauna)
Burrow	**Carbon source*** *Tetraselmis* sp.	**Fauna*** *Amphiura filiformis* (lignin)
Bulk	**No significant effect**	**Fauna*** *Nereis diversicolor* (lignin)

Lignin

Tetraselmis sp.

No fauna

oxic surface layer

A

B

anoxic sub-surface layer

Nereis diversicolor

burrow gallery

C

D

Amphiura filiformis

burrow

E disk
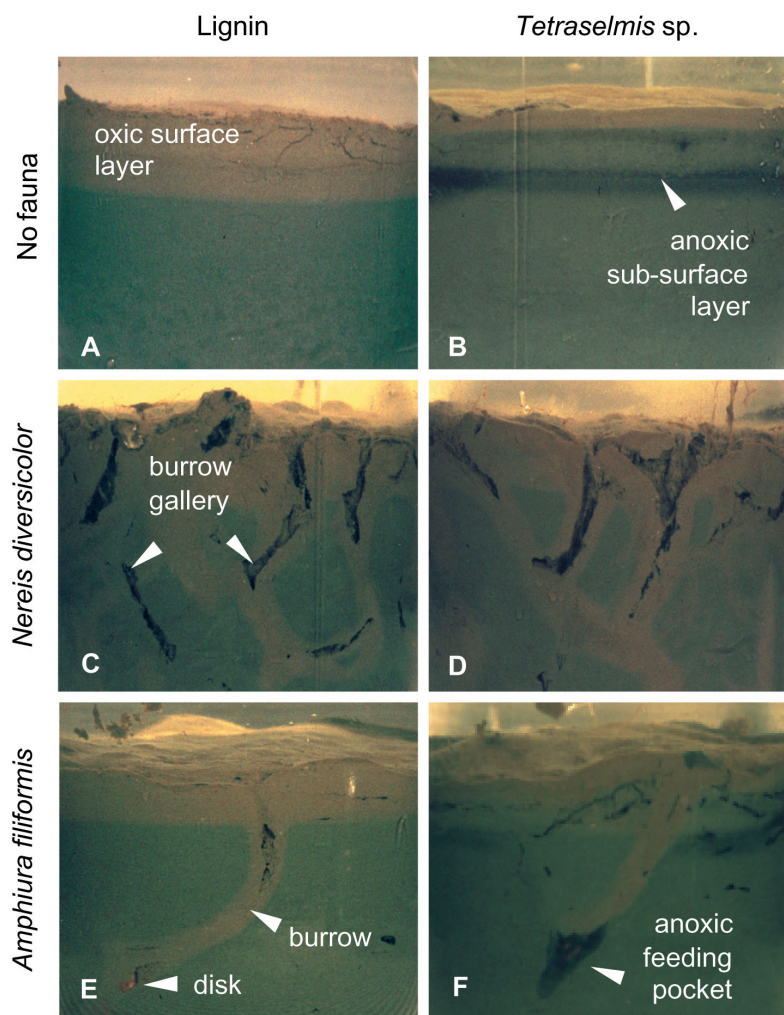
F

anoxic feeding pocket

Fig. 5. Sediment images of the different treatments taken at the end of the exposure phase

doubling of the MPM rates compared to enrichment with lignin (Table 3). As mineralization rates increased with the addition of a carbon source high in available nitrogen, bacteria are probably the main pyrene degraders, responding with enhanced pyrene mineralization to the supply of a limiting element (nitrogen). The effect of organic matter quality on MPM rates may also be interpreted in terms of contaminant bioavailability. The fraction of pyrene available for intracellular microbial mineralization may decrease due to a stronger association with refractory organic matter like lignin, compared to labile organic matter like *Tetraselmis* sp. This theory has been verified by Xing (1997), who measured increased sorption of the 2-ringed PAH naphtalene to soil organic matter as aromaticity increased, i.e. with decreasing SOM quality. Faunal presence further appeared to have suppressed MPM rates in pyrene-exposed surface

sediment enriched with *Tetraselmis* sp. (Fig. 4B,D,F). Subduction of contaminants associated with surface sediment through macrofaunal bioturbation is frequently observed in experimental systems, and has been proposed to hamper the faster aerobic microbial degradation of contaminants (Gilbert et al. 1996, Kure & Forbes 1997, Hansen et al. 1999). Resuspension, subduction and macrofaunal consumption of pyrene-contaminated organic particles may have resulted in lower exposure concentrations of both pyrene and organic matter in surface sediment with time, where macrofauna was present, thus reducing MPM rates. High SOM quality may further have stimulated surface deposit feeding, which would explain the absence of faunal impact on MPM rates in lignin-enriched treatments where the SOM quality was low (Fig. 4A,C,E). The lack of impact of either fauna or organic matter enrichment on MPM rates in uncontaminated surface sediment suggests that most microbes were probably using carbon sources other than pyrene, or that the majority of microbes present were lacking the ability to mineralize polyaromatic compounds. Although not mineralized, degradation of pyrene may still have occurred through the production of CYP metabolites, which were not monitored in this experiment. Association of metabolites with decaying organic matter may further hamper subsequent mineralization of pyrene metabolites, a process reported to be particularly pronounced in microbially-active, previously uncontaminated soils (Guthrie & Pfaender 1998).

Burrow sediment

Intriguingly, microbial communities inhabiting sediment from *Amphiura filiformis* and *Nereis diversicolor* burrows contributed significantly to the overall biodegradation of sedimentary pyrene. Similar observations have to our knowledge only previously been made by Chung & King (1999), who measured the microbial degradation potential of various other PAHs in field-collected burrow sediment of 4 marine macroinvertebrates. Selck et al. (2004) do, however, also emphasize the importance of burrows for contaminant degradation, when reporting greater amounts of fluo-

ranthene metabolites relative to the parent compound in *A. filiformis* burrow linings compared to surface sediment. It is important to bear in mind that pyrene and organic matter was only added to the surface sediment in our experiment. Consequently, in order for microbes in burrows and deeper parts of the sediment to react to surface conditions, they are entirely dependent on pyrene and organic matter being transferred to them from the surface sediment. Pyrene, with an octanol-water equilibrium partitioning constant (log K_{ow}) of between 4.50 and 5.32 (Meador et al. 1995) has a high affinity for particles, organic particles in particular, and through adsorption, particles and organic matter become vectors for pyrene transport orchestrated by macrofaunal bioturbation. In the pyrene-exposed treatments, MPM rates were dependent on macrofaunal species, with mineralization rates in *A. filiformis* burrows 1.5 to 2.5 times greater than rates measured in *N. diversicolor* burrows. *N. diversicolor* build large burrow galleries, while *A. filiformis* construct only as much burrow as needed for keeping their bodies buried at ca. 5 cm depth with 2 to 4 arms protruding from the sediment for feeding (Woodley 1975, Davey 1994). *A. filiformis* switch between filter and surface deposit feeding, and selected food particles are passed along the arms towards the mouth, generating a continuous downward flux of particles captured in the water and on the sediment surface (Woodley 1975). The brittle star is, thus, adapted for a buried life style only rarely leaving its burrow. *N. diversicolor* are omnivorous polychaetes, sometimes filter feeding within the burrow, and occasionally ascending for epibenthic feeding on everything from living prey to detritus (Fauchald & Jumars 1979). Even though downward particle transport occurs in sediment inhabited by *N. diversicolor* (Francois et al. 2002), the process is a result of burrowing and moving rather than of an essential feeding act as with *A. filiformis*. *A. filiformis* has further been shown to concentrate particle reactive contaminants such as fluoranthene and tetrachlorobiphenyl (TCB) in the burrow sediment, with TCB concentrations an order of magnitude higher in burrows compared to the surrounding bulk sediment (Gunnarsson et al. 1999a, Selck et al. 2004). *N. diversicolor* have been shown to do the same, however, to a lesser extent (Gunnarsson et al. 1999b). The lower TCB concentrations observed in *N. diversicolor* burrows may result from contaminant dilution as the wall surface area of a single *N. diversicolor* burrow is several times larger than that of a single *A. filiformis* burrow. A similar bioturbation-dependent distribution of pyrene into burrow sediment, resulting in differential pyrene exposure to microbial communities residing within the burrows, may well explain the species-specific differences in MPM rates that we observed in *N. diversicolor* and *A. filiformis* burrows.

Macrofaunal burrows in both aquatic and terrestrial systems appear to harbor distinct microbial communities, often greater in terms of biomass, diversity or activity, as compared to non-burrow soil and sediment (Dobbs & Guckert 1988, Tiunov & Scheu 1999, Bird et al. 2000). Microbial communities collected in marine macrofaunal burrows have also been reported to be highly species-specific (Steward et al. 1996). Further, Chung & King (2001) discovered 2 previously unidentified marine bacterial species residing in burrow-wall sediment of the polychaete *Nereis virens* and the mussel *Mya arenaria*. Both bacterial species were able to use PAHs as their sole carbon source, but with different efficiency. A similar scenario applied to our study provides a plausible explanation for the difference in MPM rates observed in burrow sediments from the 2 macrofaunal species.

The highest MPM rates observed in the entire experiment were measured in pyrene-exposed sediment enriched with lignin from *Amphiura filiformis* burrows, where rates were almost double compared to enrichments with *Tetraselmis* sp. and 3.8 times greater than in surface sediment of the same treatment. Oxygen availability in burrows is limited in contrast to surface sediment and therefore, oxygen-dependent PAH-degrading microbes inhabiting burrows become highly dependent on the intermittent irrigation activity of macrofauna and the reactivity of the SOM. During the experimental exposure phase, enough time was provided for specific redox conditions to develop (Fig. 5). The lability of the SOM affects sedimentary oxygen conditions as labile *Tetraselmis* sp. is degraded much faster than refractory lignin, thus consuming oxygen at a higher rate than refractory lignin. Visual observations revealed a thin black layer of reduced sediment situated at the interface between bulk and top sediment at ca. 1 cm depth when enriched with the labile carbon source *Tetraselmis* sp. in treatments with *A. filiformis* and in controls without macrofauna (Fig. 5B,F). In addition, a large reduced zone appeared associated with the disk chamber in the *Tretraselmis* sp.-enriched *A. filiformis* treatments (Fig. 5F). The observed redox patterns are supported by measurements by Vopel et al. (2003), who reported a decrease in oxygen saturation with depth in *A. filiformis* burrows, ranging from between 55 and 95% in arm channels to 40 and 44% in disk chambers. Disk-associated anoxia may well have been fuelled by a downward transport of labile organic matter (*Tetraselmis* sp.) from the sediment surface during feeding, as well as by the accumulation of partly digested reactive organic matter in feeding pockets around the disk as a result of the ruminating behavior of *A. filiformis* (M. E. Granberg

pers. obs.) where food items are repeatedly egested and reingested. Visible anoxia did not appear in any of the organic enrichments with *Nereis diversicolor*, which evidently succeed in aerating their entire burrow galleries in addition to the subsurface sediment (Fig. 5C,D). Fenchel (1996) measured oxygen saturations ranging between 50 and 80% in the lumen of small *N. diversicolor* burrows, 0.5 to 1.5 cm below the sediment surface in field-collected cores. The epibenthic feeding and defecation behavior of *N. diversicolor* should also counteract the subduction of organic matter and subsequent development of burrow sediment and subsurface anoxia. A light oxic coloration of the surface sediment and sediment adjacent to biogenic structures evolved in all treatments. Visible anoxia did not develop in any of the treatments enriched with the refractory carbon source lignin (Fig. 5A,C,E). Consequently, *Tetraselmis* sp. addition should probably have hampered microbial pyrene mineralization by creating partial oxygen deficiency where oxygen conditions were already strained, as was the case in *A. filiformis* burrows. Enrichment with *Tetraselmis* sp. also entails addition of highly available nitrogen. If white-rot fungi were active pyrene degraders in *A. filiformis* burrows, they probably reacted to the nitrogen addition by switching off their ligninolytic pyrene mineralizing enzyme system. With lignin enrichment, the burrow sediment matrix should successively become diluted with the refractory, nitrogen-poor carbon source, in turn triggering fungal ligninolytic enzyme activity in favor of pyrene mineralization. As opposed to *N. diversicolor*, that displays several different feeding modes, the monotonous subductive feeding behavior of *A. filiformis* should specifically support SOM-mediated differences in microbial pyrene mineralization in the burrows. MPM rates in uncontaminated burrow sediment were controlled by organic matter quality only (Table 4), with the highest rates observed in enrichments with *Tetraselmis* sp. (Fig. 3B,D). As mineralization rates were comparably low (Table 3), these differences may be of limited ecological significance. Nevertheless, it indicates that microbes in uncontaminated sediment and sediment with previous experience of pyrene contamination differ greatly in metabolic abilities. In uncontaminated sediments, microbial PAH-specialists or metabolic PAH-mineralizing pathways are probably not being induced, yet other microbes and metabolic pathways should be highly active as can be concluded from the development of sedimentary redox patterns (Fig. 5). When uncontaminated sediments eventually encountered pyrene in the incubation flasks, the labile *Tetraselmis* sp. probably supported both higher available nitrogen levels and greater

contaminant bioavailability compared to lignin. This should promote dormant PAH-degrading bacteria or inducible PAH-mineralization pathways to a greater extent.

Bulk sediment

Microbial pyrene mineralization rates in pyrene-exposed bulk sediments were low but measurable, while MPM rates in uncontaminated bulk sediment were almost as low as rates measured in water-only controls. Anaerobic mineralization of PAHs (nitrate- and sulfate-reducing conditions) is generally regarded as slow or incomplete compared to aerobic mineralization (Cerniglia 1993, Rockne & Strand 1998). Complete and highly efficient mineralization of several high and low molecular weight PAHs has, however, recently been observed under strict sulfate-reducing conditions in marine sediment collected from extreme sites with a history of heavy PAH contamination (Coates et al. 1997, Rothermich et al. 2002). Contaminant exposure, thus, also appears critical for microbial mineralization processes in anaerobic environments. As all incubations in our experiment, including those of bulk sediments, were carried out under oxic conditions, the main portion of the registered activity should, however, originate from aerobic microbes, in sediment bearing traces of exposure phase conditions. We, thus, register a 'priming' effect that fauna and organic matter enrichment exerts on oxic microbial pyrene mineralization rather than a true anoxic microbial mineralization in bulk sediment. The significant effect of fauna on bulk sediment MPM rates measured in the pyrene-exposed treatments, particularly pronounced with *N. diversicolor*, emphasizes the importance of bioturbation. The extensive burrow system of *N. diversicolor* along with continuous reconstruction and rerouting probably causes higher transport of contaminated oxic surface sediment along with aerobic microbes into anoxic bulk sediment compared to the smaller burrow systems and less vigorous movements of *A. filiformis*. In 2 experiments similar in design, Gunnarsson et al. (1999b) measured elevated TCB concentrations in deeper sediment layers as a result of bioturbation with both *N. diversicolor* and *A. filiformis*. In sediment inhabited by *A. filiformis*, TCB concentrations in burrows were almost an order of magnitude higher than that found in bulk sediment, while TCB concentrations in burrow sediment and bulk sediment were equally high with *N. diversicolor*. Analogously, such bioturbation-dependent distribution of pyrene would lead to elevated exposure concentrations of pyrene along with higher numbers of aerobic pyrene-mineralizing microbes in inhabited reduced bulk sediment, particu-

larly pronounced with *N. diversicolor*, thus explaining the higher MPM rates we observed in our incubations.

Experimental considerations: flask budget

When summarizing the extracted radioactivity from all parts of the incubation flask system, the average total recovery was 52% (SD ± 15%). The possibility of $^{14}CO_2$ escaping the incubation flasks when released from the sediment is strongly contradicted by the 95% trapping efficiency obtained when testing the $^{14}CO_2$ incubation systems with $Na_2^{14}CO_3$ as described above. The total recovery values showed no correlation with the MPM rates but were significantly correlated with the sediment radioactivity values (Pearson correlation, $r^2 = 0.764$, $p \ll 0.001$). Due to the lack of correlation between total recovery and MPM rates, $^{14}CO_2$ values can be safely interpreted. Total recovery values were also shown to be significantly dependent on the quality of the sedimentary organic matter, with lower radioactivity constantly obtained from lignin-enriched systems in both pyrene-exposed (2-way ANOVA $F_{1,30} = 35.3$, $p < 0.001$) and uncontaminated treatments (2-way ANOVA $F_{1,30} = 27.7$, $p < 0.001$). Most likely, the extraction efficiency of pyrene and metabolites from the sediment is dependent on the SOM quality in that pyrene associates more strongly with the refractory lignin compared to the labile *Tetraselmis* sp., as has been discussed previously in relation to bioavailability (Xing 1997). However, the most likely explanation for the low total recovery values is related to a methodological problem. At the termination of the incubation phase, the flask sediments were oven-dried at 105°C. Since pyrene has a melting point of only 150°C, the drying process may unfortunately have stimulated escape or combustion of the contaminant prior to a radioactive quantification of the sediment, thus giving erroneously low sediment radioactivity values.

CONCLUSIONS

Along with surface sediment, macrofaunal burrows are to be regarded as important sites for sedimentary pyrene degradation. Macrofauna, thus, actually increase the 'functional surface' of the sediment in terms of PAH degradation through their sedimentary constructions, extending the inter-phase between sediment and water. Yet, burrow-associated PAH mineralization appears to be highly species-specific and also dependent on the trophic state of the system, which complicates a direct translation between burrow and surface sediment function. Consequently, in order not to miscalculate fate and effects of PAHs in marine

sediment systems, the present focus on whole sediment or 2-compartment (surface and bulk) models needs to change towards considering biogenic structures as well. Furthermore, as SOM quality and contaminant fate appear so tightly coupled, both geochemically and through organism identity and activity, changes in organic matter input (i.e. due to seasonal phytoplankton blooms or eutrophication) should be taken into account when contaminant fate and effects are assessed in marine systems as a whole.

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