Meiofauna enhances organic matter mineralization in soft sediment ecosystems

Francisco J. A. Nascimento,* Johan Näslund,[1] and Ragnar Elmgren

Department of Systems Ecology, Stockholm University, Stockholm, Sweden

Abstract

We investigated the influence of meiofauna on the benthic decomposition of a radiolabeled diatom bloom by measuring the production of $^{14}CO_2$ in a laboratory microcosm. Mineralization of the diatom bloom material in the sediment was significantly enhanced in the treatment with high meiofauna abundance, with cumulative mineralization values, on average, 50% greater in the treatment with high meiofaunal abundance after 17 d, compared to sediments with low meiofauna abundance. In addition, bacteria species composition in the treatment with high meiofauna abundance was significantly different from the treatment with low meiofauna abundance, indicating that the activities of meiofauna in the sediments had an effect on the bacterial community composition. Meiofauna can enhance the mineralization of organic matter, probably by stimulating the activity of sediment bacterial community, indicating that positive biological interactions such as facilitation from meiofauna are important for ecosystem processes in soft sediments.

Marine sediments are a globally important reservoir of organic matter, and how this organic matter is mineralized is a central issue in marine ecology, because mineralization and nutrient recycling are essential for sustaining primary and secondary production in marine ecosystems (Gage and Tyler 1991; Middelburg et al. 1993). Organic matter mineralization in marine sediments occurs mostly near the surface of the sediments (Kristensen and Mikkelsen 2003) and is driven mainly by microbial processes with bacteria as the primary mediators (Gage and Tyler 1991; Marinelli and Waldbusser 2005). Bacteria in marine sediments are estimated to represent around three-quarters of all bacteria on Earth and can be responsible for about 85% of benthic biomass and oxygen consumption in deep-sea sediments (Rowe et al. 1991; Turley 2000). Among the factors controlling the rates and pathways of mineralization are the biological interactions between animals that inhabit aquatic sediments and the microbial community, because animals cause physical and biogeochemical changes that structure the landscape where microbes live (Meysman et al. 2006) and facilitate their access to oxygen and organic matter (Aller and Aller 1992). Such positive interactions are today considered essential in structuring both population- and community-level dynamics (Bruno et al. 2003; Bulleri et al. 2008). Studies performed in a variety of habitats have shown that such facilitation strongly affects parameters ranging from individual fitness to community dynamics (Bertness et al. 1999; Bruno et al. 2003) to ecosystem functions (Danovaro et al. 2008).

Biological interactions between metazoans and the microbial community are important in structuring food webs in aquatic sediments. Studies of interactions between microbes and higher trophic levels have mostly focused on the relationships between bacteria and macrofauna, with a number of studies demonstrating the importance of macrofauna for the microbiology and biogeochemistry of aquatic sediments (Kristensen et al. 1992; Kristensen 2000; Kristensen and Mikkelsen 2003). Even though meiofauna (benthic invertebrates between 1000 μm and 42 μm) are orders of magnitude more abundant and much more diverse than macrofauna in most benthic habitats, interactions between meiofauna and the microbial component of the benthic ecosystem are still poorly understood. Such interactions may be especially important for crucial ecosystem processes mediated by microbes, such as recycling of organic matter. Although meiofauna are known to be relatively important in freshwater systems (Goedkoop and Johnson 1996; Schmid-Araya et al. 2002), data from a few marine systems studies estimate their direct importance to be limited (De Mesel et al. 2006; Urban-Malinga and Moens 2006) and their direct grazing rates on settled organic matter to be low (Ólafsson et al. 1999; Nascimento et al. 2008). Although some meiofaunal groups such as nematodes have the potential to indirectly influence the activity and/or species composition of microbial communities (De Mesel et al. 2004; Moens et al. 2005), empirical studies evaluating the effects of meiofauna on benthic ecosystem functions are scarce.

In this study, we address the question of how microbial degradation of organic matter is affected by the presence of meiofauna. We followed the mineralization of a ^{14}C-labeled diatom bloom in sediments with experimentally manipulated levels of meiofauna abundance and measured the production of $^{14}CO_2$ over 17 d. We specifically tested the following hypotheses: (1) Mineralization of organic matter is not enhanced in sediments with higher abundance of meiofauna; (2) Different levels of meiofauna abundance do not affect the bacterial community diversity; (3) The presence of the macrofaunal bivalve *Macoma balthica* does not further enhance mineralization of organic matter.

Methods

Sediment sampling—Sediment was collected from a depth of 28 m at a temperature of 5°C and salinity 6 in Hållsviken (58°50′N, 17°32′E) in the northern Baltic

* Corresponding author: francisco@ecology.su.se
[1] Present address: AquaBiota Water Research, Löjtnantsgatan 25, Stockholm Sweden

proper on the 23 May 2008 using a Jonasson–Olausson box corer (sampling area 0.04 m²). Box cores were subsampled using round Perspex tubes (inner area 17 cm²), height 40 cm. Seawater from a depth of 20 m (filtered through a sand filter and a 40-μm sieve) was collected at Askö Laboratory on the same day. The cores and water were then transported to the laboratory and placed in a constant-temperature room at 5° ± 1°C with aeration, until the meiofauna were extracted. Individuals of the facultative suspension-deposit-feeding bivalve *Macoma balthica* were collected at the same site as the sediment and stored in sediment at 5° ± 1°C until the start of the experiment.

Culturing and labeling algae—The diatom *Thalassiosira weissflogii* was grown continuously in artificial water (salinity 10) with added nutrients (f/2 plus Si), at a temperature of 20°C under a 16:8 h light:dark cycle. The diatoms were labeled by adding 0.33 mCi of NaH^{14}CO$_3$ (Amersham; specific activity 54.0 mCi mmol^{-1}) to the f/2 medium (Guillard 1975). After 1 week of incubation, the diatoms were harvested by centrifugation at 2600 × g for 15 min. After centrifugation the diatoms formed a pellet at the bottom and the supernatant was discarded. To remove nonincorporated radioactivity present in the interstitial water between the diatom cells, the diatom pellet was rinsed and resuspended in fresh brackish water (salinity 10), centrifuged again, and the supernatant water was checked for radioactivity after the addition of 5 mL of Ultima Gold scintillation cocktail (PerkinElmer). This procedure was repeated until the radioactivity of the rinsing water was below 0.05% of that incorporated in the diatoms. Samples of the diatoms were dried for 24 h at 60°C and weighed. Their radioactivity was measured in a liquid scintillation counter (LKB Wallac Rackbeta 1214) after the addition of scintillation cocktail (Ultima Gold). The final radioactivity of the diatom suspension was 54.0 ± 1.4 Bq mg C^{-1}.

Meiofauna extractions—The meiofauna extractions were done between 30 May 2008 and 01 June 2008 by slicing off the top 6 cm of the cores, and sieving the sediment through a 1-mm and 40-μm sieve. The sediment and meiofauna retained on the 40-μm sieve were then immersed in an isotonic solution of 0.74 mol L^{-1} MgCl$_2$ for 5 min, to anesthetize the animals, detach them from the sediment particles, and prevent them from swimming down to the sediment during the density extraction. The sediment and animals were thereafter rinsed in brackish water of salinity 6 and washed into a 500-mL E-flask with Levasil® 200A 40% colloidal silica solution (H.C. Starck SilicaSol GmbH) with a density of 1.3. We used Levasil rather than the more commonly used Ludox colloidal silica solution for the extractions, because it is not harmful to the fauna, meaning we could expect low mortality associated with the extraction procedure. The E-flask filled with Levasil® was vigorously shaken several times. Due to the higher density of the Levasil® solution, most meiofauna animals floated while the sediment particles sank to the bottom. Low extraction efficiency for ostracods has been reported by

other studies that used density-based extraction techniques to separate meiofauna from sediment particles (Ólafsson et al. 1999). In order to increase the extraction efficiency for meiofauna ostracods, the Levasil® solution was aerated with an airstone for 20 s, creating air bubbles that helped ostracod shells to float. After aeration, the solution was left to settle for 5 min. The top 100 mL of the Levasil® solution was sieved through a 40-μm sieve and rinsed thoroughly in seawater. The remaining sediment was re-extracted twice using the same procedure (5-min and then 30-min settling time). The sediment left after the last extraction was rinsed several times in brackish seawater to remove any Levasil® residues and was added to the top of the corresponding experimental units of all the treatments except the N treatment (*see* below), followed by 300 mL of filtered brackish seawater from the same site as the sediment. Meiofaunal abundances at the start of the experiment were not assessed, because it would require destructive sampling. Based on previous observations, we expected a large variability within treatments regarding the production of ^{14}CO$_2$ as a result of the degradation of the added labeled material. As such, we prioritized a high replication level for our experiment to get good statistical power and enable the detection of significant differences among treatments.

Experimental design—Our experiment had four main treatments: (1) extracted sediment with high meiofauna abundance (HM, $n = 7$), (2) extracted sediment with low meiofauna abundance (LM, $n = 7$), (3) extracted sediment with high meiofauna abundance plus macrofauna (Mac, $n = 7$), and (4) undisturbed natural cores (N, $n = 7$). We included the N treatment with unsieved sediment as an experimental control. Although they are not directly comparable to the other three manipulated treatments (HM, LM, and Mac), it is useful to assess how the processes studied in our experiment occur in more field-like conditions and possible to identify experimental biases.

The meiofauna obtained from the extraction of two cores was added to each replicate of the HM and Mac treatments to achieve a high meiofaunal abundance. In addition, three individuals of *Macoma balthica* were placed in each core of the Mac treatment, resulting in a density of 1875 ind. m^{-2}, a density commonly reported in the area (Ankar and Elmgren 1976). For the LM treatment, extracted sediment without added meiofauna was used. An extra treatment with the same faunal community as the Mac treatment and autoclaved sediment (30 min at 121°C) was used to estimate the direct contribution of benthic fauna to the mineralization. This treatment ran for 4 d only.

Sieving removed the finer sediment particles from the experimental sediment; therefore, the settled clay particles (< 40 μm) left from the sieving water were added to each experimental unit (containing 1.7 g ± 0.2 g dry weight [dry wt] particles per experimental unit, $n = 4$), in order to try to restore the original sediment particle size. Before the start of the experiment, the experimental units were left to equilibrate for ~ 4 weeks at 5° ± 1°C with aeration and a natural light:dark cycle similar to in situ conditions with low-irradiance green light (< 0.5 μmol quanta ms^{-2}, predominantly 530 nm).

The cores used in the experiment were equipped with air-tight corks (Eslövs Korkfabrik, Sweden) at the top, each containing two drilled holes, one for oxygenating the water and at the same time creating an air pressure causing an outflow of air through the other hole. The outflowing air was led into 50-mL Falcon vials containing 25 mL of Carbosorb E (PerkinElmer) in order to trap CO_2 in the outflowing air. Just before the addition of the labeled diatoms, three sediment cores containing two extractions, identical to the HM treatment replicates, were sampled to make sure that the meiofauna were alive and active before the start of the experiment.

Addition of diatoms and start of the experiment—After homogenization of the diatom suspension, each replicate received an addition of bloom material corresponding to 0.78 g C m^{-2} (about 1–3 d of peak sedimentation of phytoplankton material during a spring bloom in the area [Ankar and Elmgren 1976; Blomqvist and Larsson 1994]). The diatoms were spread evenly over the sediment surface with a pipette and left to settle overnight, after which the aeration was restarted and the microcosm was immediately connected to its corresponding CO_2 trap.

Termination—The experiment ran for 17 d. Afterward, the upper centimeter of sediment of each experimental unit was sectioned and preserved in 4% formalin and then sieved through 1000-μm and 40-μm sieves. The meiofauna were extracted from the 40-μm sediment fraction as described in the *Experimental Setup* section. The remaining sediment was again sieved through 160 μm and checked for ostracods under a 50× binocular stereomicroscope. Extracted meiofauna were counted and identified to major taxa or to species level for harpacticoid copepods and ostracods under a 50× binocular stereomicroscope. Biomass estimations of the meiofauna community were made using length–weight relationships according to Ólafsson and Elmgren (1997). After counting, all meiofauna individuals from each replicate were placed in a scintillation vial and analyzed for ^{14}C activity after addition of Ultima Gold XR. The *M. balthica* individuals were put in filtered seawater for 24 h to empty their gut, rinsed in distilled water, dried at 60°C, weighed (without shell), and placed in a scintillation vial for analysis of ^{14}C activity after addition of Ultima Gold XR.

Depth profiles of radioactivity left in the top 4 cm of sediment were analyzed by sampling each replicate with a cut-off syringe (diameter 5 mm). Five-milliliter water samples were also taken from each experimental unit, filtered through 0.2-μm Millipore filters and measured for radioactivity as described below.

Respiration measurements—The radioactivity in the CO_2 traps was measured daily for 17 d, except for day 11 to 13, by taking a 1-mL sample from the traps, adding 10 mL scintillation liquid (PermaFluor E+, PerkinElmer), and counting in a LKB Wallac Rackbeta 1214 Liquid Scintillation Counter. The CO_2 traps were replaced every other day to avoid saturation.

Scintillation counting—For samples of water, animals, and sediment, Ultima Gold XR scintillation liquid was used. Solid samples were first dissolved in 1 mL Soluene (PerkinElmer) for 12 h at 50°C. All samples were stored for $\geq$ 24 h before counts to eliminate the risk for chemoluminescence, counted for 600 s, and compared to a standard to determine counting efficiency and to an external standard to determine background. Samples were corrected for background, counting efficiency, and color quenching. All samples had counts that were at least three times the background level (but usually more). Counting efficiencies (average ± SD) were: Carbosorb 90.4% ± 0.1%; sediments 70% ± 6%; water 82% ± 1%; animals 85% ± 3%.

Bacterial community profiling (Terminal restriction fragment length polymorphism (T-RLFP))—Sediment samples were taken at the end of the experiment using a cut-off syringe and kept frozen at −80°C until analyzed with an approach similar to that described by Edlund and Jansson (2008). We sampled the top 0.5 cm for bacteria, because this is where the highest content of added organic matter was found and where bacteria associated with the degradation of this organic matter were most likely to be present. Total environmental DNA was extracted from 150-mg homogenized sediment from the top 0.5-cm layer using the FastDNA spin kit for Soil (MP Biomedicals). Extracted DNA was used in a polymerase chain reaction (PCR) with the bacteria-specific 16S rDNA gene primer pair, Forward fD1, 5′-AGA GTT TGA TCM TGG CTC AG-3′ (Weisburg et al. 1991) and Reverse rD1, 5′-CCG TCA ATT CCT TTR AGT TT-3′ (Muyzer et al. 1995). The fD1 primer was labeled with hexachlorofluorescein, Blue 6-FAM, in the 5′-end (Cybergene AB). PCR amplification reactions contained 15 pmol of each primer, 2.5 units of Taq-polymerase (Fermentas Gmbh), 2.5 μL 10 × PCR buffer (1.5 μmol L^{-1} MgCl$_2$), 200 μmol L^{-1} dNTPs (Fermentas Gmbh), 1 ng DNA template and ddH2O, giving a final volume of 25 μL. Conditions for PCR cycling were: 5 min at 94°C followed by 30 cycles of: 94°C 40 s, 56°C 40 s, 72°C 60 s, and a final 7 min at 72°C. Each sample was amplified three times with PCR and pooled together and, thereupon, cut with HhaI [GCGC] MspI restriction enzyme at 37°C for 4 h according to the manufacturer's instructions (Fermentas Gmbh). Fourteen microliters of each digestion product was precipitated with 10% 3 mol L^{-1} NaAc and two volumes 99% EtOH at −20°C for > 12 h. The precipitation mixture was centrifuged at 20,000 × g for 20 min and the supernatant discarded. The remaining DNA pellet was washed twice using 70% ice-cold EtOH, air-dried in darkness for 1 h, and solubilized in ddH2O. Fluorescently labeled fragments were size-determined on an ABI3730XL (Applied Biosystems) by comparison with the internal MapMarker® 1000 Size Standard (Bioventures). The lengths of the fluorescent TRFs were determined using Peak Scanner 1.0 software (Applied Biosystems) and the relative peak areas were determined by dividing individual TRF area with the total area of peaks within the set threshold of 50–900 base pairs. Peaks with relative fluorescence intensities < 50 units and < 1% of total fluorescence were omitted from the analyses, to remove background noise.

Table 1. Abundance and biomass of meiofauna (ind. 10^{-3} m^{-2}; μg C) in the top 1 cm of the sediment and of macrofauna (ind. m^{-2}; mg C) in the different treatments. Values are mean $\pm$ SD ($n = 7$). HM = high meiofauna treatment; LM = low meiofauna treatment; Mac = treatment *with Macoma balthica* and high meiofauna; N = unmanipulated treatment.

Meiofauna	HM Abundance (ind. 10^{-3} m^2)	HM Biomass (μg C)	LM Abundance (ind. 10^{-3} m^2)	LM Biomass (μg C)	Mac Abundance (ind. 10^{-3} m^2)	Mac Biomass (μg C)	N Abundance (ind. 10^{-3} m^2)	N Biomass (μg C)
Nematodes	482±82	61.7±11.7	81±17	2.2±0.5	346±81	45±8	285±123	36.4±8.3
Ostracods	64±5	267±38	24±8	100±37.6	51±12	216±52	49±22	206±61
Harpacticoid copepods	7±3	12.0±6.0	—	—	5±1.2	8.6±3	6±5	10.5±8.6
Kinorhyncha	14±7	6.9±3.3	2±3	0.9±1.5	15±4	7.74±3.7	17±12	8.7±5.4
Oligochaetes	12±4	214±74	—	—	5±1.1	89±75	4±3	61±42
Macoma balthica spats	2±1	4.3±2.6	1±1	2.1±2.7	1.3±0.3	2.64±0.7	2±1	4.6±2.2
Total	585±89	569±88	108±22	109±39	427±87	372±109	365±160	328±91

Macrofauna	HM Abundance (ind. m^2)	HM Biomass (mg C)	LM Abundance (ind. m^2)	LM Biomass (mg C)	Mac Abundance (ind. m^2)	Mac Biomass (mg C)	N Abundance (ind. m^2)	N Biomass (mg C)
Marenzelleria sp.	—	—	—	—	—	—	2238±831	1.7±0.9
Macoma balthica	—	—	—	—	1721±228	1.61±0.74	430±455	15.8±11.9
Total	—	—	—	—	1721±228	1.61±0.74	2668±975	17.5±16.3

Statistical analyses—Data from the diatom mineralization measurements were expressed as percentage of added ^{14}C captured per 24 h. Differences in mineralization of organic matter among treatments throughout the 17 d were analyzed using Repeated Measures Analysis of Variance (RM ANOVA). Differences between treatments in abundances of meiofauna were tested with 1-way ANOVA. Paired a posteriori comparisons were carried out with the Tukey test using 95% confidence limits and were Bonferroni-corrected in the repeated-measures ANOVA (due to the large number of tests). Prior to the analysis of variance, Cochran's C-test was used to check the assumption of homoscedasticity. In cases of unequal variances, data were log10 (x + 1) -transformed prior to the ANOVA, which resulted in homogeneity of variance. The T-RFLP data analyses were done as recommended by Rees et al. (2004), using nonmetric multidimensional scaling (nMDS) and pair-wise comparisons between treatments using m permutation test for analysis of variance (PERMANOVA 1.6), utilizing Monte-Carlo sampling to obtain *p*-values (Anderson 2001). The nMDS and PERMANOVA analyses were both performed with Bray–Curtis similarity index as distance measure. All tests were conducted in Statistica 8.0 (StatSoft) or Palaeontological Statistics (PAST; Hammer and Harper 2001).

Results

Composition of the meiofauna at the end of the experiment—The abundance and biomass of the major meiobenthic groups in the first centimeter of sediment in each treatment are presented in Table 1. The meiofauna present in the LM treatment were mostly small nematodes and ostracods that the extraction failed to remove. The resulting meiofauna abundance and biomass in the HM and Mac treatment were, on average, 5 times higher than in the LM treatment. When compared to the unmanipulated controls, meiofaunal abundances were significantly higher in the HM and Mac and lower in the LM (ANOVA: $F_{1,2}$ = 115.7, p = 0.00001). Meiofaunal biomasses in the HM, Mac, and N treatments were significantly higher than in the LM treatment ($F_{1,2}$ = 7.3, p = 0.005), but not significantly different from each other. As expected, nematodes were the dominant major taxon in all treatments, comprising, on average, 84% ± 15%, 81% ± 14%, and 77% ± 17% of the total abundance in the HM, Mac, and N treatments, respectively (Table 1). Ostracods were the second most common group (average 11% ± 2% and 13% ± 4% for the HM and the N treatments, respectively) and Kinorhyncha the third most abundant taxon (average 2.4% ± 1.1% and 4.8% ± 2% for the HM and the N treatments, respectively). Other groups of some importance were oligochaetes and harpacticoids (average 2.1% ± 0.7% and 0.9% ± 0.6%; and 1.2% ± 0.03% and 1.6% ± 0.06%, in the HM and N treatments, respectively). The meiofaunal community composition of the sediment used in this experiment is similar to that found in previous field studies in the area, but even in the HM treatment, the absolute abundances are below those reported by previous studies (Ankar and Elmgren 1976; Ólafsson and Elmgren 1997).

The macrofaunal community in the N treatment was composed by the polychaete *Marenzelleria* spp., and *Macoma balthica* (Table 1), with *Marenzelleria* dominating abundance and *Macoma balthica* responsible for the major part of the biomass.

Composition of bacterial communities at the end of the experiment—The bacterial community, as characterized by T-RFLP analysis of the 16S rRNA gene pool in the sediment, showed a significant difference between treatments (PERMANOVA, p = 0.02). Interestingly, we find that the bacterial community in the HM treatment is
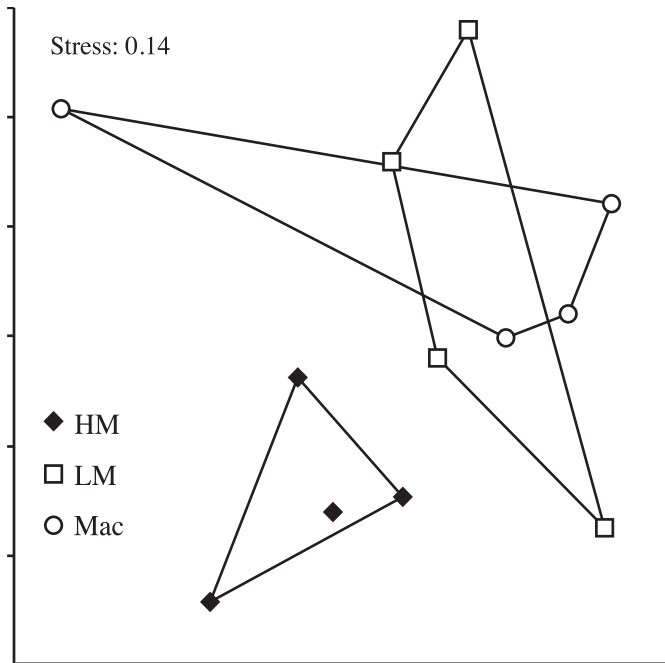
Fig. 1. nMDS plots using Bray–Curtis similarity index showing T-RFLP community genomic profiling for Eubacteria. At the end of our experiment, HM cluster apart from the other treatments, indicating a different eubacterial species composition. HM = high meiofauna treatment; LM = low meiofauna treatment; Mac = treatment with *Macoma balthica* and high meiofauna.

clustered separately from the bacterial community in the LM and Mac treatments in the nMDS plot (Fig. 1). PERMANOVA pair-wise comparisons showed a significant difference between HM and LM ($p = 0.02$). We did not assess the bacterial composition at the beginning of the experiment. However, because the initial sediment for the HM, LM, and Mac treatments was treated the same way, except for the different additions of fauna, we find it highly likely that the differences in bacterial community composition at the end of the experiment are related to the activities of the infauna in the experimental sediments.

Diatom carbon distribution—At the end of the experiment we found 0.9%, 0.08%, 17%, and 11% in animal tissue for the HM, LM, Mac, and N treatments, respectively (Table 2). In the water column we found only 4.9% of the carbon in the HM treatment and 8.1%, 1.7%, and 0.9% in the LM, Mac, and N treatments, respectively (Fig. 2A). The sediment contained 50%, 30%, 27%, and 49% of the added radioactivity for the HM, LM, Mac, and N treatments, respectively. Figure 2B shows how the radioactivity was distributed vertically within the first 4 cm of the sediment, 17 d after the settling of the labeled phytoplankton. In the HM and LM treatments, almost all the labeled carbon in the sediment was found in the top centimeter (91% ± 3% and 87% ± 4%, respectively). Most of the labeled material was found in the top centimeter also in the Mac and N treatments (52% ± 8% and 55% ± 5%,

Table 2. Percentage of added label in meiofauna and macrofauna tissue and percentage of added carbon mineralized in the first 3 d for all the treatments. Values represent average ± SD ($n = 7$). HM = high meiofaunal abundance and biomass treatment; LM = low meiofauna abundance and biomass treatment; Mac = treatment with *Macoma balthica* and high meiofaunal abundance and biomass; N = unmanipulated treatment; and A = treatment with high meiofauna abundance and biomass plus *Macoma balthica* in autoclaved sediment.

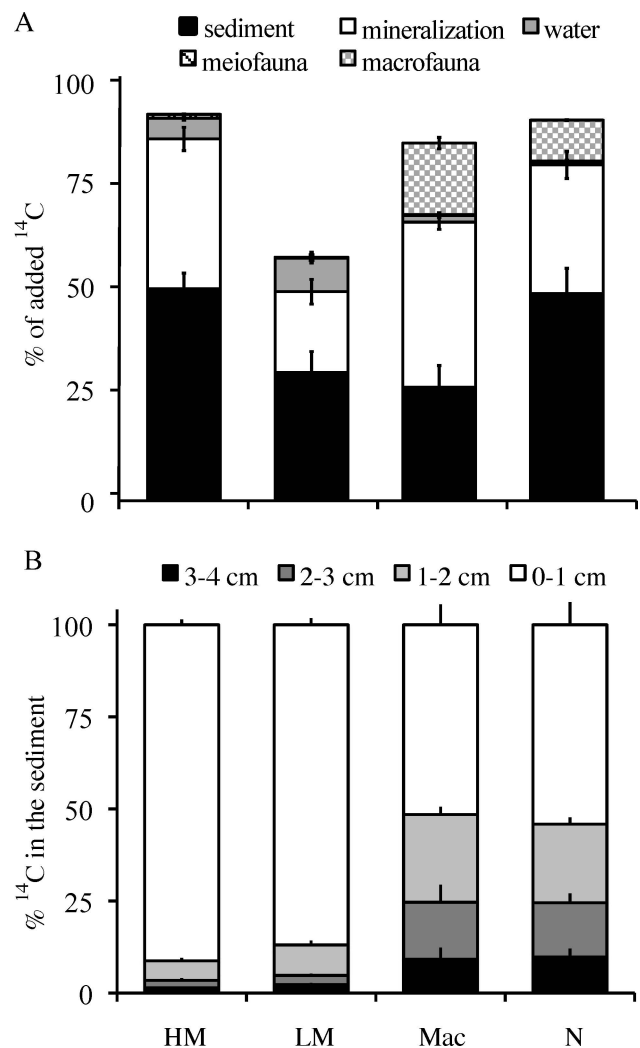| Treatment	% of label in meiofauna	% of label in macrofauna	% mineralized in the first 3 d
HM	0.90±0.30	—	10±3
LM	0.08±0.08	—	4±4
Mac	0.14±0.13	17±3	15±3
N	0.23±0.30	11±2	12±5
A	—	—	1±1

Fig. 2. (A) Recovery of added diatom carbon at the end of the experiment, (B) Vertical distribution of diatom carbon in the sediment from 1 cm to 4 cm at the end of the experiment. HM = high meiofauna treatment; LM = low meiofauna treatment; Mac = treatment with *Macoma balthica* and high meiofauna; N = unmanipulated treatment. Values are means ± SE ($n = 7$).
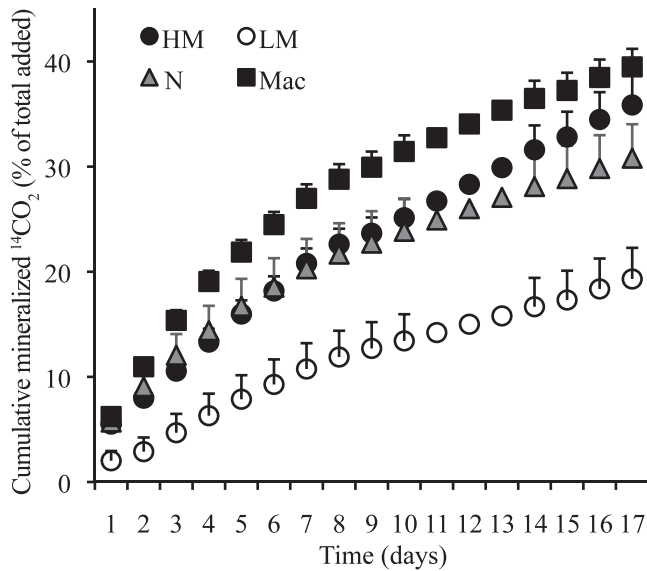
Fig. 3. Cumulative amount of mineralized ^{14}C in the three treatments. HM = high meiofauna treatment; LM = low meiofauna treatment; Mac = treatment with *Macoma balthica* and high meiofauna; N = unmanipulated treatment. Values are means ± SE (n = 7).

Fig. 4. Linear regression between mineralized ^{14}C and meiofaunal (A) biomass, and (B) abundance.

respectively) but here macrofauna bioturbation buried more labeled carbon below 1 cm. The recovery of added label was 85–92% for all treatments except the LM treatment, where it was only 58% (Fig. 2A). We have no obvious explanation for the lower recovery in the LM treatment, but the amount of label recovered in the sediment in the LM treatment was, on average, lower than the other treatments (Fig. 2B). It is reasonable to assume that the added label in the LM treatment was less evenly distributed on the sediment surface than in the other treatments, due to the low abundance of animals and consequent low bioturbation in the LM treatment. It would, then, be easy to underestimate the radioactivity left in the sediment with the sampling technique used in this study.

Mineralization of the diatom bloom—As seen in Table 2, meiofauna in the different treatments incorporated only small amounts of carbon of diatom origin. On the other hand, meiofauna had a clear effect on the mineralization of the diatom bloom, with the amount of $^{14}CO_2$ produced in the HM treatment being significantly higher than in the LM treatment (RM ANOVA, $F_{1,24} = 7.7$, $p = 0.012$). On average, the cumulative mineralization of the settled diatom bloom after 17 d was about 50% higher in the HM treatment than in the LM (Fig. 3). Furthermore, there was a significant positive correlation (Fig. 4) between the amount of diatom bloom that was mineralized in our experimental units and meiofaunal abundance ($r^2 = 0.49$; $p = 0.0006$) and biomass ($r^2 = 0.41$; $p = 0.0017$). The mineralization of added organic matter in the Mac treatment did not differ statistically from the HM treatment, although higher, on average, but was significantly higher than in the LM treatment (RM ANOVA, $F_{1,24} = 7.7$, $p = 0.006$).
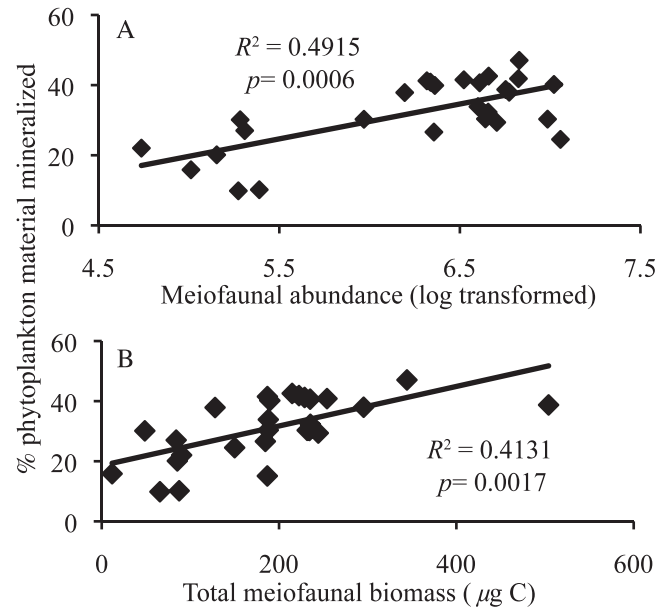
Discussion

Our results show clearly that the presence of meiofauna affects how labile organic matter is mineralized in marine sediments, as shown by the difference in $^{14}CO_2$ production between the treatments with low and high meiofauna abundance (Fig. 3).

Table 2 shows that the production of $^{14}CO_2$ during the first 3 d in cores with both *M. balthica* and high meiofauna abundance in autoclaved sediment was only 0.9% of the added radioactivity, a small fraction of what was mineralized in the HM and Mac treatments during the same time period, indicating that most of the respiration in our test sediments is done by the microbial community. The mineralization values in the cores with autoclaved sediment should, however, be analyzed cautiously, due to the highly artificial sediment used in these corers. Autoclaving the sediment causes radical changes in its chemical, physical and biological properties, which may affect the activity of the animals and, hence, the mineralization rates. Nevertheless, it indicates that direct mineralization by infauna was small compared to that of the microbial community. This suggests that the increased mineralization in the HM treatment is not a result of increased direct mineralization by a higher meiofauna abundance, but instead a result of positive interactions between meiofauna and the microbial community.

Such interactions between nematodes and bacteria, mediated by grazing, have been shown to increase nutrient mineralization in terrestrial systems (Abrams and Mitchell 1980; Anderson et al. 1981; Ingham et al. 1985), but are not as well-documented in marine systems (De Mesel et al. 2003). Näslund et al. (2010) tested the effects of meiofauna on the degradation of a polyaromatic hydrocarbon (PAH) pollutant in a system similar to ours, and found that high meiofauna abundance inhibits degradation of PAH,

presumably through grazing on PAH-degrading bacteria. In contrast, the increase of nearly 50% in cumulative production of $^{14}CO_2$ after 17 d in sediments with high meiofaunal abundance in our experiment, together with the strong correlation between both meiofauna abundance and biomass with the amount of spring bloom mineralized (Fig. 4), show that meiofaunal interactions with the microbial community had a positive effect on the degradation of the diatom bloom. Several studies have proposed mechanisms through which meiofauna can stimulate bacterial degradation of organic matter (Cullen 1973; Findlay and Tenore 1982; Moens et al. 2005). Among them, grazing by meiofauna has been suggested to be important due to keeping the bacterial community in an active growth phase and increasing its demand for nutrients, which may speed up the decomposition of the organic matter (Miller and Jumars 1986; Alkemade et al. 1992; Lillebo et al. 1999). In addition, bioturbation by meiofauna provides the sediment with electron acceptors such as oxygen, which are vital for high microbial decomposition rates (Aller and Aller 1998; Kristensen 2000). Furthermore, meiofauna have faster turnover rates and are, therefore, able to return nutrients to sediments faster than macrofauna, increasing their availability to bacteria (Coull 1999). The experimental design used here does not allow us to identify the relative importance of these mechanisms for stimulating the degradation of organic matter. Nevertheless, the increase in mineralization with higher meiofauna abundance was clear, and it seems likely that all the mechanisms mentioned above contributed to this increase.

Indications of an effect of meiofauna on the microbial community were found in the significant difference in eubacteria community species composition between the HM and LM treatments at the end of the experiment (Fig. 1). This shows that the presence of meiofauna in the sediments influenced the bacterial community composition. Other studies have found that nematodes and protozoa can significantly influence bacterial community structure (Van Hannen et al. 1999; Hahn and Hofle 2001; Ronn et al. 2002). Our results indicate that grazing by meiofauna on microbes have also influenced ecosystem functions, such as degradation of organic matter, which are performed by a large number of bacterial species. However, a similar grazing pressure may be detrimental to ecological processes mediated by more specialized bacteria, as found by Näslund et al. (2010).

It is interesting to note that addition of macrofauna to sediments with high meiofauna abundance did not significantly stimulate the mineralization of organic matter in our experiment, although, on average, mineralization was slightly higher in the Mac treatment. This was surprising, given that macrofauna are known to stimulate microbial activity (Lopez and Levinton 1987; Kristensen et al. 1992). The effect of macrofauna on microbial organic matter degradation depends on sediment characteristics, as well as on the functional group and abundance of macrofaunal individuals (Kristensen and Mikkelsen 2003) and on interactions between animals and microorganisms. Kristensen et al. (1992) hypothesized that deposit-feeding

macrofauna compete with the microbial community for freshly deposited and labile detritus, diminishing their share in the degradation of these compounds. Indeed, the relatively large proportion of labeled carbon found in *M. balthica* tissue (17% of added carbon), shows that macrofauna utilized considerable quantities of the added diatoms. It is, therefore, likely that *M. balthica* reduced the availability of freshly deposited diatoms for microorganisms. In addition, it is possible that *M. balthica* counteracted the stimulation of the microbial community by meiofauna suggested above. Macrofauna has been shown to reduce meiofauna activity in the sediment and interfere with their access to freshly deposited organic matter (Nascimento et al. 2011). Our results also show that meiofauna incorporated less labeled carbon in the Mac than in the HM treatment (Table 2). In addition, we see in Fig. 1 that, in contrast to the HM treatment, the species composition of the bacterial community in the Mac treatment did not differ significantly from the LM, suggesting that the meiofauna-mediated structural effects on the microbial community was reduced by the presence of *M. balthica*. Thus, it seems that the expected stimulation of microbial activity by the presence of macrofauna is counteracted by competition with bacteria for freshly deposited organic matter and by interference from macrofauna reducing the activity of meiofauna in the sediment, thus reducing their stimulation of bacterial activity. In any case, we did not find significant enhancement of mineralization by macrofauna addition when meiofauna abundance was high. This suggests that the meiofauna community can mediate organic matter mineralization in sediments with little or no macrofauna. The importance of meiofauna for a number of ecosystem processes, including the transfer of organic matter between trophic levels has been demonstrated by Danovaro et al. (2008), who found deep-sea ecosystem functioning and efficiency to be related to high nematode diversity.

In deep-sea sediments, meiofauna dominate over macrofauna both in biomass and abundance, much as in our HM treatment. Benthic ecosystems are currently under strong anthropogenic pressure and vulnerable to biodiversity loss (Glover and Smith 2003); therefore, the activity of meiofaunal assemblages can increase the resilience of benthic ecosystem processes essential for the functioning of the aquatic ecosystems, such as nutrient regeneration and energy transfer to higher trophic levels.

In conclusion, meiofauna are important in stimulating the mineralization of freshly deposited detritus by the microbial community and, thus, mediating the fate of organic matter in aquatic sediments.

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