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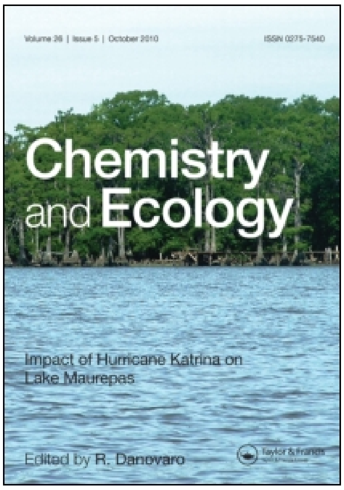

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SHORT-TERM RESPONSE OF BENTHIC BACTERIA AND NANOFLAGELLATES TO SEDIMENT RESUSPENSION: AN EXPERIMENTAL STUDY

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We investigated benthic bacterial abundance, biomass and activities and heterotrophic nanoflagellate (HNF) abundance and biomass during sediment resuspension and after a resilience period through short-term laboratory experiments. The strong sediment resuspension increased bacterial carbon production only slightly (Tukey's test, $P < 0.05$) but somehow interfered with microbial growth. During sediment resuspension, after an initial increase (at 48 and 72 h) bacterial abundance and biomass decreased until the end of resuspension. Sediment resuspension also altered the relationship between HNFs and bacteria (increase in the ratios of bacterium to HNF abundance and biomass after sediment resuspension; Tukey's test, $P < 0.01$). After sediment resuspension, the fraction of bacterial C production removed by flagellates decreased, whereas bacterial turnover did not change significantly, suggesting that HNF pressure on bacteria decreased. These results are consistent with the significant decrease in both HNF abundance and biomass (Tukey's test, $P < 0.01$) and suggest that this small benthic component is less resilient to sediment resuspension than bacteria. Sediment resuspension also determined significant changes in sediment organic matter pools (proteins and carbohydrates). Sediment resuspension determined a decrease in total protein concentrations, whereas total carbohydrate concentrations did not change significantly. Sediment resuspension had significant effects on extracellular enzymatic activities, as aminopeptidase increased and β-glucosidase activity decreased. Based on total protein and carbohydrate concentrations and enzymatic activities, we observed that sediment resuspension resulted in an increase in protein turnover and a decrease in carbohydrate turnover. These results indicate that sediment resuspension plays a major role in the early diagenesis of sediment organic matter.

Keywords: Resuspension; Benthic bacteria; Heterotrophic nanoflagellates; Sedimentary organic matter; Microcosms

1 INTRODUCTION

Several physical factors such as wind, tides and currents have the potential of affecting pelagic and benthic biological dynamics. Unpredictable factors such as sediment resuspension events, induced by episodic benthic storms or bottom-trawling activities, might have a major impact on biological components but have not been investigated to any great extent.

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The intensity and frequency of benthic disturbance caused by repeated trawling are likely to be spatially variable, and their effects may change with time (Watling and Norse, 1998). Moreover, fishing by bottom trawling has been identified as a new potential threat for marine coastal ecosystems functioning (Jennings and Kaiser, 1998) as also suggested by several investigations carried out on macro- and meiobenthic assemblages (see Hall, 1999 and Kaiser and de Groot, 2000, for extended reviews; Smith *et al.*, 2000; Jennings *et al.*, 2001; 2002; Thrush *et al.*, 2001; Bradshaw *et al.*, 2002; Schratzberger *et al.*, 2002; Schratzberger and Jennings, 2002; Smith *et al.*, 2003). These studies pointed out a significant impact of bottom trawling on macrofaunal communities, but results dealing with meiobenthos are still controversial.

There is a number of studies dealing with factors controlling the relationships among the different pelagic microbial components. Among these, bottom-up (*i.e.* C bioavailability) and top-down controls and the significance of physical factors such as hydrodynamics, benthic turbulence, storms, tides, shear stress near the seafloor and local resuspension have received some attention (Karl, 1999; Nimmo Smith *et al.*, 1999; MacKenzie, 2000; Arin *et al.*, 2002; Maar *et al.*, 2002). However, little information is available on the significance of sediment resuspension induced by benthic storms or bottom trawling on benthic heterotrophic micro-organisms (bacteria and smaller protozoa).

In this work, we investigated through laboratory experiments the response of benthic bacteria and nanoflagellates to episodic events of sediment resuspension to provide useful quantitative information for modelling the impact of bottom trawling on C mobilization mediated by bacteria.

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2 MATERIALS AND METHODS

2.1 Sediment Sampling

The experiments were carried out using surface sediments (top 1 cm), collected by means of a multiple corer, in the Thermaikos Gulf (north-western Aegean Sea, eastern Mediterranean). Sediments from five different localities, at a depth of about 30 m, were mixed together and stored at *in situ* temperature (11 ± 1 °C) until microcosm preparation in the laboratory. Sediments used for the experiments were characterized by the dominance of the clay fraction (40–50%), followed by the sandy fraction (10–20%). At each station, seawater was also collected about 1 m above the bottom, immediately filtered through a 200-μm mesh to eliminate larger mesozooplankton organisms, and stored at *in situ* temperature until microcosm preparation.

2.2 Experimental Design

The effects of sediment resuspension were studied in short-term laboratory experiments carried out during February 2002. The experiment was designed to simulate an intense and continuous sediment-resuspension event, induced by persistent strong trawling activities or extremely severe stormy conditions, followed by a resilience period, for a total experimental course of 15 d.

Aliquots of the mixed sediments and 1l of 0.2 μm-pre-filtered seawater (1:2, V:V) were inoculated in three Nalgene jars (5 dm^3), previously acid-washed and autoclaved to avoid any contamination. Each microcosm was left undisturbed for 2 d in thermostatic chambers (11 °C, in the dark). During the experimental course, each microcosm was kept in the dark and at *in situ* temperature (11 ± 1 °C).

Two jars were resuspended for 8 d (‘treated’), and a third untreated jar served as control. Resuspension of the sediment was induced using a horizontal shaker (model SO2) at 120 rpm corresponding to a motion of 2-cm oscillation amplitude. Such motion continuously resuspended the first centimetre of the sediment in the jar. Jars (1.07 kg) were subjected to an oscillation frequency of 2 s^{-1}, amplitude (d) of 2 cm and a period (τ) of 0.50 s.

The energy dissipation rate (ε) in our experiments was calculated in accordance to Moeseneder and Herndl (1995), after MacKenzie and Leggett (1993). ε ($cm^2\,s^{-3}$) was calculated using the following formula:

$$\varepsilon = \frac{P}{m}\left(\frac{\text{power}}{\text{mass}}\right),$$

where power is W/τ, work is $F \times 2d$, $2d$ is the total distance per oscillation, and F is calculated as mass per acceleration (a). The acceleration (a) is defined as $\Delta v/t = v_{\max-0}/t$, where v is the velocity ($m\,s^{-1}$), and t is the time (in seconds) in which the maximum velocity is reached. During our experiments, ε was equal to 512 $cm^2\,s^{-3}$.

2.3 Sampling

Sediment samples were collected before starting the sediment resuspension, 12, 24, 48, 72, 96, 168 and 192 h during sediment resuspension and after the end of sediment resuspension at 216, 240, 264 and 360 h. At each sampling time, the following variables have been determined: bacterial and heterotrophic nanoflagellate (HNF) abundance and biomass, bacterial carbon production, extracellular enzymatic activities (aminopeptidase and β-glucosidase) and biochemical composition of sedimentary organic matter (in terms of total proteins and carbohydrates). All sediment samples were collected using a sterile tip (1 ml) in triplicate from each microcosm at each sampling time for each variable.

Sediment samples for bacterial and HNF counting were immediately fixed with formaldehyde (2% in sterile sea water) and glutaraldehyde (1.5% in sterile sea water), respectively. Aliquots of sediment for bacterial carbon production and extracellular enzymatic activity determinations were immediately treated as described below. For the analysis of the organic matter biochemical composition, sediment samples were frozen at −20 °C until analysis.

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2.4 Biochemical Composition of Sedimentary Organic Matter

Total protein concentrations were determined according to Hartree (1972) and modified by Rice (1982) to compensate phenol interference. Concentrations are reported as bovine serum albumin equivalents. Total carbohydrate concentrations were assessed according to Gerchacov and Hatcher (1972) and reported as D(+)-glucose equivalents. Protein and carbohydrate contents were converted into carbon equivalents using 0.49 and 0.40 mg of C mg^{-1} as conversion factors, respectively (Fabiano *et al.*, 1995).

2.5 Bacterial Abundance, Biomass and C Production

For bacterial counting, sediment samples were diluted 500–800 times with sterile, 0.2 μm pre-filtered water and stained for 5 min with Acridine Orange (final concentration 5 mg l^{-1}), then filtered on 0.2-μm black Nuclepore polycarbonate filters and analysed by epifluorescence microscopy (Zeiss Axioskop2, at 1000×). Bacterial biovolume was estimated according to Danovaro and Fabiano (1995) and converted to carbon content assuming

310 fg C μm^{-3} (Fry, 1990). Bacterial counts were normalized to sediment dry weight after desiccation (60 °C, 24 h).

Bacterial carbon production was measured by [^{3}H]-leucine incorporation following the procedure described by Van Duyl and Kop (1994). Sediment sub-samples (200 μl), added with an aqueous solution of ^{3}H-leucine (0.2 nmol final concentration, specific activity of 72 Ci mmol^{-1}), were incubated for 1 h in the dark at *in situ* temperature. After incubation, samples were added with ethanol (80%) before scintillation counting. The radioactivity (as dpm) was measured with a liquid scintillation counter (Liquid Scintillation Analyzer Packard). Sediment blanks were made adding ethanol immediately before [^{3}H]-leucine addition. Data were normalized to sediment dry weight after desiccation (60 °C, 24 h).

2.6 Extracellular Enzymatic Activities

β-D-Glucosidase (MFU-β-glucopyranoside, Glu-MUF) and aminopeptidase (L-leucine-4-methylcoumarinyl-7-amide, Leu-MCA) activities were determined immediately after sediment retrieval according to Hoppe (1993). One hundred and fifty microlitres of Glu-MUF and Leu-MCA were added to slurry sub-samples (final concentration 300 μM; Meyer-Reil, 1987). Incubation was performed in the dark, at *in situ* temperature for 1 h. After incubation, samples were centrifuged (3000 rpm, 5 min), and the release of the fluorescent dye was measured fluorometrically (at an excitation of 380 nm and emission of 440 nm for Leu-MCA; at an excitation of 365 nm and emission of 455 nm for Glu-MFU). Data were normalized to dry weight (60°C, 24 h) and reported as nanomoles of fluorescent dye released per gram of sediment dry weight per hour. Aminopeptidase and β-glucosidase activity values were converted into equivalents of C mobilized, assuming that 1 nmol of substrate enzymatically hydrolysed corresponded to 72 ng of mobilized C.

2.7 Heterotrophic Nanoflagellates

HNF extraction from the sediment was carried out using a Percoll gradient centrifugation, according to Epstein (1995). HNF counting was carried out by epifluorescence microscopy (1000×) using the double staining (DAPI and FITC) technique (Sherr *et al.*, 1993). Only cells with a major axis comprised between 2 and 20 μm and with a definite nucleus were counted. Cells with bizarre shapes, cells associated with other cells or cells having a harder outer membrane or shell were excluded (Bak and Nieuwland, 1989). HNF cell size was measured on all counted cells (as maximal length and width) and utilized for specific individual biovolume estimation assuming the analogy of HNF cells to the geometrical model of a compressed ellipsoid. HNF biomass was estimated, converting cell biovolumes using 200 fg C μm^{-3} as the conversion factor (Børsheim and Bratbak, 1987; Ekebom, 1999).

2.8 Statistical Analyses

The effects of sediment resuspension on benthic variables investigated during the experiments were assessed using a serial two-way analysis of variance, with microcosm (control and treated) and time (sampling) as sources of variation. When significant differences were observed, a post-hoc Tukey's comparison test was performed. In order to obtain an overview of tested effects, following a before-after design, we have also carried out a serial two-way analysis of variance on mean values of the control (12–360 h) and the treated jars during (12–192 h) and after (216–360 h) sediment resuspension.

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3 RESULTS

3.1 Sediment Organic Matter

Sediment protein concentrations displayed significant temporal changes and significant differences between treated and control sediments (ANOVA, $P < 0.001$).

In control sediments, total protein concentrations (Fig. 1a) ranged from 2.5 $\pm$ 0.3 to 4.1 $\pm$ 1.0 mg g^{-1} (at 24 and 168 h). During sediment resuspension, total protein concentrations (Fig. 1a) in treated sediments ranged from 2.1 $\pm$ 0.0 to 3.7 $\pm$ 0.6 mg g^{-1} (at 192 and 24 h). After 96 h, a general decreasing pattern in protein concentrations was observed in treated sediments until the end of sediment resuspension (192 h), with values significantly lower than in the control (Tukey's test, $P < 0.01$). Both during and after sediment resuspension, protein concentrations were on average lower in treated than in control sediments (3.0 $\pm$ 0.2, 2.7 $\pm$ 0.3 and 3.4 $\pm$ 0.2 mg g^{-1} for those treated during and after sediment resuspension, and for control sediments, respectively).

Total carbohydrate concentrations (Fig. 1b) did not display any difference between treated and control sediments. During and after sediment resuspension, total carbohydrate concentrations did not significantly change in treated sediments and displayed mean values of 2.3 $\pm$ 0.1 and 2.2 $\pm$ 0.6 mg g^{-1}, respectively, similar to those in control sediments (on average 2.2 $\pm$ 0.2 mg g^{-1}).

3.2 Bacterial Parameters and Extracellular Enzymatic Activities

During sediment resuspension, bacterial abundance in treated sediments (Fig. 2a) displayed a significant increase at 48 and 72 h (Tukey's test, $P < 0.001$), then decreased at 192 h (2.11 $\pm$ 0.17 $\times$ 10^9 cells g^{-1}). Opposite peaks occurred at the same sampling times in the control sediments. During and after sediment resuspension, bacterial abundance in treated sediments was on average 4.12 $\pm$ 0.60 and 3.42 $\pm$ 0.31 $\times$ 10^9 cell g^{-1}, respectively, similar to those in control sediments (3.86 $\pm$ 0.39 $\times$ 10^9 cell g^{-1}).

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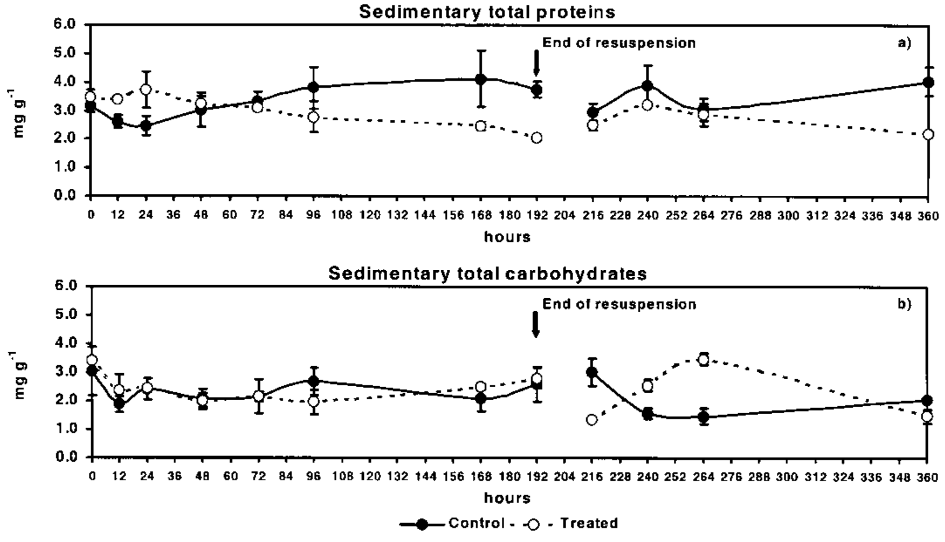

FIGURE 1 (a) Total sedimentary protein and (b) carbohydrate concentrations in control and treated sediments during the experimental course. Standard deviations are also reported.

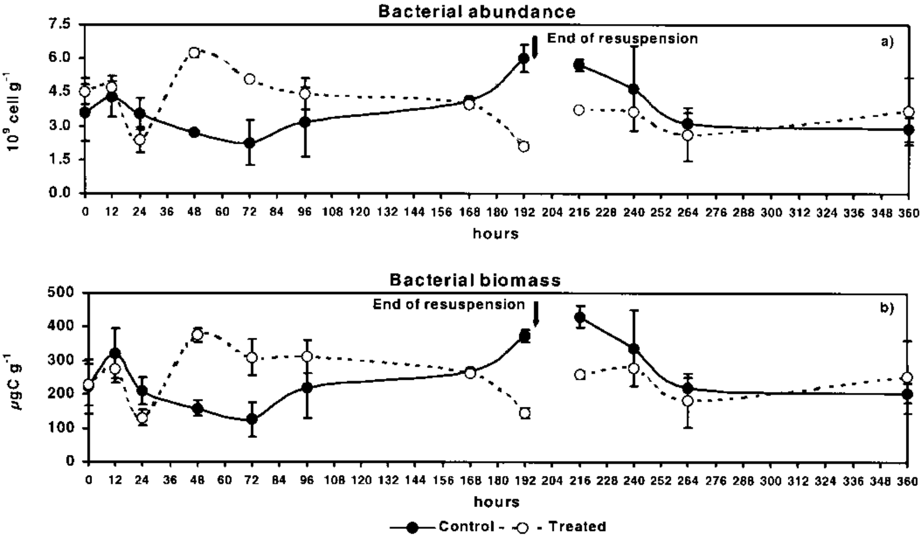

FIGURE 2 (a) Bacterial abundance and (b) biomass in control and treated sediments during the experimental course. Standard deviations are also reported.

Bacterial biomass (Fig. 2b) displayed temporal patterns similar to those of bacterial abundance. During and after sediment resuspension, bacterial biomass in treated sediments displayed mean values of 258.2 ± 36.7 and 242.9 ± 24.1 μg C g^{-1}, respectively.

Bacterial C production and extracellular enzymatic activities during the experiment displayed significant temporal changes and significant differences between treated and control sediments (all ANOVA, $P < 0.001$, $P < 0.01$ for β-glucosidase activity).

During the experimental course, bacterial carbon production (Fig. 3a) in the control sediments ranged from 0.58 ± 0.15 to 1.21 ± 0.11 μg C g^{-1} h^{-1} (at 360 and 48 h). During sediment resuspension, bacterial carbon production in treated sediments displayed mean values significantly higher than in control sediments (0.89 ± 0.06 μg C g^{-1} h^{-1}) and higher than those after sediment resuspension (1.14 ± 0.06 and 0.86 ± 0.14 μg C g^{-1} h^{-1}, respectively; Tukey's test, $P < 0.05$).

During sediment resuspension, aminopeptidase activity (Fig. 3b) yielded values significantly higher in treated than in control sediments (on average 727.6 ± 57.9 and 398.4 ± 62.9 nmol g^{-1} h^{-1}, respectively; Tukey's test, $P < 0.01$). The difference in aminoapeptidase activity values between treated and control sediments also persisted after the end of the resuspension (Tukey's test, $P < 0.05$). By contrast, both during and after sediment resuspension, β-glucosidase activity (Fig. 3c) displayed lower values in treated than in control sediments (on average 12.8 ± 1.4, 10.8 ± 1.5 and 15.8 ± 0.7 nmol g^{-1} h^{-1} in treated during and after resuspension and control sediments, respectively).

3.3 Heterotrophic Nanoflagellates Abundance and Biomass

HNF abundance and biomass displayed significant temporal changes and significant differences between control and treated sediments (all ANOVA, $P < 0.001$).

In control sediments, HNF abundance (Fig. 4a) ranged from 62 ± 18 (at 72 h) to 221 ± 35 (at time 0) $\times 10^3$ cells g^{-1}. During sediment resuspension, HNF abundance did not display significant differences between treated and control sediments (102 ± 18 and 86 ± 7 $\times$ 10^3 cells g^{-1}, respectively). By contrast, after sediment resuspension, HNF abundance in

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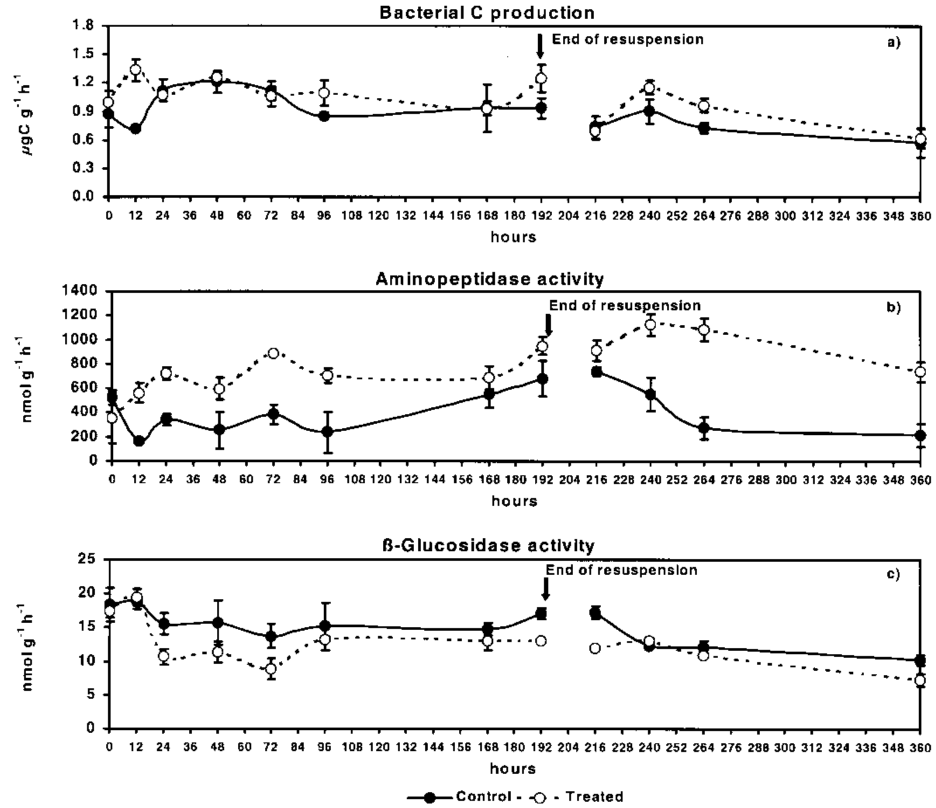

FIGURE 3 (a) Bacterial carbon production and extracellular enzymatic activities: (b) aminopeptidase and (c) β-glucosidase in control and treated sediments during the experimental course. Standard deviations are also reported.

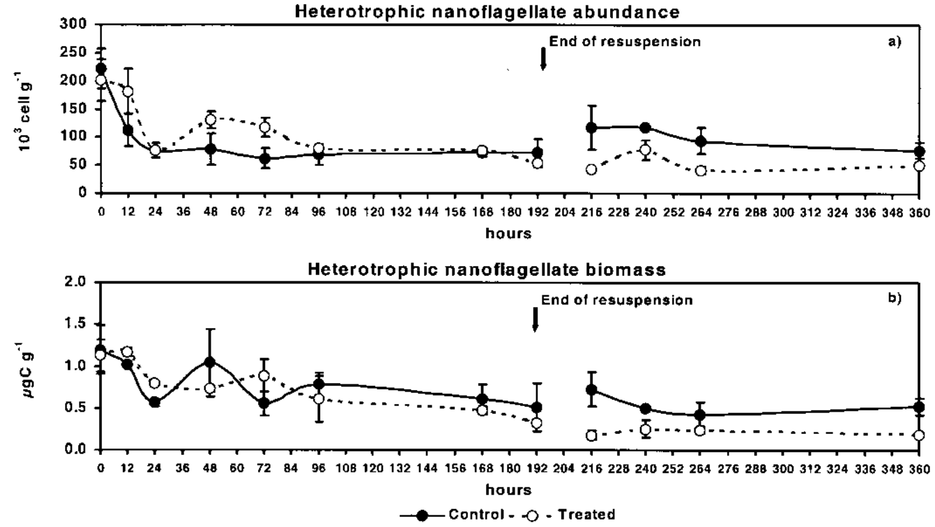

FIGURE 4 (a) Heterotrophic nanoflagellate abundance and (b) biomass in control and treated sediments during the experimental course. Standard deviations are also reported.

treated sediments was significantly lower than in control sediments (on average 53 ± 10 and $86 \pm 7 \times 10^3$ cells g^{-1}, respectively; Tukey's test, $P < 0.05$) and even lower than during resuspension (Tukey's test, $P < 0.01$). During sediment resuspension, HNF biomass did not display any significant difference between control and treated sediments, which both exhibited mean values of 0.7 ± 0.1 μg C g^{-1} (Fig. 4b), whereas after sediment resuspension, HNF biomass in treated sediments displayed mean values significantly lower than in control sediments (0.2 ± 0.0 and 0.7 ± 0.1 μg C g^{-1}, respectively) and even lower than during resuspension (Tukey's test, $P < 0.01$ for both).

4 DISCUSSION

4.1 Kinetic Energy Involved During Sediment Resuspension

It is not easy to compare the intensity of experimental induced resuspension with normal trawling practice. The effects of bottom trawling on bottom characteristics are well known (Rosenberg *et al.*, 2003; Smith *et al.*, 2003). However, even in experimental trawling carried out *in situ* (Thrush *et al.*, 1995; Currie and Parry, 1996), there is no information on the actual energy (as dissipation energy) involved during bottom trawling.

The rate of dissipation, ε ($cm^2\ s^{-3}$), of turbulent kinetic energy is considered a key parameter for the description of turbulent mixing and can be considered an estimate of the produced turbulent kinetic energy (Moseneder and Herndl, 1995). Although a comparison is difficult because of differences in the formulae utilized for calculation and because of different assumptions and turbulence conditions, we compared ε values during our experiments with values reported under different conditions and sources of turbulence (Tab. I). In the present study, the kinetic energy ($\approx 500\ cm^2\ s^{-3}$) was generally higher than previous literature values but similar to values reported by Moeseneder and Herndl (1995). They investigated changes of heterotrophic bacterioplankton production induced by water turbulence, testing levels of turbulence higher than those found in the sea under stormy conditions (Peters *et al.*, 1998).

In the present study, during simulation of sediment resuspension, we obtained a maximum velocity of $0.08\ m\ s^{-1}$, which is indeed similar to the velocity reported by Christiansen *et al.* (1997) ($0.10\ m\ s^{-1}$) to cause resuspension of fine-grained sediments (91% silt and clay) with a high organic matter content (about 13% dry wt.).

Although no direct measurements are available, it could be hypothesized that trawling can induce levels of turbulence comparable with those occurring during extremely severe stormy conditions, like those imposed in our experiments.

4.2 Effects of Sediment Resuspension on Microbial Communities

Advances in understanding bacterial interactions in marine systems have often been obtained from studies conducted in experimental meso- or microcosms (Wainright, 1987; Shiah and Ducklow, 1995; Garstecki and Wickham, 2001; Arin *et al.*, 2002; Maar *et al.*, 2002). However, comparisons between different experimental approaches are difficult because of the varying experimental conditions (Sanford *et al.*, 2001). This is particularly true for our study, since information on the benthic microbial response to sediment resuspension is almost lacking (Watling *et al.*, 2001).

Stimulation of bacteria- and nanoplankton growth after sediment resuspension events has been demonstrated in field and laboratory studies (Wainright, 1987; 1990; Ritzrau and Graf,

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TABLE I Energy-dissipation rates (ε) under different turbulence sources and conditions.

Source of turbulence	*Site*	*Energy-dissipation rate ε (cm^2 s^{-3})*	*Authors*
Tidal mixing	Water column	>40	Bowers and Simpson (1987)
Wind	Surface water (0.2 m depth)	2.9×10^{-7}*	MacKenzie and Leggett (1993)
Wind	Water column (150 m depth)	4.8*	MacKenzie and Leggett (1993)
Experimental shaking	Microcosms	420	Moeseneder and Herndl (1995)
Wind	Surface water (0.1 m depth)	0.11	Moeseneder and Herndl (1995)
Stormy conditions	Coastal waters	0.07–11	Peters *et al.* (1998)
Different atmospheric forcing (calm, wind, thermal convection)	Switzerland lake	10^{-7}–10^{-1}*	Kocsis *et al.* (1999)
Strong forcing	Water column	0.5*	Thwaites *et al.* (2000)
Plume shear stress	Eel river flood plume	0.1–1*	Hill *et al.* (2000)
Moderate wind	Georges bank (North America)	10^{-4}–10^{-2}*	Incze *et al.* (2001)
Experimental vertical oscillation	Coastal waters	0.055	Arin *et al.* (2002)
Experimental vertical oscillation	Coastal waters	0.055	Maar *et al.* (2002)
Artificial turbulence	Microcosms	0.135	Delaney (2003)
Experimental horizontal shaking	Microcosms	512	Present study

*Original ε values reported in data source as W m^{-3} or W kg^{-1} have been converted to cm^2 s^{-3}.

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1992; Cotner *et al.*, 2000; Garstecki and Wickham, 2001). Cotner *et al.* (2000) also provided field evidence that resuspended sediments, rich in organic and inorganic nutrients, may stimulate planktonic heterotrophic production on a large scale, even at low temperatures. The effects of sediment resuspension on benthic microbial production are not equally documented (Fiordelmondo *et al.*, 2003).

Sediment resuspension may alter relationships among different benthic components (bacteria and flagellates). Garstecki and Wickham (2001), studying experimental resuspension effects on trophic interactions in benthic microbial communities that were artificially assembled, found that during resuspension, trophic coupling between flagellates and ciliates can become closer when the food supply is sufficient. Our results indicate that sediment resuspension increased bacterial carbon production slightly, but we did not observe a related significant increase in bacterial or nanoflagellate biomass.

In natural systems, mechanical resuspension can have impact on smaller components (such as bacteria and protozoa) by displacement from the benthic domain (Watling *et al.*, 2001; Fiordelmondo *et al.*, 2003). During our resuspension experiments, we subjected microbes to a high mechanical energy ($\varepsilon \approx 500$ cm^2 s^{-3}) that might have interfered with microbial growth stimulation due to altered nutrient availability. This is in agreement with experimental evidence provided by Moeseneder and Herndl (1995), demonstrating that bacterial growth did not increase at high levels of turbulence.

The results from our experiments suggest that the relationship between HNFs and bacteria was altered after sediment resuspension. Indeed, the ratios of bacterium to HNF abundance and biomass increased significantly (Tukey's test $P < 0.01$; Fig. 5) after sediment resuspension. HNFs are consumers of bacteria (Azam *et al.*, 1983; Hondeveld *et al.*, 1994),

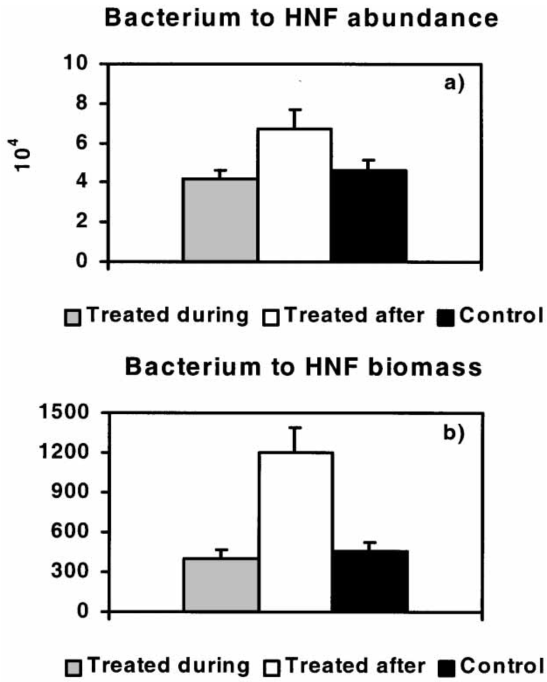

FIGURE 5 (a) Mean values of the bacterial to heterotrophic nanoflagellate abundance and (b) biomass ratios during (12–192 h) and after (216–360 h) sediment resuspension in treated sediments in comparison with control sediments.

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being able to ingest on average 20 bacteria $\mathrm{HNF}^{-1}\,\mathrm{h}^{-1}$ (Starink *et al.*, 1996; Wieltschnig *et al.*, 2003). Using such a grazing rate, we extrapolated the bacterial C production fraction removed by flagellates. We observed that in treated sediments, this fraction displayed slightly higher values during resuspension (11%) than those after sediment resuspension (8%). Our values were similar to those reported by Wieltschnig *et al.* (2003) in freshwater sediments (accounting for 0–14% of bacterial secondary production). Since the bacterial biomass doubling time (on average about 10 d, calculated as the ratio of bacterial biomass to bacterial C production) did not display any significant change in treated sediments during and after resuspension, it may be hypothesized that HNF pressure on bacteria, recently reported to be rather weak (Wieltschnig *et al.*, 2001; 2003), may be even lower after sediment resuspension. Our experiments suggest that, after mechanical interference of sediment resuspension, benthic components, especially HNFs, lack resilience. Indeed, HNF abundance and biomass decreased significantly (Tukey's test, $P < 0.01$, Fig. 6) after sediment resuspension.

4.3 Effects of Sediment Resuspension on Sediment Organic C Availability and Mobilization

The accumulation of organic matter on the sea surface is the result of the balance between *in situ* production, allochthonous inputs, utilization/degradation and export. All these processes also influence organic-matter composition which, in turn, plays an important role from both a biogeochemical (as organic matter degradation rates might affect carbon burial in the sediments; Buscail *et al.*, 1995; Hartnett *et al.*, 1998) and trophodynamic

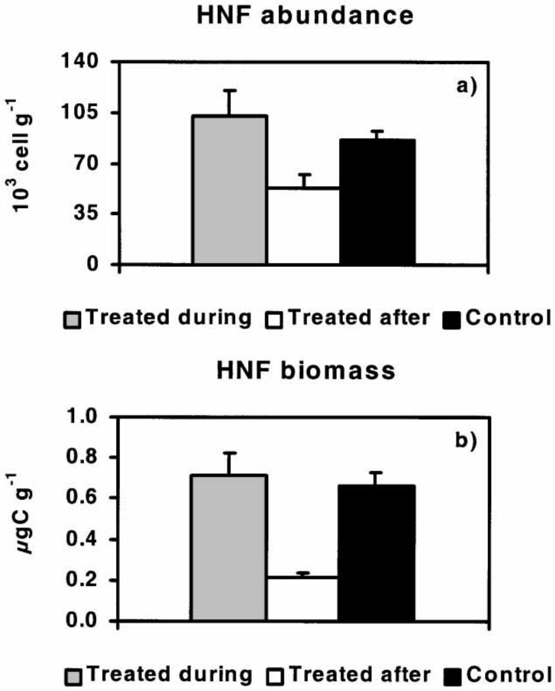

FIGURE 6 (a) Mean values of the heterotrophic nanoflagellate abundance and (b) biomass during (12–192 h) and after (216–360 h) sediment resuspension in treated sediments in comparison with control sediments.

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perspective (influencing feeding strategies and the distribution of benthic organisms; Danovaro *et al.*, 1999; Dell'Anno *et al.*, 2002; Grémare *et al.*, 2002).

Pusceddu *et al.* (2003) report that strong resuspension events, such as bottom trawling or stormy conditions, alter the diagenesis of sediment organic matter. We observed that sediment resuspension caused the decrease in total sedimentary protein concentrations, while carbohydrate concentrations remained fairly constant, suggesting that sedimentary proteins are more easily removed from the sediment. In the present experiments, sediment resuspension significantly increased aminopeptidase but decreased β-glucosidase activity (Fig. 7a and b), and this effect was also observed after sediment resuspension. Indeed, the aminopeptidase-to-β-glucosidase activity ratio was always higher in treated than in control sediments (about two and three times, respectively, during and after resuspension; Tukey's test, $P < 0.01$; Fig. 7c). Such a difference has significant effects on the diagenesis of sediment organic matter, stimulating labilization of more labile compounds (*e.g.* proteins more than carbohydrates). During the course of the experiments, the total protein turnover time (calculated as the ratio of total protein content to proteins enzymatically mobilized by aminopeptidase) was about threefold higher in control than in treated sediments (3 and 1 d, respectively), whereas the opposite was observed for carbohydrates.

Changes in extracellular enzymatic activities observed in this experimental study are in accordance with field results reported by Pusceddu *et al.* (2003). They reported that bottom trawling increased the fraction of bioavailable organic carbon (enzymatically hydrolysable proteins and carbohydrates), suggesting that sediment reworking can facilitate the enzymatic transformation of complex organic matter into more labile compounds (particularly proteinaceous compounds).

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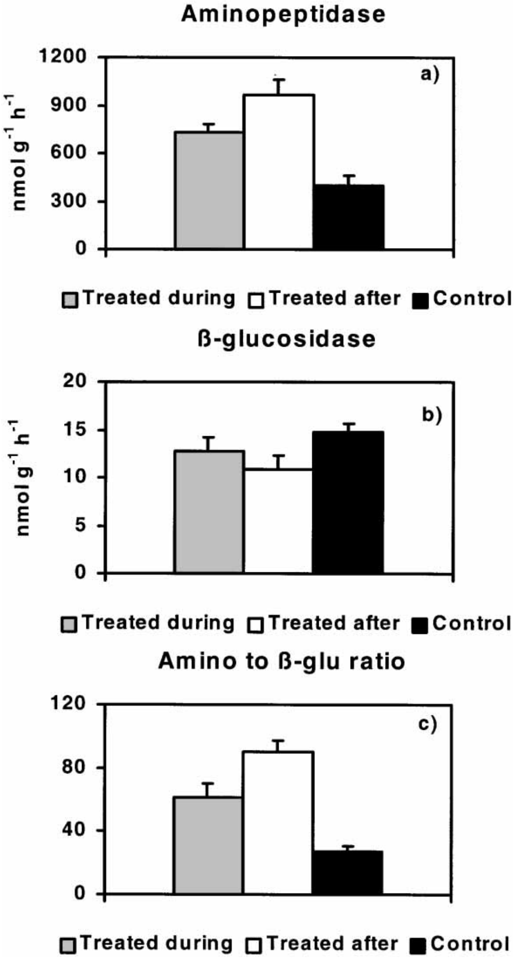

FIGURE 7 (a) Mean values of aminopeptidase and (b) β-glucosidase activity and (c) aminopeptidase-to-β-glucosidase ratio during (12–192 h) and after (216–360 h) sediment resuspension in treated sediments in comparison with control sediments.

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