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ORIGINAL ARTICLE

Meiofauna reduces bacterial mineralization of naphthalene in marine sediment

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The role of sediment-living meiofauna, benthic invertebrates smaller than 1000 μm such as nematodes and ostracods, on the mineralization of naphthalene, a common polycyclic aromatic hydrocarbon (PAH) in marine sediments, was studied in microcosms using radiorespirometry. A method to extract live meiofauna was developed and used in order to experimentally manipulate meiofauna abundance and group diversity. Higher abundances of meiofauna were found to significantly decrease naphthalene mineralization. Furthermore, a change in the bacterial community composition (studied using terminal restriction fragment length polymorphism) was also observed in presence of higher meiofauna abundance, as well as a lower number of cultivable naphthalene-degrading bacteria. The reduced mineralization of naphthalene and the altered bacterial community composition in the presence of increased meiofauna abundance is likely the result of top-down control by meiofauna. This study shows that higher abundances of meiofauna can significantly decrease the microbial mineralization of PAHs such as naphthalene and also significantly modify the bacterial community composition in natural marine sediments.
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Introduction

Biodegradation of anthropogenic contaminants, such as polycyclic aromatic hydrocarbons (PAHs), is a crucial ecosystem service achieved mainly through the action of microorganisms. The aerobic microbial transformation pathways of PAHs with less than five aromatic rings have been intensely studied, mostly using isolated cultures (Peng *et al.*, 2008). The degradation rates of PAHs in sediment can be influenced by several environmental variables, such as temperature, O_2 level, redox state and sediment grain size (Delaune *et al.*, 1981; Hinga, 2003; Lei *et al.*, 2005). Not much is known about how PAH-degrading microorganisms interact with other sediment-living organisms under natural conditions. In comparison, mineralization of various types of organic matter by microbial communities is well studied and is known to increase in the presence of microbial grazers such as ciliates and macrofauna (Fenchel and Harrison, 1976; Gerlach, 1978; Wieltschnig *et al.*, 2008). Several mechanisms have been proposed to explain the positive effect of grazing on bacteria, for example, an increased nutrient turnover to bacteria, which allows a higher heterotrophic bacterial growth, or by preventing bacterial stasis, which helps sustain an exponential growth. It is also known from soil studies that predation pressure can prevent successful colonization of inoculated bacteria (van Veen *et al.*, 1997). Predation effects on natural populations of pollutant-degrading bacteria, however, are relatively poorly studied considering their potential importance. The role of meiofauna, such as nematodes, copepods and ostracods, on bacterial PAH degraders in sediments has, to our knowledge, not been studied previously. Top-down effects from meiofauna on bacterial PAH degraders are possible both directly through predation, and/or indirectly through trophic cascade interactions in the microbial food web.

Sediment-living organisms are often grouped on the basis of their respective size, coupled to a general increase in trophic position with increasing size. Rough size categories can be summarized as bacteria (<2 μm), heterotrophic flagellates (2 to >50 μm), ciliates (10 to >300 μm), meiofauna (40–1000 μm, metazoans) and macrofauna (>1 mm) (Leadbeater and Green, 2000; Lynn, 2008; Giere, 2009). Exceptions to the size categories exist, as several groups have a wide size range, for example, some ciliates can be over 3000 μm long (Giere, 2009), in addition to many species being omnivores

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(Leadbeater and Green, 2000), enabling a shift in trophic position depending on food availability. However, the groups mentioned above are useful as a generalized descriptor of the major organism groups of the benthic food web.

Trophic cascade chains are known in the pelagic environment, where high abundances of copepods decrease the number of ciliates, leading to an increased abundance of flagellates and resulting in a lower abundance but a higher diversity of bacterioplankton (Zöllner *et al*., 2009). Ciliate grazing has previously been observed to increase bacterial mineralization of naphthalene (Tso and Taghon, 2006) and it is possible that benthic meiofauna also may affect mineralization. This could occur both directly (through bacterial grazing) and indirectly (for example, through predation on protozoa or by altered biogeochemistry through burrowing activities). Thus, meiofauna may be an important regulating factor for the microbial mineralization of biodegradable organic contaminants such as PAHs.

The aim of this study was to investigate the role of meiofauna on PAH mineralization using radiolabelled [1-^{14}C]-naphthalene as a model compound and by experimentally manipulating meiofauna abundance, using a density extraction method. We also included a natural control treatment (that is, with macrofauna and unsieved sediment) in order to be able to compare our manipulated treatments to an undisturbed sediment system, and possibly identify experimental biases. The main aim of the study was to investigate the significance of meiofauna for bacterial community structure and function (in the form of PAH mineralization) in marine sediments.

Materials and methods

Sediment collection

Surface sediment was collected on the 23 May 2008, from a depth of 28 m in Hållsviken (58° 50′N, 17° 32′ E), in the northern Baltic Proper using a box corer (area 0.04 m^2). Intact sediment cores were subsampled from the box cores using round Perspex tubes (inner area 17 cm^2), height 40 cm. Seawater (filtered through a sand filter and a 40 μm sieve) was collected from Askö Marine station at a depth of 20 m on the same day (salinity 6.0). The cores and water were immediately transported to the laboratory and placed in a room held at *in situ* conditions, that is, at a temperature of 5 ± 1 °C and a natural light/dark cycle with low-irradiance green light (<0.5 μEms^{-2}, predominantly 530 nm). The collected sediment was characterized as silty clay with a high porosity (0.81 ± 0.01, $n = 3$) and rich in organic matter (3.1 ± 0.0% TOC; 3.6 ± 0.0% TC; 0.5 ± 0.0% TN; with a C/N ratio of 7.8, $n = 3$). The density of the sediment was 1.2 ± 0.1 gcm^{-3}, $n = 3$. Content of C and N content was analyzed by a Leco-CHN 932 analyzer (St Joseph, MI, USA) according to Hedges and Stern (1984), using EDTA as standard.

Meiofauna extractions

Meiofauna abundances were manipulated by extracting the meiofauna from the top 6 cm of surface sediment from collected cores. The extractions of meiofauna were performed by sieving the sediment sequentially through 1000 and 40 μm sieves. The sediment retained on the 40 μm sieve containing meiofauna was gently wiped-off from exceeding water at the bottom of the sieve with tissue paper and put into a 0.74 M $MgCl_2$ bath for 5 min to anesthetize the meiofauna (Giere, 2009). The sieved sediment fraction was rinsed in seawater and put into a 500 ml Erlenmayer flask. Meiofauna was extracted using density extraction by LEVASIL 200A 40% (H.C. Starck SilicaSol GmbH, Leverkusen, Germany) diluted to a density of 1.3. The E-flask containing the LEVASIL/sediment suspension was inverted several times, and was left for 5 min, to let the sediment settle and the meiofauna float up. This is similar to the standard procedure used in meiobenthology (Giere, 2009) but LEVASIL is used instead of LUDOX (which is toxic to fauna). To increase the extraction efficiency for ostracods, the LEVASIL/sediment suspension was aerated using an airstone for 20 s before it was left to settle. This resulted in air bubbles attached onto the ostracod shells, thus increasing their buoyancy. Following the density gradient separation, approximately 100 ml of the suspension was decanted (the fraction containing floating meiofauna). It was sieved through a 40 μm sieve and rinsed thoroughly in seawater. The remaining fraction was re-processed twice using the same procedure (5 min and then 30 min time for the buoyancy separation) to further increase the extraction efficiency of meiofauna. The sediment left after the last extraction was rinsed extensively several times in seawater on a 40 μm sieve to remove LEVASIL residues and was re-added to the surface sediment in each of the 14 sliced cores, thus replacing the 6 cm removed top slice. Pooled meiofauna extracted from two sediment cores were added to the extracted sediment in 7 of these 14 cores (high meiofauna treatment, HM), and 7 cores were left with only the extracted sediment (low meiofauna treatment, LM). The extraction procedure, apart from removing meiofauna, also removed a part of the finer sediment particle fraction. In order to compensate for this loss, particles lost during sieving (<40 μm) were added back to both LM and HM treatments (1.7 ± 0.2 g d.w. suspended in seawater).

Experimental design

The experiment contained three treatments with seven replicates (sediment cores) per treatment ($N = 21$): (1) 'LM': low meiofauna abundance, that is,

sediment cores which had much of its meiofauna extracted; (2) 'HM': high meiofauna abundance, that is, sediment cores which first had its meiofauna extracted, whereupon meiofauna was re-added; (3) 'N': non-manipulated control cores, that is, non-sieved sediment with natural faunal densities. The LM and HM constitutes the two main experimental treatments in the experiment, and were treated exactly the same way, except for the addition of meiofauna in the HM treatment. The N treatment's main function was to provide an experimental control, and enable a comparison to more field-like conditions.

Experimental setup

Two days after the meiofauna extractions, the water columns of all 21 cores was replaced carefully with fresh seawater, whereupon 0.3 g Cm^{-2} of the microalgae *Thalassiosira weissflogii* (from a continuous culture grown in F/2 medium, Guillard (1975)) was added to the surface of each core to provide fresh food for the fauna. The cores were left to equilibrate for 4 weeks with gentle aeration in the water column to re-establish the sediment redox gradients within the LM and HM treatments.

Sediment spiking with ^{14}C-naphtalene

Sieved clay particles (<40 μm) in a sediment suspension, from the sediment collection, were autoclaved at 121 °C. A total of 1.85 Mbq of [1-^{14}C]-naphthalene (American Radiolabeled Chemicals, St Louis, MO, USA) with a specific activity of 1.998 MBqμmol^{-1} (dissolved in methanol) was added to 40 ml of autoclaved sediment in a 50 ml sterilized glass bottle. The sediment bottle was shaken for 1 day at 5 °C to homogenize the sediment suspension. A total of 12.1 ± 0.4 mg d.w. sediment ($n = 4$) (1 ml sediment suspension) containing 48.1 ± 1.85 kBq ($n = 4$) was added to the surface sediment of each replicate, using a 1 ml glass pipette (30 cm length). The amount of naphthalene added to each core was 3 μg per core, far below reported toxic concentrations to benthic fauna (Carman *et al.*, 1995; Lotufo, 1997). Our aim was not to simulate a highly polluted environment, such as those found in harbors, but rather a contamination level that might normally be found in a continental shelf. Total PAH concentrations in Baltic Sea surface sediment vary depending on location, but a typical range is 125–3624 ng/g d.w. (Pikkarainen, 2004). The system was left for 1 day in order to let the sediment settle, whereupon the aeration was turned on. The system was left to equilibrate for 1 more day, after which the mineralization measurements were started.

Naphthalene mineralization measurements

The top of the Perspex cores were sealed with air-tight cork stoppers, each with two holes, one to let air in for oxygen supply and one to let the exceeding air out. The outflowing air was led through poly-tetra-fluoroethylene tubing (ϕ: 1.1 mm) into 50 ml Falcon vials, containing Carbosorb (PerkinElmer, Waltham, MA, USA) in order to trap CO_2 in the outflowing air. One ml of Carbosorb was subsampled every day. The Carbosorb was replaced every other day. In addition to the 21 experimental replicates, two additional non-manipulated replicates were set up with polyurethane filters (placed between the sediment cores and Carbosorb) for the outflowing air in order to estimate naphthalene evaporation. The evaporation was very low (<1%) and was omitted from further calculations and analyses (similar evaporation rates were determined by Tso and Taghon (2006), who also used a higher ambient temperature, 16 °C). A schematic figure over the experimental system is presented in Figure 1, and a summary over the full experimental timeline is presented in Table 1.

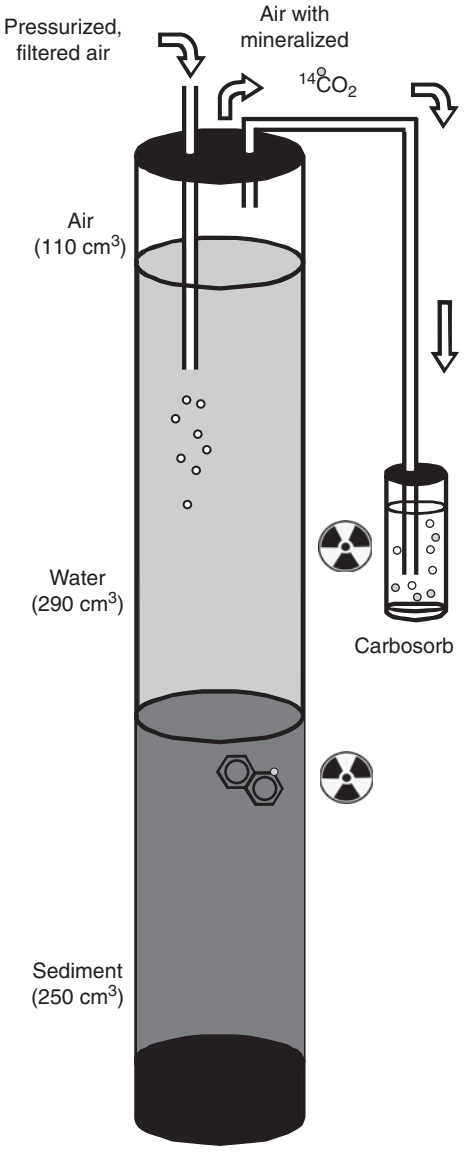

Figure 1 Schematic figure of the experimental system used in the experiment. Arrows indicate the direction of the airflow.

Table 1 A summary of the experimental timeline

Day	*Event*
−35	Collection in the field (sediment cores)
−28 to −26	Meiofauna extractions
−24	Water exchange and phytoplankton addition
−23	Aeration was turned on
−3	Spiking of silt/clay with ^{14}C-naphthalene
−2	Addition of spiked sediment
−2 to −1	Settling of spiked sediment
−1	Equilibration period
0	Start of mineralization measurements
1 to 11	Daily sampling of Carbosorb
11	Termination of experiment

Termination
The experiment was ended after 11 days, when the trapped $^{14}CO_2$, in some of the Carbosorb samples was approaching detection limits (that is, three times above the background radioactivity level). Water samples (10 ml) were taken from the water column to quantify the remaining ^{14}C in the water column. Sediment cores were subsampled with a cutoff syringe (diameter 5 mm, length 4 cm) and were sliced every cm for the measurement of ^{14}C depth profiles, that is, the amount of naphthalene and naphthalene-derived carbon buried in the sediment. The surface sediment layer (0–1 cm, and 1–4 cm) of each core was sliced off, subsamples from the 0–1 cm layer were taken for terminal restriction fragment length polymorphism (T-RFLP) analysis, see below (kept frozen at −80 °C until processed) and plate counts of naphthalene-degrading bacteria were determined. After subsampling, both sediment layers were sieved sequentially through 1000 and 40 μm sieves for macro- and meiofauna samples. The meiofauna sediment samples were preserved in 4% buffered formalin before extracting the meiofauna, using the method described above. Meiofauna was sorted and counted under a ×50 binocular stereomicroscope.

Radioactivity measurements
The radioactivity of samples was determined using an adapted protocol from Olafsson *et al.* (1999) using an LKB Wallac Rackbeta 1214 Liquid Scintillation Counter (Turku, Finland) and 20 ml glass scintillation vials. For counting Carbosorb samples, Permafluor E+ (PerkinElmer) was used, Ultima Gold (PerkinElmer) was used for the water, animal and sediment samples. Sediment and animal samples were dissolved in 1 ml Soluene (PerkinElmer) for 12 h at 50 °C. All samples were stored for at least 24 h before counts to reduce the risk for chemoluminescence (not observed), counted for 600 s or 3 million counts and compared both to an internal standard to determine counting efficiency and to an external standard to determine background.

Table 2 Average recovery of ^{14}C in the three treatments, ± s.e.

Compartment	*LM*	*HM*	*N*
Fauna	0.0 ± 0.1	0.0 ± 0.1	0.7 ± 0.4
Water	5.2 ± 0.6	5.1 ± 0.7	1.3 ± 0.1
Sediment	23.9 ± 3.9	43.2 ± 3.2	36.9 ± 6.2
CO_2	55.2 ± 4.7	43.7 ± 7.6	53.4 ± 3.9
Total	92 ± 8	84 ± 6	92 ± 6

Abbreviations: HM, high meiofauna; LM, low meiofauna; N, no manipulation.

Samples were corrected for background and counting efficiency and were controlled for color quenching (not observed). All samples had counts that were at least three times the background. Counting efficiencies were as follows (average ± s.d.): Carbosorb 90.4% ± 0.1, sediments 70.1% ± 5.9, water 82.3% ± 1.3, animals 85.4% ± 2.5. Total radioactivity recovery was 89.5 ± 17% of added ^{14}C-Naphthalene, with no difference in recovery among treatments (one-way analysis of variance (ANOVA), $F_{(2,\ 18)} = 0.45$, $P = 0.64$). Recovery of ^{14}C in different compartments for each treatment is presented in Table 2.

Plate counts of naphthalene-degrading bacteria
Plate counts of culturable naphthalene-degrading bacteria were performed after the termination of the experiment by using the method described by Alley and Brown (2000). Mineral medium agar plates streaked with dilution series of bacteria extracted from sediments according to Tso and Taghon (2006) were coated with sublimed naphthalene using a sand bath ($n = 4$, for each dilution), and incubated for 10 days in 5 °C, upon which the number of colonies with surrounding clear zones were counted.

Community profiling (T-RFLP)
The diversity of the bacterial community was analyzed using T-RFLP of a fragment of the 16S rRNA gene, according to Näslund *et al.* (2008), except that the DNA extractions were performed using the Powersoil kit (MO BIO Laboratories, Carlsbad, CA, USA) and a FastPrep beadbeater (MP Biomedicals, Solon, OH, USA) at a speed of 5.5 ms^{-1} for 1 min. *Msp*I (Fermentas Gmbh, St Leon Rot, Germany) was used for the restriction enzyme cutting and that the size determination of labeled fragments was carried out using an ABI3730XL DNA analyzer (Applied Biosystems, Foster City, CA, USA) at Uppsala Genome Center, Sweden. The diversity of the Ciliophora community was analyzed as described by Dopheide *et al.* (2008), using the primer pair 384F and 1147R, with the same exceptions as for the bacterial community analysis. To confirm that Ciliophora genes were amplified, the PCR product was cloned and sequenced according

to Edlund and Jansson (2008), with the exception that an ABI3730XL (Applied Biosystems) was used. The resulting partial sequences of the 18S rRNA gene were checked for chimeric sequences (Huber *et al.*, 2004) and deposited in the EMBL nucleotide sequence database under the accession numbers FN555293–FN555300. The sequences were classified using RDP-II (release 10.13) (Cole *et al.*, 2009) and analyzed using the NCBI Basic Local Alignment Search Tool (Altschul *et al.*, 1990) to find the closest matches. The results confirmed that Ciliophora genes had been amplified, and that *Euplotes* spp., *Moneuplotes crassus*, *Euplotopsis encysticus* and uncultured ciliates were the closest matches.

Isolation and sequencing of naphthalene degraders
Twelve random colonies (four from each treatment) from the plate counts were picked out and streak-plated five times, whereupon they were screened for similarity using Amplified Ribosomal DNA Restriction Analysis of a fragment of the 16S rRNA gene (using *Hha*I and *Msp*I) (Vaneechoutte *et al.*, 1995), separating the restriction fragments on 1% agarose gels. Three distinctly different isolates were found, from which DNA was extracted and used as a template in PCR using the same conditions as for the bacterial T-RFLP analysis (but using unlabeled primers). The resulting PCR products were sent to Macrogen (Seoul, Korea) for sequencing. The resulting sequences were analyzed equivalent to the Ciliophora sequences and deposited in the EMBL nucleotide sequence database under the accession numbers FN641887–FN641889.

Calculations
Total meiofaunal biomass was determined using the average individual biomass for each meiofaunal group from a previous study in the same area (Olafsson *et al.*, 1999) and multiplying the average biomass with the total abundance counted for each group in the respective samples. Owing to the fact that the meiofauna extractions efficiently extracted all large nematodes ($>200\,\mu m$), the average biomass for smaller nematoda ($<200\,\mu m$) was used in the biomass calculations for the LM treatment. Macrofaunal biomass was determined by gravimetry (shell-free dry weight for adult bivalves), and the carbon content of the fauna was assumed to be 40% (Karlson *et al.*, 2008).

Statistical analyses
Naphtalene mineralization was expressed as DPM (desintegrations per min) captured per 24 h and analyzed using repeated measures ANOVA. The total amounts of mineralized naphthalene and total meiofaunal biomass were further analyzed in a linear regression analysis. Plate counts of naphthalene-degrading bacteria were compared using one-way ANOVA and were square root transformed to meet the assumptions for the ANOVA. Levene's test was used to test the assumption of homogeneity of variances and residuals were inspected visually. The T-RFLP data were plotted graphically using non-metric multidimensional scaling analysis in PAST (Hammer *et al.*, 2001) and pairwise comparisons between treatments were made using PERMANOVA 1.6, using Monte-Carlo sampling to obtain *P*-values (Anderson, 2001; McArdle and Anderson, 2001). The non-metric multidimensional scaling and PERMANOVA analyses were both performed using Bray-Curtis similarity index as distance measure, and using 9999 permutations. The ANOVA analyses were performed in Statistica 7.0 (Statsoft, Tulsa, OK, USA). In all tests the statistical significance level was set at 0.05.

Results

Mineralization of naphthalene
There was a significant difference in mineralization of naphthalene among treatments (repeated measures ANOVA, $F_{(2,\ 18)}=4.6$, $P=0.025$), time ($F_{(10,\ 180)}=59.2$, $P<0.001$) with a nonsignificant interaction ($F_{(20,\ 180)}=1.2$, $P=0.299$). A Tukey HSD *post hoc* test for the treatment factor showed that the HM had a significantly lower naphthalene mineralization than the LM treatment ($P=0.0199$) and that there was no significant difference between N and LM ($P=0.212$) or N and HM ($P=0.443$). The cumulative amount of mineralized naphthalene in the three treatments is shown in Figure 2. A linear regression analysis showed a significant negative regression between the total amount of mineralized naphthalene and total meiofaunal biomass ($r^2=0.34$, $P=0.005$, Figure 3).

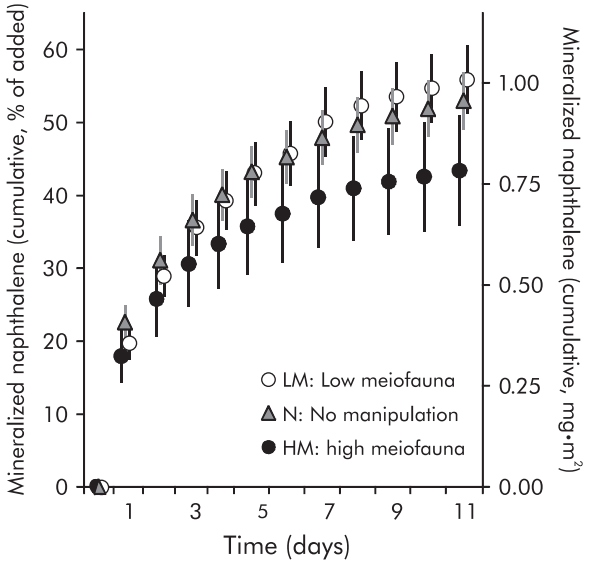

Figure 2 Cumulative amounts of mineralized ^{14}C-Naphthalene in the three treatments. Error bars denote s.e. ($n=7$).

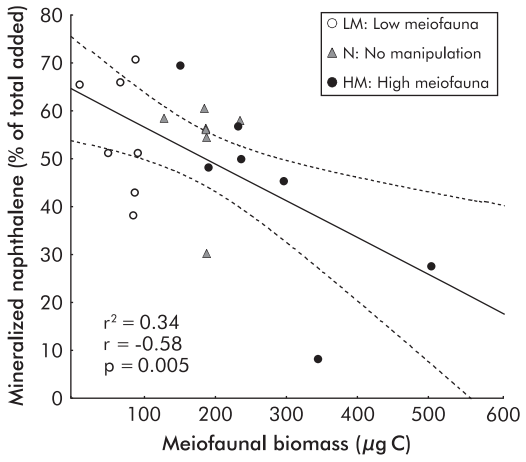

Figure 3 Linear regression between mineralized ^{14}C-Naphthalene and total meiofaunal biomass. Dotted lines denote 95% confidence interval for the regression line.

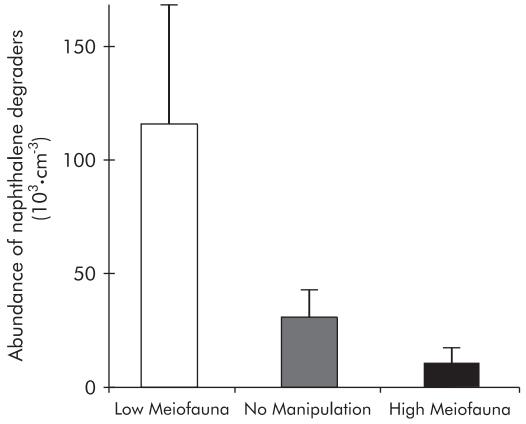

Figure 4 Number of cultivable naphthalene-degrading bacteria (cm^{-3}) in the sediment surface layer (0–1 cm) at the end of the experiment. Error bars denote s.e. ($n = 4$).

Naphthalene-degrading bacteria

The plate counts of naphthalene-degrading bacteria for the three treatments are presented in Figure 4. Significant different abundances of plate-cultured naphthalene-degrading bacteria were found among treatments (ANOVA, $F_{(2,\ 9)} = 4.27$, $P = 0.049$). The abundances were significantly higher in the LM treatment compared with the HM treatment (Tukey HSD, $P = 0.0338$), however, N did not differ significantly from the LM and HM treatments ($P = 0.23$ and 0.52, respectively). Sequencing of a fragment of the 16S rRNA gene allowed classification of the three isolates as members of the Pseudomonadaceae family, of which two were most closely matched to the genus *Flavimonas* and one was classified as *Pseudomonas* sp.

Bacterial communities

Non-metric multidimensional scaling plots of the bacterial T–RFLP community analyses are shown

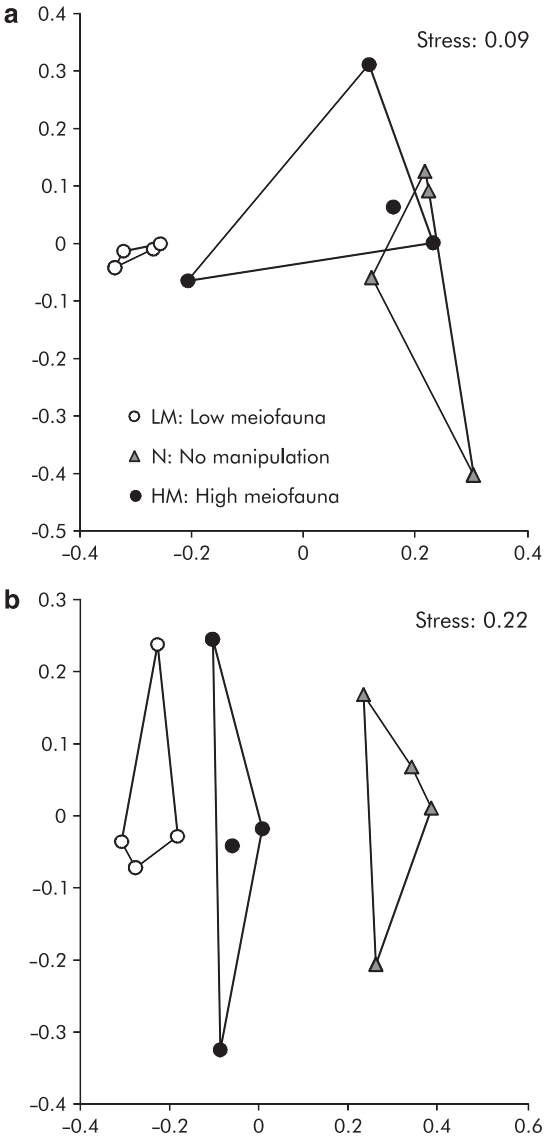

Figure 5 Non-metric multidimensional scaling plots illustrating the T–RFLP community profiles of (**a**) Bacteria and (**b**) Ciliophora.

in Figure 5a. PERMANOVA pairwise comparisons showed that the treatments had significantly different communities of Bacteria ($P = 0.003$). The LM treatment was significantly different from the N ($P = 0.002$) and HM ($P = 0.019$) treatments, but there was no difference between the N and HM treatments ($P = 0.375$).

Faunal communities

The meio- and macrofauna biomass and abundances in the different treatments are presented in Table 3. The LM treatment contained the lowest amounts of meiofauna, containing small nematodes and some ostracods that remained after the extractions. The HM and N treatments also contained Copepoda, Kinoryncha and low abundances of Oligochaeta, in addition to higher abundances of nematodes and

Table 3 Faunal abundances and total biomass for each meio- and macrofauna group in the treatments (mean ± s.e., per core for macrofauna and 10^3 per m^2 for meiofauna)

Group	*Individual biomass (μg C)*	*Abundance LM*	*Abundance HM*	*Abundance N*
Macrofauna				
Marenzelleria arctia	1560	0 ± 0	0 ± 0	4.3 ± 0.6
Macoma balthica	3090	0 ± 0	0 ± 0	1.0 ± 0.5
Monoporeia affinis	800	0 ± 0	0 ± 0	0.4 ± 0.4
Total macrofaunal abundance		0 ± 0	0 ± 0	5.7 ± 0.6
Total macrofaunal biomass (mg C)		0 ± 0	0 ± 0	10.4 ± 1.6
Meiofauna				
Nematoda	0.18 (>200 μm) 0.02 (<200 μm)	98 ± 34	702 ± 227	486 ± 181
Ostracoda				
Candona neglecta	1.7	2 ± 1	11 ± 5	7 ± 1
Paracyprideis fennica	2.8	12 ± 6	38 ± 20	22 ± 5
Heterocyprideis sorbeyana	2.6	10 ± 5	28 ± 16	19 ± 3
Copepoda; Harpacticoida				
Microarthridion littorale	1.4	0 ± 0	3 ± 2	2 ± 1
Pseudobradya spp.	0.3	0 ± 0	4 ± 3	3 ± 3
Kinorhyncha	0.3	1 ± 1	22 ± 8	15 ± 5
Oligochaeta	10.4	0 ± 0	2 ± 1	2 ± 1
Macoma balthica (spat)	1.2	0 ± 1	2 ± 0	3 ± 2
Total meiofaunal abundance		124 ± 16	832 ± 94	558 ± 68
Total meiofaunal biomass		68 ± 11	290 ± 46	193 ± 12

Abbreviations: HM, high meiofauna; LM, low meiofauna; N, no manipulation.

ostracods. Meiofauna biomass was in average 4.3 times higher in the HM compared with the LM treatment. The abundances of meiofauna in all three treatments are relatively low but within the same order of magnitude, as reported field abundances of meiofauna in the area (Ankar and Elmgren, 1978). The N treatment additionally included three macrofaunal species, the polychaete *Marenzelleria arctia*, the bivalve *Macoma balthica* and the amphipod *Monoporeia affinis*. Non-metric multidimensional scaling plots of the ciliophora communities are shown in Figure 5b. The Ciliophora communities were significantly different among treatments (PERMANOVA, $P = 0.009$), where the N treatment was significantly different from the LM ($P = 0.009$) and HM ($P = 0.042$) treatments, but there was no significant difference between the LM and HM treatments ($P = 0.51$).

Discussion

A comparison between the LM and HM treatments showed that both the mineralization of naphthalene and the number of cultivable naphthalene-degrading bacteria were significantly lower with higher meiofauna abundances (HM). To our knowledge, the effect from meiofauna on PAH mineralization has not been studied previously but studies on the effects of protozoans on degradation of contaminants have been conducted (Rogerson and Berger, 1983; Kota *et al*., 1999; Tso and Taghon, 2006). In these studies, protozoans had a stimulatory effect on the bacterial degradation of contaminants, possibly by increasing the turnover of nutrients. The relationship between bacterial degradation of contaminants and protozoa has been studied using grazing inhibitors such as cytochalasin (which inhibits eukaryote actin filament elongation and suppresses movement (Sampath and Pollard, 1991)) or cycloheximide (inhibits eukaryote protein synthesis (Vazquez, 1974)). One drawback with grazing inhibitors is that they may affect other eukaryotes present and not just the targeted protozoa. If meiofauna can affect contaminant degradation, as found in our study, results from previous studies with grazing inhibitors and protozoans may have been biased by negative effects on metazoans such as meiofauna.

The mineralization of naphthalene in our experiment was relatively fast, as expected from the low-molecular weight of the PAH used and the oxic conditions prevailing in our sediment. Furthermore, with the relatively high pollution load of the Baltic Sea, it is likely that these sediments have been previously exposed to PAHs, allowing for a fast mineralization response from a dormant or already active microbial PAH-mineralizing population (Carmichael and Pfaender, 1997).

A significantly different bacterial diversity was found in HM compared with the LM treatment, revealing that differences in meiofauna abundance also caused a difference in the bacterial community. Cuny *et al*. (2007) observed similar changes in the bacterial community due to the presence of the polychaete *Nereis diversicolor* in oil-polluted

microcosms. Interestingly, this change was not observed in microcosms not polluted by oil. Castle *et al.* (2006) also observed that an addition of naphthalene can cause changes in the bacterial community, showing that PAH pollution by itself can be a significant driver in community interactions.

Eukaryotes present in the sediment are not likely to directly influence the mineralization rate of naphthalene due to the fact that contrary to many bacterial biotransformation pathways, eukaryotic biotransformation pathways do not cleave the aromatic ring structure (Cerniglia and Heitkamp, 1989). The eukaryotic biotransformation pathway functions rather as a detoxification mechanism by creating water-soluble metabolites (McElroy, 1990; Christensen *et al.*, 2002). These metabolites have in turn been shown to be difficult to use for PAH-degrading bacteria, thereby inhibiting mineralization rather than promoting it (Giessing and Johnsen, 2005). Despite this, the presence of fauna, such as polychaetes, is known to increase PAH mineralization (Gardner *et al.*, 1979; Bauer *et al.*, 1988; Granberg *et al.*, 2005) through increased oxygen and nutrient fluxes due to bioturbation. Such stimulatory effects from fauna are likely to have occurred in all treatments, as meiofauna also is known to increase oxygen fluxes (Aller and Aller, 1992)

Anaerobic mineralization of PAHs can also occur in sediments (Coates *et al.*, 1997), although it is not likely to be important in this study due to the fact that the majority of the remaining ^{14}C in the sediment was present in the top centimeter of the sediment (average 78%, data not shown), and that the water column was continuously aerated.

The number of cultured naphthalene degraders found in our study is comparable to, but generally lower than those found by Tso and Taghon (2006) (for example, an average of 1.8×10^5 colony-forming units cm^{-3} in their control treatment), possibly indicating a higher predation pressure in our experiment. It is important to note that the abundances are based on plate count estimations, which may be biased due to the possible presence of uncultivable bacteria, making it difficult to draw definite conclusions on the total number of naphthalene degraders. An alternative approach could have been real-time PCR quantification of functional genes coding for PAH-metabolizing enzymes. However, this was not possible in this study due to logistical constraints. Furthermore, a higher abundance of bacteria does not necessarily equal a higher community activity as the activity per bacterial cell could differ between treatments, as grazed bacterial populations have lower abundances but higher activity per cell (Traunspurger *et al.*, 1997). This could be true for our experiment as well considering the number of naphthalene degraders in the N versus the LM treatment and the total naphthalene mineralization in each treatment. It is also possible that the depth distribution of naphthalene degraders is greater in the N treatment, considering that the presence of macrofauna provides higher bioturbation rates, and thus an increased burial depth for both the added naphthalene-spiked sediment and naphthalene-degrading bacteria.

The isolated naphthalene-degrading bacteria were matched to a group containing known marine PAH degraders (Garcia-Valdes *et al.*, 1988; Niepceron *et al.*, 2009), which are susceptible to grazing (Brettar *et al.*, 1994). The isolates were also analyzed with T-RFLP but the corresponding TRFs were not found in the community profiles, suggesting that their abundance are below the detection limit for T-RFLP or that more species than the ones isolated are responsible for the mineralization in our microcosms. We did not observe any significant differences in the Ciliophora community (assessed by T-RFLP) between the LM and HM treatment. The N treatment, however, was significantly different from both LM and HM treatments, caused either by the presence of macrofauna or due to experimental artefacts in the LM and HM treatments. On the other hand, T-RFLP only estimates relative abundances and it is, despite the T-RFLP results, possible that the absolute ciliate abundances were affected by meiofauna, as this is known to occur in sediments (Hamels *et al.*, 2001).

As approximately half of the added naphthalene was metabolized by bacteria and as predation on these bacteria likely had a significant effect, a question that remains is how much carbon that was assimilated for possible further transfer in the sediment food web. Studies of pure cultures of naphthalene degraders have shown that approximately 20% of the carbon from added naphthalene is assimilated into bacterial biomass (Annweiler *et al.*, 2000). Nuclear magnetic resonance analyses of pyrene-degrading bacteria also show that carbon from PAHs is assimilated into the bacterial biomass (Nieman *et al.*, 2007), demonstrating that PAH-derived carbon is used as a carbon source, and not only respired. This carbon is naturally available for transfer to higher trophic levels by predation. However, the distribution in the sediment food web after initial predation is a complex question, and our knowledge of the sediment microbial food web is far from complete. Top-down control (predation pressure) on bacteria from higher trophic levels can also function as a strong evolutionary driver on the bacterial communities, causing, for example, larger cell sizes, filamentous growth forms, aggregation and attachment to particles (Jurgens and Sala, 2000; Matz and Kjelleberg, 2005; Pernthaler, 2005), further highlighting the importance of trophic interactions for bacterial activity and ecosystem functioning. Previous studies found clear links between meiofauna and organic matter mineralization (see for example, Findlay and Tenore, 1982; Alkemade *et al.*, 1993; Coull, 1999), which similar to degradation of pollutants is a crucial ecosystem function and service largely driven by microbial activities.

Our results show that top-down control from meiofauna can significantly affect bacterial PAH mineralization. This may have implications for bioremediation measures and our understanding of the fate of contaminants in the aquatic environments. By considering multiple trophic levels and their interactions, a more holistic understanding of contaminants is achieved; with lower risk for misjudging degradation potential or time needed for natural attenuation of PAHs in contaminated sediments.

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References

Alkemade R, Wielemaker A, Hemminga MA. (1993). Correlation between nematode abundance and decomposition rate of *Spartina anglica* leaves. *Mar Ecol Prog Ser* **99**: 293–300.

Aller RC, Aller JY. (1992). Meiofauna and solute transport in marine muds. *Limnol Oceanogr* **37**: 1018–1033.

Alley JF, Brown LR. (2000). Use of sublimation to prepare solid microbial media with water-insoluble substrates. *Appl Environl Microbiol* **66**: 439–442.

Altschul SF, Gish W, Miller W, Meyers EW, Lipman DJ. (1990). Basic local alignment search tool. *J Mol Biol* **215**: 403–410.

Anderson MJ. (2001). A new method for non-parametric multivariate analysis of variance. *Austral Ecology* **26**: 32–46.

Ankar S, Elmgren R. (1978). *The Benthic Macro-and Meiofauna of the Askö-Landsort Area (Northern Baltic Proper): A Stratified Random Sampling Survey*. 2nd edn. Askö laboratory: Sweden.

Annweiler E, Richnow HH, Antranikian G, Hebenbrock S, Garms C, Franke S *et al.* (2000). Naphthalene degradation and incorporation of naphthalene-derived carbon into biomass by the thermophile *Bacillus thermoleovorans*. *Appl Environl Microbiol* **66**: 518–523.

Bauer JE, Kerr RP, Bautista MF, Decker CJ, Capone DG. (1988). Stimulation of microbial activities and polycyclic aromatic hydrocarbon degradation in marine sediments inhabited by *Capitella capitata*. *Mar Environ Res* **25**: 63–84.

Brettar I, Ramos-Gonzalez MI, Ramos JL, Höfle MG. (1994). Fate of *Pseudomonas Putida* after release into lake water mesocosms—different survival mechanisms in response to environmental-conditions. *Microb Ecol* **27**: 99–122.

Carman KR, Fleeger JW, Means JC, Pomarico SM, McMillin DJ. (1995). Experimental investigation of the effects of polynuclear aromatic hydrocarbons on an estuarine sediment food web. *Mar Environ Res* **40**: 289–318.

Carmichael LM, Pfaender FK. (1997). Polynuclear aromatic hydrocarbon metabolism in soils: relationship to soil characteristics and preexposure. *Environ Toxicol Chem* **16**: 666–675.

Castle DM, Montgomery MT, Kirchman DL. (2006). Effects of naphthalene on microbial community composition in the Delaware estuary. *FEMS Microbiol Ecol* **56**: 55–63.

Cerniglia CE, Heitkamp MA. (1989). *Microbial degradation of polycyclic aromatic hydrocarbons (PAH) in the aquatic environment*. CRC Press: Boca Raton, Florida.

Christensen M, Banta GT, Andersen O. (2002). Effects of the polychaetes *Nereis diversicolor* and *Arenicola marina* on the fate and distribution of pyrene in sediments. *Mar Ecol Prog Ser* **237**: 159–172.

Coates JD, Woodward J, Allen J, Philp P, Lovley DR. (1997). Anaerobic degradation of polycyclic aromatic hydrocarbons and alkanes in petroleum-contaminated marine harbor sediments. *Appl Environ Microbiol* **63**: 3589–3593.

Cole JR, Wang Q, Cardenas E, Fish J, Chai B, Farris RJ *et al.* (2009). The Ribosomal Database Project: improved alignments and new tools for rRNA analysis. *Nucl Acids Res* **37**: D141–D145.

Coull BC. (1999). Role of meiofauna in estuarine soft-bottom habitats. *Aust J Ecology* **24**: 327–343.

Cuny P, Miralles G, Cornet-Barthaux V, Acquaviva M, Stora G, Grossi V *et al.* (2007). Influence of bioturbation by the polychaete *Nereis diversicolor* on the structure of bacterial communities in oil contaminated coastal sediments. *Mar Poll Bull* **54**: 452–459.

Delaune RD, Patrick WH, Casselman ME. (1981). Effect of sediment Ph and Redox conditions on degradation of Benzo(a)Pyrene. *Mar Poll Bull* **12**: 251–253.

Dopheide A, Lear G, Stott R, Lewis G. (2008). Molecular characterization of ciliate diversity in stream biofilms. *Appl Environ Microbiol* **74**: 1740–1747.

Edlund A, Jansson J. (2008). Use of bromodeoxyuridine immunocapture to identify psychrotolerant phenanthrene-degrading bacteria in phenanthrene-enriched polluted Baltic Sea sediments. *FEMS Microbiol Ecol* **65**: 513–525.

Fenchel T, Harrison P. (1976). The significance of bacterial grazing and mineral cycling for the decomposition of particulate detritus. In: Anderson J and Macfadyen A (eds). *The Role of Terrestrial and Aquatic Organisms in Decomposition Processes*. Blackwell Scientific: Oxford. pp 285–299.

Findlay S, Tenore KR. (1982). Effect of a free-living marine nematode (*Diplolaimella chitwoodi*) on detrital carbon mineralization. *Mar Ecol Prog Ser* **8**: 161–166.

Garcia-Valdes E, Cozar E, Rotger R, Lalucat J, Ursing J. (1988). New naphthalene-degrading marine Pseudomonas strains. *Appl Environ Microbiol* **54**: 2478–2485.

Gardner WS, Lee RF, Tenore KR, Smith LW. (1979). Degradation of selected polycyclic aromatic hydrocarbons in coastal sediments: importance of microbes and polychaete worms. *Water Air Soil Poll* **11**: 339–347.

Gerlach SA. (1978). Food-chain relationships in subtidal silty sand marine sediments and the role of meiofauna in stimulating bacterial productivity. *Oecologia* **33**: 55–69.

Giere O. (2009). *Meiobenthology: The Microscopic Motile Fauna of Aquatic Sediments*, 2nd edn. Springer-Verlag: Berlin Heidelberg.

Giessing AMB, Johnsen AR. (2005). Limited microbial degradation of pyrene metabolites from the estuarine polychaete Nereis diversicolor. *Chemosphere* **61**: 1281–1287.

Granberg ME, Hansen R, Selck H. (2005). Relative importance of macrofaunal burrows for the microbial mineralization of pyrene in marine sediments: impact of macrofaunal species and organic matter quality. *Mar Ecol Prog Ser* **288**: 59–74.

Guillard R. (1975). Culture of phytoplankton for feeding marine invertebrates. In: Smith WL CM (ed). *Culture of marine invertebrate animals*. Plenum Press: New York. pp 29–60.

Hamels I, Moens T, Mutylaert K, Vyverman W. (2001). Trophic interactions between ciliates and nematodes from an intertidal flat. *Aquatic Microb Ecol* **26**: 61–72.

Hammer Ø, Harper D, Ryan P. (2001). PAST: Paleontological Statistics Software Package for Education and Data Analysis. *Palaeontologia Electronica* **4**: 4.

Hedges JI, Stern JH. (1984). Carbon and nitrogen determinations of carbonate-containing solids. *Limnol Oceanogr* **29**: 657–663.

Hinga KR. (2003). Degradation rates of low molecular weight PAH correlate with sediment TOC in marine subtidal sediments. *Mar Poll Bull* **46**: 466–474.

Huber T, Faulkner G, Hugenholtz P. (2004). Bellerophon: a program to detect chimeric sequences in multiple sequence alignments. *Bioinformatics* **20**: 2317–2319.

Jurgens K, Sala MM. (2000). Predation-mediated shifts in size distribution of microbial biomass and activity during detritus decomposition. *Oikos* **91**: 29–40.

Karlson AML, Nascimento FJA, Elmgren R. (2008). Incorporation and burial of carbon from settling cyanobacterial blooms by deposit-feeding macrofauna. *Limnol Oceanogr* **53**: 2754–2758.

Kota S, Borden RC, Barlaz MA. (1999). Influence of protozoan grazing on contaminant biodegradation. *FEMS Microbiol Ecol* **29**: 179–189.

Leadbeater BSC, Green JC (eds). (2000). *Flagellates: Unity, Diversity and Evolution*. Taylor & Francis: London.

Lei L, Khodadoust AP, Suidan MT, Tabak HH. (2005). Biodegradation of sediment-bound PAHs in field-contaminated sediment. *Water Res* **39**: 349–361.

Lotufo GR. (1997). Toxicity of sediment-associated PAHs to an estuarine copepod: Effects on survival, feeding, reproduction and behavior. *Mar Environ Res* **44**: 149–166.

Lynn DH. (2008). *The ciliated protozoa: characterization, classification, and guide to the literature*, 3rd edn. Springer: Dordrecht.

Matz C, Kjelleberg S. (2005). Off the hook—how bacteria survive protozoan grazing. *Trends Microbiol* **13**: 302–307.

McArdle BH, Anderson MJ. (2001). Fitting multivariate models to community data: a comment on distance-based redundancy analysis. *Ecology* **82**: 290–297.

McElroy AE. (1990). Polycyclic aromatic hydrocarbon metabolism in the polychaete *Nereis virens*. *Aquat Toxicol* **18**: 35–49.

Näslund J, Hedman JE, Agestrand C. (2008). Effects of the antibiotic ciprofloxacin on the bacterial community structure and degradation of pyrene in marine sediment. *Aquat Toxicol* **90**: 223–227.

Nieman JKC, Holz RC, Sims RC. (2007). 13C NMR Analysis of biologically produced pyrene residues by *Mycobacterium sp*. KMS in the presence of humic acid. *Environ Sci Technol* **41**: 242–249.

Niepceron M, Portet-Koltalo F, Merlin C, Motelay-Massei A, Barray S, Bodilis J. (2009). Both Cycloclasticus spp. and Pseudomonas spp. as PAH-degrading bacteria in the Seine estuary (France). *FEMS Microbiol Ecol* **71**: 137–147.

Olafsson E, Helene M, van de Bund WJ. (1999). Species specific uptake of radio-labelled phytodetritus by benthic meiofauna from the Baltic Sea. *Mar Ecol Prog Ser* **177**: 63–72.

Peng R-H, Xiong A-S, Xue Y, Fu X-Y, Gao F, Zhao W *et al.* (2008). Microbial biodegradation of polyaromatic hydrocarbons. *FEMS Microbiol Rev* **32**: 927–955.

Pernthaler J. (2005). Predation on prokaryotes in the water column and its ecological implications. *Nat Rev Microbiol* **3**: 537–546.

Pikkarainen AL. (2004). Polycyclic aromatic hydrocarbons in Baltic Sea sediments. *Polycyclic Aromatic Compounds* **24**: 667–679.

Rogerson A, Berger J. (1983). Enhancement of the microbial-degradation of crude-oil by the ciliate *Colpidium Colpoda*. *J Gen Appl Microbiol* **29**: 41–50.

Sampath P, Pollard TD. (1991). Effects of cytochalasin, phalloidin, and pH on the elongation of actin filaments. *Biochemistry* **30**: 1973–1980.

Traunspurger W, Bergtold M, Goedkoop W. (1997). The effects of nematodes on bacterial activity and abundance in a freshwater sediment. *Oecologia* **112**: 118–122.

Tso SF, Taghon GL. (2006). Protozoan grazing increases mineralization of naphthalene in marine sediment. *Microb Ecol* **51**: 460–469.

Vaneechoutte M, Dijkshoorn L, Tjernberg I, Elaichouni A, de Vos P, Claeys G *et al.* (1995). Identification of Acinetobacter genomic species by amplified ribosomal DNA restriction analysis. *J Clin Microbiol* **33**: 11–15.

van Veen J, van Overbeek L, van Elsas J. (1997). Fate and activity of microorganisms introduced into soil. *Microbiol Mol Biol Rev* **61**: 121–135.

Vazquez D. (1974). Inhibitors of protein synthesis. *FEBS Lett* **40**: S48–S62.

Wieltschnig C, Fischer U, Velimirov B, Kirschner A. (2008). Effects of deposit-feeding macrofauna on Benthic Bacteria, Viruses, and Protozoa in a Silty freshwater sediment. *Microb Ecol* **56**: 1–12.

Zöllner E, Hoppe HG, Sommer U, Jürgens K. (2009). Effect of zooplankton-mediated trophic cascades on marine microbial food web components (bacteria, nanoflagellates, ciliates). *Limnol Oceanogr* **54**: 262–275.