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Benthic microbial abundance and activities in an intensively trawled ecosystem (Thermaikos Gulf, Aegean Sea)

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Abstract

Abundance of benthic bacteria, heterotrophic nanoflagellates and ciliates, extracellular enzymatic activities, bacterial C production, C mineralisation and sediment community oxygen consumption rates were measured in the Thermaikos Gulf (Northeastern Mediterranean), before (September 2001), and during intense trawling activities (October 2001 and February 2002). The biochemical composition of sedimentary organic matter has revealed that bottom trawling had an effect on the trophic state of Thermaikos Gulf. Changes on the benthic microbial food web were also recorded, during the three sampling seasons. Even though trawling-induced sediment resuspension did not alter significantly the abundance of the microbial components, with the exception of the most impacted station, it determined changes regarding their relative importance. Thus, the ratios of bacterium to nanoflagellates and ciliate to nanoflagellates abundance increased in the trawled stations, causing a sudden increase in bacterial C production, in comparison to the non-trawled station. Four months later, the effects of trawling on the microbial food web were less evident, masked possibly by the drastic decrease in the water temperature. The results of the present work suggest that bottom trawling induces alteration of the sedimentological variables and can be considered as a factor affecting the function of the microbial food web in marine coastal ecosystems. These alterations cause faster mobilisation of organic C buried in the sediment and increase nutrient concentrations and availability in the system, thus inducing an effect that could lead to coastal eutrophication.
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1. Introduction

Several shallow sectors of the Mediterranean Sea (e.g. the northern Adriatic Sea and the northern Aegean Sea) are experiencing a wide variety of anthropogenic impacts that, acting synergistically, are increasingly threatening the ecological integrity of marine coastal environments (Turley, 1999). For example, the overexploitation of fishing resources is often associated with fishing practices, such as bottom trawling, which may severely alter the environment (Sánchez et al., 2004).

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Recent investigations undertaken in a variety of coastal oceans world-wide, have demonstrated that bottom trawling may cause wide-spread disturbance to the seabed, by means of sediment resuspension (for a review see, Stergiou et al., 1997). In the last decade, many attempts have been made to study the impact of trawling activities on the abundance, biomass, diversity and community structure of macro- and meio-benthic assemblages (de Groot and Lindeboom, 1994; Dayton et al., 1995; Lindeboom and de Groot, 1998; Gislason et al., 2000; Schratzberger and Jennings, 2002; Schratzberger et al., 2002). Schratzberger et al. (2002) have shown that seabed disturbance, induced by trawling, has a limited impact on the overall abundance of small-sized organisms such as meiofauna (Schratzberger and Jennings, 2002; Schratzberger et al., 2002). In addition, Sparks-McConkey and Watling (2001) have reported a significant decrease in the numbers of infaunal species, species abundance and diversity, immediately after the trawling disturbance of a soft-sediment system.

A major effect of bottom trawling on benthic communities consists of the direct impact of fishing gears on benthic animals, resulting in a massive mortality of macro-benthic fauna (Thrush and Dayton, 2002). A side effect is the increase of available food for other (smaller) benthic organisms less impacted by trawling (Pusceddu et al., 2005; Schratzberger et al., 2002). Therefore, it could be hypothesised that bottom trawling may also play a significant role on the dynamics of the benthic microbial community.

The effects of sediment resuspension induced by tides, winds and other physical processes on the microbial food web dynamics have been very well documented for the plankton (Garstecki et al., 2002). Field and experimental studies have shown that resuspension may increase bacterioplankton abundance and biomass (Ritzrau and Graf, 1992), as well as enhance C remineralisation rates and increase dissolved nutrient and particulate organic carbon concentrations in the water column (Arfi and Bouvy, 1995; Wainright, 1990).

Recently, field and laboratory investigations carried out on the response of the benthic microbial loop to sediment resuspension have demonstrated significant changes in benthic bacterial activities and C mineralisation rates (Fiordelmondo and Pusceddu, 2004; Fiordelmondo et al., 2003). These studies suggested also that wide-spread sediment resuspension events, such as those induced by bottom dredging and/or trawling, might have severe implications on the biogeochemistry of coastal oceans.

Watling et al. (2001) observed a decrease in microbial abundance and activity after a scallop dragging experiment undertaken in an undisturbed area, by commercial draggers. Nonetheless, the present knowledge on the impacts of anthropogenic sediment disturbance, induced by bottom trawling, on the dynamics of benthic microbial communities and especially in areas such as Thermaikos Gulf, which has been impacted for decades, is still largely unknown (Pilskaln et al., 1998; Pusceddu et al., 2005).

The main objective of the present study was to investigate changes in the structure and functioning of the benthic microbial food web, due to bottom trawling activities, in order to assess the consequences of these activities on the biogeochemistry of the intensively trawled ecosystem of Thermaikos Gulf (North Aegean Sea).

2. Study area

The Gulf of Thermaikos (Fig. 1), is situated in the NW Aegean Sea (Eastern Mediterranean) and in its northeast corner lies the city of Thessaloniki (population >1.5 million). It is an inland Gulf area (open to the Aegean Sea, to the south), extending $39°30'$N to $40°38'$N and $22°30'$E to $23°19'$E and comprised also by a continental shelf, with depths exceeding 200 m. It is characterised by strong anthropogenic influences from Thessaloniki harbour and the city's adjacent industrial zone whilst, at the same time, it receives riverine inputs that have passed through a hydroelectric power dam-construction (Karageorgis and Anagnostou, 2001). Three major rivers (Axios, Aliakmon and Pinios) discharge into the Gulf along its northwestern coastline, introducing significant amounts of particulate matter, as well as nutrient loads in the marine environment (Karageorgis and Anagnostou, 2001; Lykousis and Chronis, 1989). Due to these continental inputs, the Gulf is rather fertile and is one of the major sites for trawling activities in Greece (Stergiou et al., 1997). Thermaikos Gulf is a microtidal environment, in which water movements are influenced largely by thermohaline circulation, mixing of different water masses and the prevailing winds. Generally, more saline waters enter the outer shelf of the Gulf over the eastern part and turn

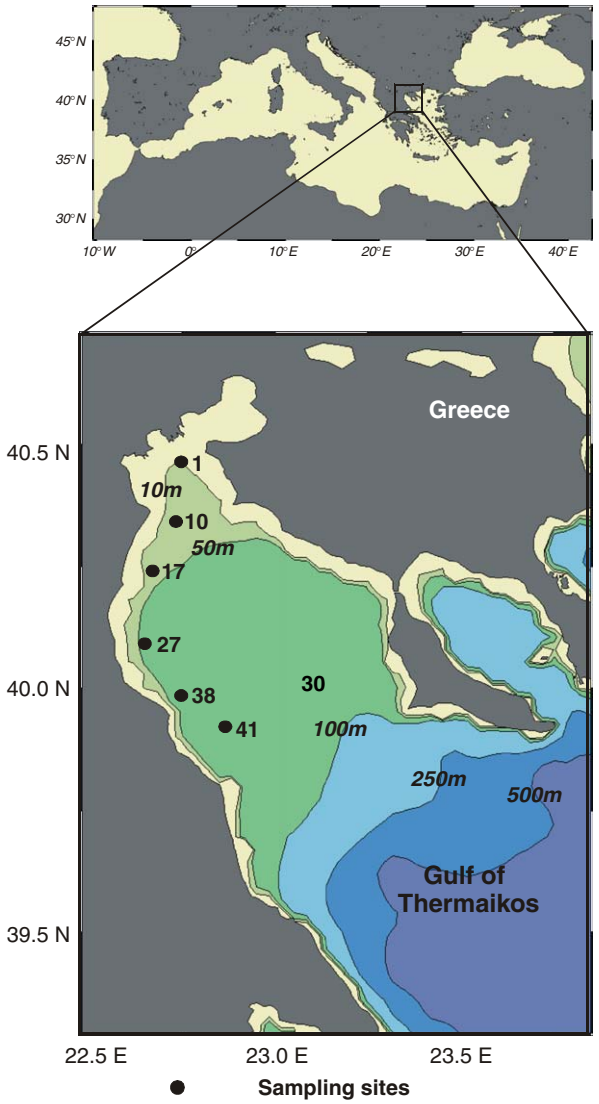

Fig. 1. Map showing the sampling stations in Thermaikos Gulf (Eastern Mediterranean).

towards the northwest in the inner part, whereas less saline waters flow southerly along the western coastline (Poulos et al., 2000).

Sediment cores were collected on board the *R/V Aegaeo* during the EU research programme INTERPOL. Three oceanographic cruises were carried out during September, October 2001 and February 2002. Sampling was undertaken along 7 stations (Fig. 1) and each sampling period was selected in order to obtain information on the benthic microbial community before (September 2001), and during the onset of trawling, at 15 days (October 2001) and 120 days (February 2002) from the initiation of trawling. The trawling season in the Gulf is limited to 8 months, from October to May, so the sampling in September was carried out after 90 days or more of trawling cessation. During September and October 2001, the temperature at the water–sediment interface remained almost constant at all the sampling stations (ranging between 16 and 21 °C), dropping significantly during February 2002 (9–10 °C). Six stations (01, 10, 17, 27, 38, 41 at 30, 41, 55, 64, 51, 80 m water depth, respectively) were located along a north–south transect, near the western intensively trawled area of the Gulf, whilst one station (30 at 86 m depth) in the east was used as the reference station (Fig. 1), since it had never been subjected to trawling. During the INTERPOL project a series of physical and geological measurements were also carried out in order to investigate the degree of sediment resuspension imposed by the trawling activities. All the coastal stations near the western area of the Gulf were found to be highly impacted by the trawling activities, whereas sediment resuspension events were more intense at stations 01, 10 and 17. All the data concerning physical forcing and resuspension events during the sampling periods are presented in the relevant papers of this Special Issue.

3. Materials and methods

3.1. Chemical analysis

A Bowers and Connelly Multiple-corer (8 cores, i.d. 9.0 cm) (Barnett et al., 1984) was used to collect undisturbed sediment samples from Thermaikos Gulf. Mixed surface sediment samples (0–1 cm) and the resuspended "fluff" layer, ~0.5 cm above the surface (bottom viscous layer—BVL) were collected under sterile conditions and used for all the analyses. Redox potential (Eh) was measured using calibrated combined redox electrodes (Russell pH, Scotland, type no. CMPT 11/280/SA1.5), as described by Pearson and Stanley (1979).

Sediment chlorophyll *a* (Chl*a*) and phaeopigment concentration were determined fluorometrically (Lorenzen and Jeffrey, 1980; Yentsch and Menzel, 1963), using a Turner TD-700 fluorometer. Chloroplastic pigment equivalents (CPE) were assumed as the sum of chlorophyll *a* and phaeopigment concentrations. Total organic carbon (TOC) and nitrogen (TON) sediment contents were analysed according to Hedges and Stern (1984), using a

Perkin–Elmer CHN 2400 analyser. Total proteins (PRT) were determined according to Hartree (1972) and Rice (1982). Total carbohydrates (CHO) were analysed, following the method described by Gerchacov and Hatcher (1972) and the total lipids (LIP) were analysed according to Bligh and Dyer (1959) and quantified by the method of Marsh and Weinstein (1966). All the measurements were carried out in triplicate, from at least two independently obtained replicate samples, since the Multiple-corer was deployed two times at each sampling station and in each season. For each biochemical assay, blanks were obtained using pre-combusted sediments (450 °C for 4 h). Carbohydrate, protein and lipid concentrations were converted into carbon equivalents using the conversion factors 0.40 and 0.49 and 0.75 mg C mg^{-1}, respectively, and normalised to sediment dry weight (Pusceddu et al., 2005). The sum of total protein, carbohydrate and lipid carbon equivalents was reported as biopolymeric organic carbon (e.g. Bacterial and Protozoan Counting, Pusceddu et al., 2005).

3.2. Bacterial and protozoan counting

Surface sediment samples (0–1 cm), diluted in 0.2 μm filtered seawater, were fixed with 0.2 μm-filtered formaldehyde (2% final concentration) and stored at 4 °C, until analysis in the laboratory. A series of dilutions were undertaken and bacterial counting was performed after being sonicated (Branson Sonifier 2200; 60 W for 3 min), stained for 5 min with acridine orange (final concentration 0.01%) (Montagna, 1982) and filtered on black Nucleopore polycarbonate 0.2-μm-pore-size filters, at ⩽100 mm Hg. Filters were analysed using an epifluorescence microscope (magnification, ×1000; filter beam splitter, 510 nm; long-pass, 520 nm; Zeiss Universal Microscope). At least 10 microscope fields were counted (at least 400 cells), in each filter (Luna et al., 2002). Benthic heterotrophic nanoflagellates (HNAN) and ciliates were extracted from the sediments using density-gradient centrifugation in Percoll gradients, as described by Epstein (1995). HNAN counting was carried out under epifluorescence microscopy (Axioscope Zeiss) 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, whereas cells with bizarre shapes, cells associated with other cells or cells having a harder outer membrane or shell were excluded (Bak and Nieuwland, 1989). HNAN cell size was measured on all the counted cells (as maximal length and width) and utilised for specific individual biovolume estimation, assuming the analogy of HNAN cells to geometrical model of a compressed ellipsoid.

3.3. Extracellular enzymatic activities

Determination of the extracellular enzymatic activities was performed at 6 stations (01, 10, 17, 27, 38, 30), according to Meyer-Reil and Koester (1992) and Hoppe (1993). Aminopeptidase and β-D-glucosidase activities were measured using the fluorogenic substrate analogues Leu-MCA (L-leucine-7-amino-4-methylcoumarin; Sigma) and MUF-β-glucoside (4-methylumbelliferyl β-D-glucoside; Sigma), respectively. Sediment slurries were prepared by using 1:1 dilution (v/v), in 0.2 μm-filtered sterile seawater. Incubations were performed in the dark, at in situ temperatures and for periods of 0, 1 and 2 h. Leu-MCA and MUF-β-glucoside were added separately, as fluorogenic substrates at 6 different substrate concentrations for enzyme kinetics determination (Leu-MCA: 50, 75, 150, 300, 600 and 900 μM; MUF-β-glucoside: 25, 40, 50, 75, 100 and 200 μM). Immediately after incubation, samples were vortexed and centrifuged for 5 min at 3000*g* and the fluorescence of the supernatant was measured with a Hitachi F-2000 spectrofluorometer (excitation 380 and 365 nm, emission 440 and 445 nm for Leu-MCA and MUF-β-glucoside, respectively). A calibration curve was performed for each sediment slice under the same conditions, using a range of known MUF (4-methylumbelliferone) and MCA (7-amino-4-methylcoumarine) concentrations. The potential activity was determined by the amount of measured fluorescence that was released with time. Data were normalised to sediment dry weight (60 °C, 24 h) and expressed as nmol of MUF or MCA g^{-1} h^{-1} (Hoppe, 1993; Meyer-Reil and Koester, 1992).

3.4. Bacterial C production

Bacterial C production was estimated from the incorporation of [^{3}H]leucine, as described by Smith and Azam (1992), Kirchman (1993) and van Duyl and Kop (1994). Surface sediment samples (0–1 cm) were collected and diluted under sterile conditions, with 0.2 μm-filtered seawater (1:1 v/v). A total of 200 μl of samples were introduced in 2 ml-Eppendorf

tubes and 50 μl of [4, 5-^{3}H]-l-leucine (Amersham TRK 636, specific activity 171 Ci mmol^{-1}) was added (20 nM final concentration). The controls received 1.7 ml of 80% ethanol, before injection of tritiated leucine. All the samples, including the controls, were incubated for 1 h in the dark and at in situ temperature. Incubations were stopped with 1.7 ml of 80% ethanol and the samples were stored at 4 °C, in the dark, until further processing in the laboratory. Samples were centrifuged for 10 min at 14,000g, the supernatants were discarded and the pellets resuspended in 1.7 ml of ice-cold 80% ethanol and filtered onto 0.2 μm-pore-size polycarbonate membrane filters (of 25 mm diameter). For protein precipitation, the filters were washed four times with 5% trichloroacetic acid, placed in sterile pyrex tubes with 2 ml of 2 M NaOH and incubated for 2 h at 100 °C in a dry bath. Samples were cooled to room temperature, centrifuged for 5 min at 14,000g and 1 ml of the supernatant mixed with 10 ml Hionic-Fluor scintillation liquid (BCS, Amersham). Samples were vortexed and radioactivity was measured with a BECKMAN LS 6500 Liquid Scintillation Counter. Bacterial production was estimated according to Kirchman (1993), following the equation $BP = L \cdot 131.2 \cdot (C/protein) \cdot ID/(\%L)$, where BP is bacterial production, L is the leucine incorporation rate in moles l^{-1} h^{-1}, 131.2 is the molecular weight of leucine, C/protein is the ratio of carbon to protein for each cell (0.86) (Kirchman, 1993), ID is the isotopic dilution (in order to calculate the ID, concentration kinetics were performed and the results showed that the degree of participation (DP) of the leucine used was always more than 90% and thus the isotopic dilution was negligible, ID = 1) and % L is the fraction of leucine in the protein (0.073) (Kirchman, 1993).

3.5. Carbon mineralisation rates

Organic carbon mineralisation was estimated at 4 of the 7 stations (01, 17, 38, 30) according to Dauwe et al. (2001), by measuring the production of CO_2 in the headspace above sediment water slurries. A total of 20 ml of fresh derived surface sediment (0–1 cm) was transferred into dark glass bottles and diluted with 10 ml of 0.2 μm-filtered seawater. Slurries were purged with CO_2-free synthetic air and incubated in the dark at in situ bottom water temperature, for 60 days, until further laboratory analysis. Bottles containing only filtered seawater and gas were used as controls. The concentrations of CO_2 in the headspace were determined on a 5890 Hewlett Packard Gas Chromatographer, equipped with a thermal conductivity detector, and a Carboxen 100G column (30 × 0.53 mm^2, Supelco). Data recovery was obtained using a HP3365 series II Chemstation (version A.03.01). Calibration standard curves were acquired using ultrapure gas carbon dioxide (Messer, product No. 1527). The total amount of carbon dioxide produced was calculated as the sum of the headspace and the dissolved carbon dioxide. All the mineralisation rates were normalised to sediment dry weight (60 °C, 24 h) and expressed as nmol CO_2 g^{-1} h^{-1}.

3.6. Sediment community oxygen consumption (SCOC)

In situ measurements at 5 stations (01, 10, 17, 38, 30) were obtained with the use of a free-falling bottom lander, which is a 3 m wide tripod supporting a floatation rack with acoustic releases and 2 single-cup sediment traps. The tripod holds two benthic chambers (32 cm Ø) each containing a magnetic stirrer, O_2-sensors (Idronaut™) and 3 pairs of syringes, each of which draw 60 ml water samples from the chamber headspace at pre-set intervals. The downward action of the chambers and, hence, the enclosed volume, are controlled by the readings of a resistivity probe in the chamber (Andrews and Bennett, 1981). When the lander was retrieved, the samples were analysed immediately for dissolved oxygen, according to the Winkler method (Carpenter, 1965). The consumption was calculated by the detected decrease in oxygen concentration, with time. All the rates were expressed as nmol O_2 ml^{-1} h^{-1}.

3.7. Potential turnover time of protein and carbohydrate pools

Total sedimentary protein and carbohydrate potential turnover time were estimated as the ratios between protein C (calculated assuming 0.49 μg C μg^{-1} protein) and aminopeptidase activity (expressed in C equivalents, assuming 72 μg C μg^{-1} substrate released) and between carbohydrate C (calculated assuming 0.4 μg C μg^{-1} carbohydrate) and β-D-glucosidase activity (expressed in C equivalents assuming 72 μg C μg^{-1} substrate released).

3.8. Bacterial C efficiency

Bacterial C efficiency was estimated, in order to obtain an impression about carbon bioavailability changes during the sampling periods. Thus, the protein plus carbohydrate carbon mobilised through extracellular enzymatic activities was estimated, then compared to the amount of C that actually turned into bacterial biomass. Bacterial C production was increased by a 40%, in order to take into account respiration (Cole and Pace, 1995). Bacterial C efficiency values were expressed as ng C incorporated/μg C degraded.

3.9. Statistical analysis

Temporal and spatial changes were assessed using two-way analysis of variance (ANOVA), with sampling dates and stations as the main sources of variability. When significant spatial differences (per station) were observed ($P<0.05$), a post hoc Newman–Keuls (NK) comparison test for homogenous groups was performed. For bacterial C production, C mineralisation and SCOC rates data, one-way ANOVA was performed using sampling date as the main source of variability. The statistical software SPSS 9.0 (1998) and STATISTICA 5.1 (1996) were used. Marked differences and correlations were significant, at a probability level of $P<0.05$. Sediment characteristics were compared by principal component analysis (PCA), using the measured sedimentological variables. PCA was carried out using the PRIMER 5.2.2 software package (Plymouth Routines In Multivariate Ecological Research).

4. Results

4.1. Redox potentials and sediment organic matter

Redox potential, TOC and TON, photosynthetic pigments, proteins, carbohydrates and lipids in the surface sediments (0–1 cm) of Thermaikos Gulf, during the 3 sampling periods, are reported in Tables 1 and 2. A more detailed description of the chemical characterisation of the sediments,

Table 1
Characteristics of the surface sediments (0–1 cm) in the Thermaikos Gulf stations

Stations	Eh (mV)	Total organic C		Total organic N		C/N ratio		Chlorophyll *a*		Phaeopigments		CPE	
		(%)	SD	(%)	SD	(%)	SD	($\mu g\,g^{-1}$)	SD	($\mu g\,g^{-1}$)	SD	($\mu g\,g^{-1}$)	SD
September 2001													
1	290	1.20	0.03	0.16	0.00	8.62	0.07	2.21	0.67	8.28	0.34	10.49	1.00
10	397	1.01	0.03	0.16	0.01	7.49	0.23	5.18	2.12	9.10	0.50	14.28	2.39
17	431	0.84	0.03	0.12	0.03	8.73	2.15	4.83	0.06	6.14	0.41	10.97	0.35
27	498	0.98	0.03	0.11	0.01	10.15	0.32	4.31	1.11	6.95	0.64	11.26	1.71
38	464	0.99	0.07	0.14	0.00	8.30	0.28	2.21	0.37	6.24	0.37	8.45	0.00
30	450	0.29	0.11	0.05	0.01	6.67	1.24	0.39	0.05	1.28	0.16	1.67	0.19
October 2001													
1	265	1.40	0.08	0.17	0.01	9.88	0.85	4.44	0.90	12.29	1.59	16.73	2.48
10	398	1.15	0.14	0.14	0.01	9.39	0.20	5.76	1.08	7.26	1.69	13.02	2.62
17	437	0.89	0.03	0.12	0.01	8.88	0.13	3.96	0.50	5.84	0.45	9.80	0.40
27	474	0.94	0.02	0.13	0.00	8.77	0.19	2.82	0.20	5.92	0.96	8.75	1.16
38	456	1.05	0.03	0.13	0.00	9.76	0.35	2.14	0.28	5.62	0.38	7.76	0.62
30	488	0.49	0.16	0.06	0.01	8.70	1.61	0.74	0.11	2.72	0.87	3.46	0.94
February 2002													
1	348	1.47	0.12	0.16	0.01	10.67	0.52	7.68	2.75	14.66	2.96	22.34	5.71
10	292	1.04	0.02	0.11	0.01	10.65	0.66	4.95	0.70	8.86	2.68	13.81	3.37
17	416	0.97	0.02	0.12	0.00	9.72	0.17	4.64	0.39	7.89	0.72	12.53	0.99
27	359	0.94	0.07	0.12	0.01	9.37	0.46	4.88	0.97	7.03	1.40	11.91	2.26
38	405	1.01	0.04	0.11	0.00	10.45	0.21	3.02	0.27	6.67	0.49	9.69	0.75
30	375	0.53	0.04	0.07	0.02	9.48	1.85	1.22	0.07	3.23	0.21	4.45	0.26

Sediment redox potential is given in mV. Total organic carbon (C) and nitrogen (N), chlorophyll *a*, phaeopigments and chloroplastic pigment equivalents (CPE) are presented relative to sediment dry weight. SD indicates standard deviations between replicate samples ($n=3$).

Table 2
Comparison of biopolymeric carbon (BPC), % contribution of proteins, carbohydrates and lipids to BPC, protein and carbohydrate turnover time (PRTt and CHOt, respectively), within the surface sediments (0–1 cm) of Thermaikos Gulf during the three sampling periods (September 2001, October 2001, February 2002)

Stations	Biopolymeric C (BPC) ($mg\,C\,g^{-1}$)	Proteins (% of BPC)	Carbohydrates (% of BPC)	Lipids (% of BPC)	PRTt (days)	CHOt (days)
September 2001						
1	3.20	26.00	56.40	17.60	5.93	206.92
10	2.90	32.93	52.45	14.62	3.74	131.16
17	2.30	33.59	53.84	12.57	2.95	124.54
27	2.67	28.91	58.71	12.38	3.34	184.15
38	2.56	28.86	58.90	12.25	3.55	114.96
30	0.40	42.50	45.51	11.99	2.79	79.68
Mean ± SD	2.73 ± 0.34	30.05 ± 3.16	56.06 ± 2.88	13.89 ± 2.29	3.90 ± 1.17	152.35 ± 40.65
October 2001						
1	4.11	53.07	45.70	1.23	13.05	167.42
10	2.98	52.19	46.33	1.47	4.10	111.66
17	2.48	53.82	44.32	1.87	4.12	97.18
27	2.66	51.23	47.78	0.99	4.42	133.75
38	3.02	49.74	46.00	4.27	3.07	178.74
30	1.21	41.48	54.13	4.39	1.77	370.76
Mean ± SD	3.05 ± 0.63	52.01 ± 1.60	46.03 ± 1.24	1.97 ± 1.33	5.75 ± 4.11	137.75 ± 35.01
February 2002						
1	1.67	74.90	19.73	5.37	14.21	30.62
10	1.90	62.87	33.53	3.60	7.09	69.34
17	1.77	52.68	45.07	2.24	4.18	493.42
27	1.62	59.12	37.91	2.96	5.29	135.64
38	2.34	57.32	37.96	4.72	10.29	236.12
30	0.99	50.33	48.31	1.36	6.42	5078.29
Mean ± SD	1.86 ± 0.29	61.38 ± 8.40	34.84 ± 9.41	3.87 ± 1.27	8.21 ± 4.07	192.99 ± 185.15

The mean for the impacted stations (1, 10, 17, 27, 38) is given. SD indicates the standard deviation.

including vertical patterns, has been reported elsewhere (Pusceddu et al., 2005).

Redox potentials of the sediment were always positive (265–498 mV), indicating the presence of permanent oxic conditions during the entire study period. TOC and TON concentrations increased significantly (ANOVA, $P<0.001$), from September to October to February. Values of the C/N ratio during trawling were significantly higher (ANOVA, $P<0.001$) than those in September (Table 1).

Chlorophyll *a* concentrations in February 2002 were significantly higher (ANOVA, $P<0.001$) than those in September and October 2001, whereas phaeopigment concentrations increased from September to October and February (Table 1). Sediment organic matter quantity and biochemical composition displayed clear changes during the study period (Table 2). Biopolymeric carbon (BPC) concentrations displayed a significant increase from September 2001 to October 2001, then decreased significantly in February 2002 (ANOVA, $P<0.001$). Lowest values were recorded at the reference station 30. In September 2001, carbohydrates were the dominant fraction of BPC (on average 56%), followed by proteins (30%) and lipids (14%). In October 2001 and February 2002, proteins became the dominant fraction of the BPC (50% and 60%, respectively), at the expense of both carbohydrates (46% and 35%, respectively) and lipids (2% and 4%, respectively) (Table 2).

Total protein concentrations increased from September to October 2001 and February 2002 (ANOVA, $P<0.001$), whereas total carbohydrate concentrations displayed an opposite temporal pattern, with lower values in February 2002 than in September and October 2001. Total lipid concentrations were significantly higher in September, than in October 2001 and February 2002 (ANOVA, $P<0.001$). During all the sampling periods, both the total protein and total

carbohydrate concentrations at Station 30 were significantly lower than those at all the other stations, whereas the highest concentrations were recorded at the northern stations, 01 and 10 (NK test, $P<0.01$, for both station and period).

In order to compare the stations during all the sampling periods, based upon their chemical characterisation, PCA was undertaken, taking into account all the environmental variables presented in Tables 1 and 2 (Fig. 2). The results indicated that 73.2% of the total variance was represented by only two principal components (PC). PC1 and PC2 explained 50.7% and 22.5% of the total variance, respectively, separating clearly the reference station (30) from all the other stations. The impacted stations of the pre-trawled period (September 2001) were also separated from their counterparts in October and February, whereas organic matter quantity and biochemical composition of the most impacted station (01) was largely modified in September and October (e.g. stations F1, O1; Fig. 2).

4.2. Bacterial and protozoan abundance

Bacteria, heterotrophic nanobenthos and ciliate abundances (Fig. 3) displayed significant temporal and spatial changes (ANOVA, $P<0.05$ for all variables). Bacterial abundance values in February 2002 were significantly lower than in the first period (ANOVA, $P<0.05$), whereas no appreciable differences were observed between the other sampling periods (Fig. 3). When compared to September, HNAN abundance decreased significantly only in February 2002 (ANOVA, $P<0.05$) (Fig. 3). Total ciliate abundance in February 2002 was significantly lower, than in September and October 2001 (ANOVA, $P<0.05$) (Fig. 3). Bacteria, heterotrophic nanoflagellate and ciliate abundances did not exhibit any clear spatial pattern, with the exception of the values at Station 01 in October 2001, which were significantly higher than those at all other stations (NK test, $P<0.01$) (Fig. 3).

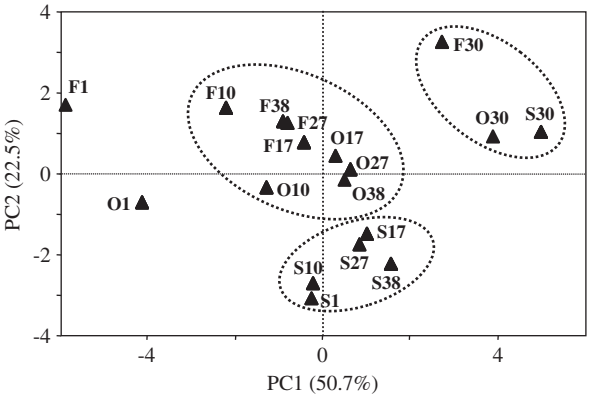

Fig. 2. Principal component analysis of the surface sedimentological variables presented in Tables 1 and 2. For resolution purposes, the station labels are accompanied by the first letter of each of the sampling months, e.g. S1, O1 and F1 indicate Station 01 in September 2001, October 2001 and February 2002, respectively.

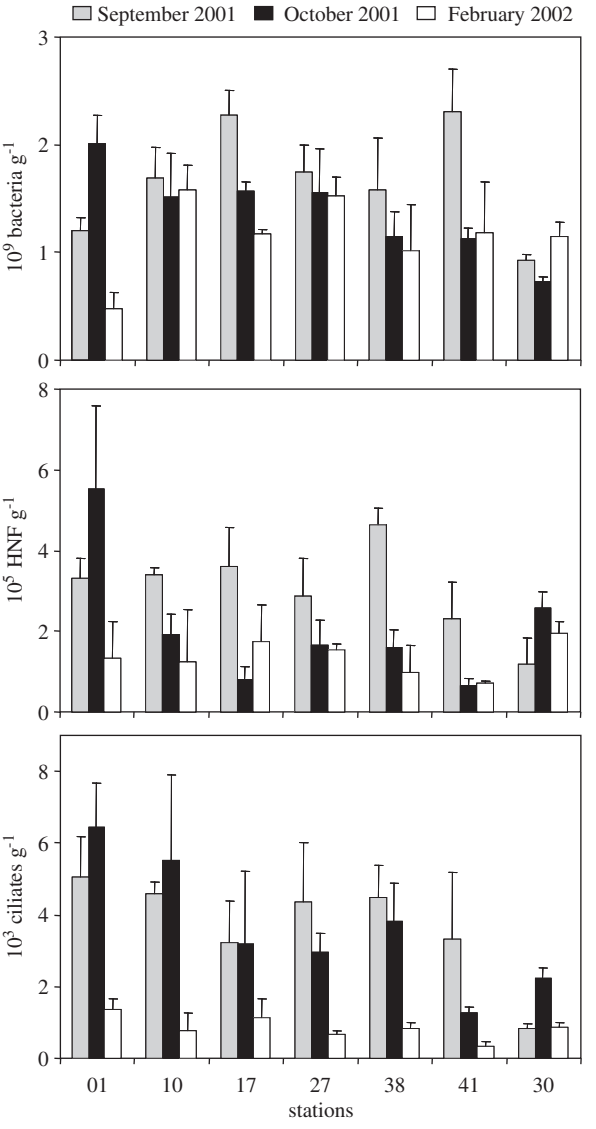

Fig. 3. Mean measurements of bacterial, heterotrophic nanoflagellates (HNAN) and ciliates abundance of the sampling stations, in September 2001, October 2001 and February 2002.

In all the sampling periods, heterotrophic nanobenthos was dominated by small cells (i.e. 2–5 μm in cell length, 74–82%); these were followed by cells in the size range of 5–10 μm (13–17%) and larger cells (10–20 μm, 4–9%). The ratio of bacterium to HNAN abundance, as well as the ratio of ciliate to HNAN abundance, increased significantly in October 2001 during intensive trawling (ANOVA, $P<0.05$; Fig. 4). At the reference station (30) the ratio of bacterium to HNAN abundance decreased (Fig. 4), whereas the maximum change in both ratios was recorded at Station 17 (Fig. 4).

4.3. Potential turnover time of protein and carbohydrate pools

Protein and carbohydrate turnover time increased during trawling (Table 2); at the reference station (30), then decreased from September to October 2001 (Table 2). The entire protein pool was degraded potentially in 4 days in September 2001, 6 days in October 2001 and 8 days in February 2002 (Table 2). Carbohydrate turnover time at the impacted stations decreased from September 2001

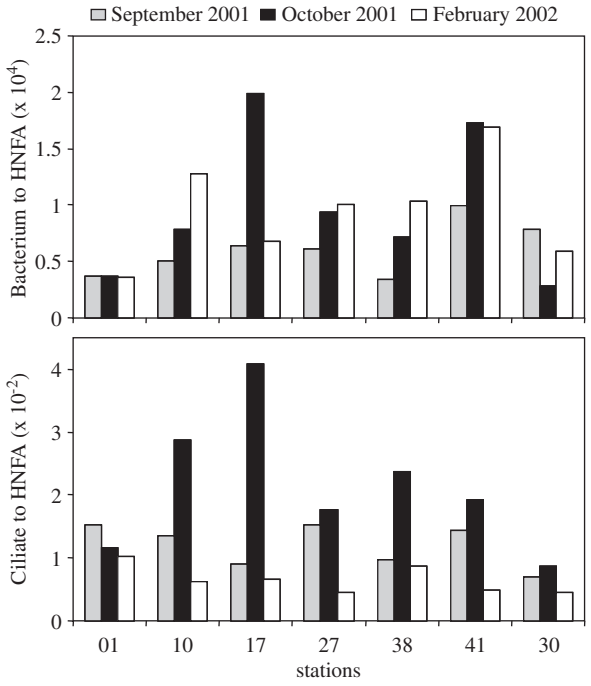

Fig. 4. Values of bacterium to HNAN abundance and ciliate to HNAN abundance ratio in the upper first centimetre of the study area sediments, during the three sampling periods (September 2001, October 2001 and February 2002).

(152 days) to October 2001 (138 days), but increased in February 2002 (193 days) (Table 2). In comparison, the reference station (30) displayed an increasing trend throughout the sampling periods (80, 370 and 5078 days in September, October 2001 and February 2002, respectively; Table 2).

4.4. Microbial activities

Aminopeptidase activity (L-MCA, Fig. 5) increased significantly from September to October 2001, then dropped significantly in February 2002 (ANOVA, $P<0.05$). β-D-glucosidase activity (β-Glu, Fig. 5) did not change between September and October 2001, but significantly decreased in February 2002 (ANOVA, $P<0.05$). The aminopeptidase to β-D-glucosidase ratio ranged from 16–27 in September 2001, to 15–160 in October 2001 and 8–824 in February 2002.

Bacterial C production values (Fig. 5) increased significantly (ANOVA, $P<0.05$) from September to October 2001, then decreased in February 2002 (ANOVA, $P<0.05$). In October 2001, Station 01 exhibited the highest recorded value of bacterial C production (Fig. 5).

Bacterial C efficiency did not change significantly between the sampling periods, with the exception of the most impacted station (01) which increased sharply in October 2001 (Fig. 5). C mineralisation and SCOC rates displayed significant temporal changes (ANOVA, $P<0.05$; Fig. 5). C mineralisation rates increased significantly (ANOVA, $P<0.05$) from September 2001 to October 2001, decreasing strongly in February 2002 (Fig. 5). Compared to the non-trawled Station 30, C mineralisation rates at both Stations 01 and 17 were significantly higher in October 2001, than in the pre-trawling period. SCOC values decreased, from September to October to February (ANOVA, $P<0.05$) (Fig. 5); whereas a strong decrease was also measured at the most impacted Station 01, in October 2001.

5. Discussion

In this paper, benthic microbial abundance and activities as well as the biochemical composition of sedimentary organic matter were measured in Thermaikos Gulf, before and during intense trawling activities. Significant changes in the relative importance of benthic microbial components were recorded during the 3 sampling periods. This

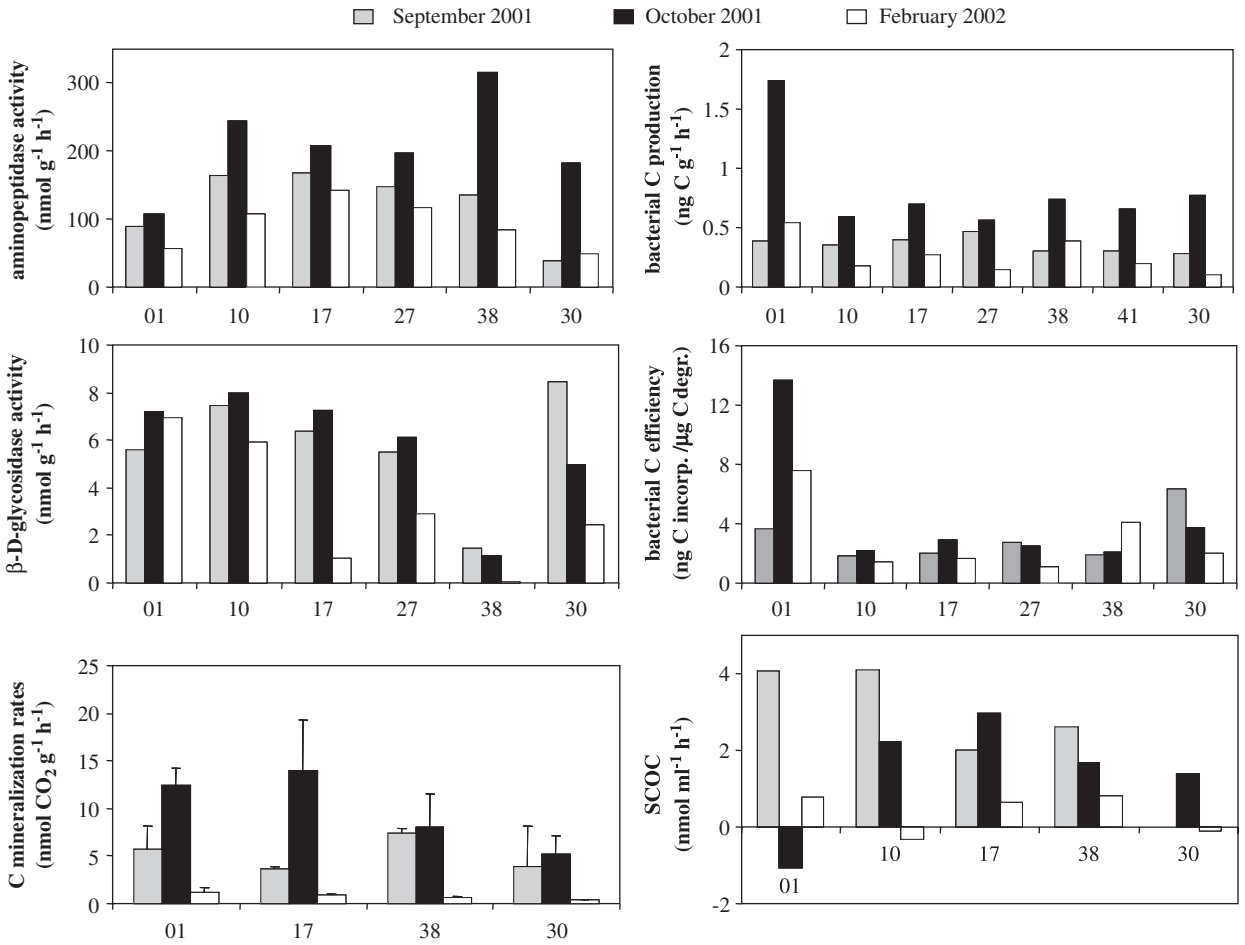

Fig. 5. Mean measurements of aminopeptidase activity, β-D-glucosidase activity, C mineralisation rates, bacterial C production, bacterial C efficiency, and sediment community oxygen consumption rates of the sampling stations, in September 2001, October 2001 and February 2002.

pattern was expressed as a sudden increase in the bacterial C production in October 2001, whereas the effects of trawling on the microbial food web were less evident in February 2002.

5.1. Organic matter availability at the seafloor

Organic matter availability is one of the most important limiting factors for benthic microbial communities (Manini et al., 2003). In this study, organic matter availability was estimated by analyzing the biochemical composition of the sediment organic matter, during the 3 sampling periods.

The accumulation of BPC is the end result of a balance between allochthonous inputs, production, degradation, mineralisation and export. These processes are regulated mostly by the biochemical composition of the BPC pools (e.g. proteins, carbohydrates and lipids) (Manini et al., 2003). TOC concentrations in the coastal sediments of Thermaikos Gulf (ranging 0.29–1.40%) displayed concentrations similar to those reported from other Mediterranean coastal areas (Buscail et al., 1995; Tselepides et al., 2000). The highest concentrations, at Station 01, were most likely the result of important riverine inputs and enhanced primary production in the northern area of the Gulf.

Comparing the 3 sampling periods, the sediments of Thermaikos Gulf displayed the highest protein concentrations in October 2001, whilst TOC and TON concentrations as well as the contribution of the different biochemical classes of organic compounds to BPC, changed significantly during trawling. In September 2001, carbohydrates represented the major biochemical class (accounting for 45.51–58.90% of the total BPC concentration); in

October and February, proteins dominated the sediments of Thermaikos Gulf (Table 2).

Food availability at the sea floor was estimated also, by analysing sediment chloroplastic pigments, indicating the input of phytodetrital matter to the benthos. During the present study, the lack of significant changes in the phytopigment content of the sediments between, the sampling periods of September and October 2001, indicates that primary production processes and/or vertical fluxes of material sinking from the upper water column did not change significantly in the Gulf.

5.2. Microbial abundance and activity

Bacteria and their protistan grazers play a major role in the functioning of the pelagic foodwebs and the regulation of the major biogeochemical cycles (e.g. carbon) (Epstein and Rossel, 1995). Phagotrophic protists, including HNAN and ciliates have been reported as the dominant bacterivores in many ecosystems (Hondeveld et al., 1995). The net growth of these communities results from the difference between the organic matter input and organic matter removal, as a consequence of grazing, mineralisation, degradation and uptake (Epping and Kühl, 2000).

Bacterial abundance in the surface sediments of Thermaikos Gulf, during all the sampling periods, exceeded largely the literature values reported for coastal sediments, as well as continental and bathyal sediments (Boetius et al., 1996; Danovaro et al., 2000) of both oligotrophic and organically enriched areas (Böttcher et al., 2000; Fabiano et al., 2003); this is probably as the result of high organic matter availability.

Small size HNAN (2–5 μm) were the dominant protozoa, at all sampling stations during the three seasons. This pattern is in accordance with previously reported data, due to the development of suitable techniques for protozoa extraction from the sediments (Hamels et al., 2004). Both HNAN and ciliate densities were comparable to those found in other sediments obtained from the North Sea (Hondeveld et al., 1995) and from a polyhaline intertidal estuary in NW Europe (Hamels et al., 2004); likewise. They were significantly higher than those obtained from a sandy tidal flat in Massachusetts (Epstein, 1997b).

The coupling between microbial parameters, during the three sampling periods was rather weak. With the exception of a marine intertidal flat composed of fine sandy sediments (Epstein, 1997a), benthic flagellates (Hondeveld et al., 1995) and ciliates (Kemp, 1988) have been shown repeatedly to be of little to moderate importance in controlling bacterial production in marine sediments (Kemp, 1990). A possible reason for the scarcity of correlation between the microbial components is the interference of covariables, such as cell size and activity, the grain size distribution and the interactions with meio- and macro-benthic organisms (Duineveld et al., 2000). Another possible explanation for the observed uncoupling between bacterial abundance and bacterial production, could be also the pitfalls in the staining procedure. Fluorochromes, such as acridine orange as well as 4′,6′-diaminodino-2-phenylindole hydrochloride (DAPI), overestimate usually the number of active bacteria due to staining of dead cells and non-bacterial particles (Novitsky, 1987). Luna et al. (2002) obtained live bacterial fractions of between 26% and 30% in coastal marine sediments, indicating that the cell-specific production obtained using total bacterial abundance is most likely underestimated.

The high levels of bacterial production in the study area suggest that a large fraction of organic matter was converted into bacterial biomass. The primary step of organic matter breakdown is mediated by extracellular enzymatic activities, that make organic macromolecules available for bacterial uptake (Manini et al., 2003). The measure of enzymatic activities provides also important evidence on the quality and quantity of organic matter available to heterotrophs. Moreover, although the rates should be taken into consideration with caution (because of the experimental conditions, i.e. optimal temperature and pH for enzymatic digestion and availability of substrate, at saturation), these measurements are a good proxy for the turnover rates of carbon- and nitrogen-enriched organic compounds (Fabiano and Danovaro, 1998). The recorded rates of enzymatic activity in our study area are comparable to those reported for the sediments of the North Aegean Sea (Bianchi et al., 2003), the deep sea sediments of the Northeastern Atlantic (Poremba, 1995) and the Celtic Sea (Poremba and Hoppe, 1995).

Values of aminopeptidase activity appear to be at least two orders of magnitude higher than those recorded for β-D-glucosidase. The prevalence of aminopeptidase activity, over that of the β-D-glucosidase has been recorded as a common feature

in oceanic oligotrophic areas (Misic et al., 2002). Previous studies undertaken in other coastal and deep-sea environments (Christian and Karl, 1995; Kim, 1990) have reported that aminopeptidase displayed generally the highest activity, at about 1000 times higher than β-D-glucosidase activity. However, in our case, the aminopeptidase activity exceeded β-D-glucosidase by a factor of 8–160, with the exception at the reference station (30) in February 2002 (with a factor of 824). Since enzyme activities are known to be stimulated by organic matter availability, this low ratio can be explained as being the result of the high nutritional quality of the carbohydrate pools (Pusceddu et al., 2005). In addition, rates of bacterial C production and C mineralisation follow a profile similar to that of aminopeptidase activity, with the maximum values recorded in October 2001.

5.3. The effect of trawling

Although Thermaikos Gulf is characterised by strong hydrographic features, bottom sediments are usually not scoured by tidal flows, due to the relatively high water column depth (up to 200 m). The study area is subjected to strong trawling for most of the year (up to 8 months), resulting in sediment resuspension throughout most of the sampling time. Thus, due to the difficulties involved of selecting an appropriate reference area, a station distinct from all other stations was finally selected. Although the reference station is located away from the riverine-influenced flow and all the trawled stations, concurrent studies during the INTERPOL project have revealed that, during the pre-trawled and trawled season, the physico-chemical conditions of the water column, the riverine inputs and the sediment properties did not change significantly (Lampadariou et al., 2005; Pusceddu et al., 2005; and Zervakis et al., 2005). These observations suggest that the non-trawled Station 30 can be used as a reference station.

For the most part, there were no significant differences between the trawled stations, with respect to sediment chemistry, microbial abundance and activities (Figs. 2–5), whereas the environmental parameters of the non-trawled station were generally low. Since organic matter quantity and biochemical composition of the reference station (30) did not change significantly between the sampling periods (Fig. 2), the recorded changes in the sedimentological variables of the impacted stations in October 2001, compared to the pre-trawled season, could be attributed possibly to the influence exerted by trawling. These analyses have revealed that bottom trawling possibly had an effect on the trophic state of the sediment in Thermaikos Gulf.

The significant increase in the ratio of bacterium to HNAN abundance and in the ratio of ciliate to HNAN abundance, recorded in October 2001 (during the intensive trawling), suggests that among the microbial components investigated HNAN were the most sensitive fraction. This finding confirms the results of previous studies (Fiordelmondo and Pusceddu, 2004; Wieltschning et al., 2001) and suggests that heterotrophic nanobenthos are affected particularly by sediment particle resuspension (i.e. mechanical disturbance) induced by the trawlers.

Statistical analysis revealed also that among the measured biological variables, bacterial C production, aminopeptidase activity and C mineralisation rates changed significantly during trawling; the highest recorded values were at stations located in the northwestern region of Thermaikos Gulf (e.g. Stations 01, 10, 17), where the trawling activity was more intense. The highest aminopeptidase activities in the sediments of Thermaikos Gulf were recorded in October 2001. Bacterial C production and C mineralisation rates, in the sediments of Thermaikos Gulf, displayed also maximum values in October 2001; this suggests that trawling had 'positive' effects on food availability, for the benthic microbial assemblages. On the other hand, all the variables of the microbial food web were found to be significantly lower in February 2002; this is due probably to the rapid decrease in temperature, which is an important factor which controls microbial activity (Mayer, 1989).

The increase in turnover time, of both protein and carbohydrate pools during trawling, can be related to the 'uplift' of deposited organic C from the deeper sediment layers; that indicates that the increased degradation rates of organic C were stimulated mostly by the availability of sediment organic matter. Thus, the effect of sediment resuspension caused by trawling events has a positive feedback on the trophic state of the system. In fact, the trawling activities may stimulate nutrient cycling through the acceleration of organic matter degradation rates and, at the same time, enhance the amount of available organic C to the surface layers of the seabed. The latter phenomenon may affect benthic oxygen consumption and, as a

consequence, the oxygen availability at the sediment water interface may be diminished; this could lead to possible hypoxic or anoxic episodes, which could be stressful to the benthos.

Oxygen is the terminal electron acceptor with the highest free energy yield; until it is consumed entirely, it is the most important variable controlling the redox chemistry of surface sediments (Warnken et al., 2003). Rates of oxygen consumption represent also the summation of carbon consumption, by all benthic communities. Therefore, investigating the effect of trawling on oxygen consumption rates is of great importance, since a significant change in oxygen availability, as well as the removal of the upper oxic sediment layers, can lead to changes in benthic–pelagic coupling (Warnken et al., 2003).

The oxygen results from the in situ benthic chamber deployments indicated that trawling reduced SCOC rates in October 2001. This effect was more evident at the stations heavily impacted upon by trawling (stations 01, 10), indicating that aerobic respiration diminished probably due to increased oxygen consumption, caused by C mineralisation. The measured rates were reduced also in February 2002 but, most likely, as a direct result of the lack of benthic biological activity due to the rapid decrease in temperature. Conversely, the fraction of C incorporated into the bacterial biomass to the amount of C that can be degraded enzymatically (bacterial C efficiency; Fig. 5), increases during October 2001; this indicates that the immediate effects of trawling are the facilitation of bacterial activities and C incorporation rates.

Schratzberger and coworkers (Schratzberger and Jennings, 2002; Schratzberger et al., 2002) have suggested that the food consumption rates of macrobenthic communities, after trawling events, decrease significantly due to mortality; this implies that production (fixed carbon), arriving from the photic zone, is processed by other organisms more resistant to trawling (Schratzberger et al., 2002). Lampadariou et al. (2005), in their analysis of the impacts of trawling on the meiobenthic assemblages of Thermaikos Gulf, indicate a lack of response at the major taxon level. In fact, trawling could be beneficial to the very small-sized animals and microbiota, which can survive the passage of the trawl and take advantage of the release of organic matter and nutrients (Lampadariou et al., 2005). Our results indicate that the difference in trophic state, imposed by the trawling activities, has an effect on the relationships between microbial components, as well as on bacterial production and degradation ability in the sediments. These results demonstrate the key role of sediment resuspension in the early diagenesis of sediment organic matter.

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References

Andrews, D., Bennett, A., 1981. Measurements of diffusivity near the sediment–water interface with a fine scale resistivity probe. Geochimica et Cosmochimica Acta 45, 2169–2175.

Arfi, R., Bouvy, M., 1995. Size, composition and distribution of particles related to wind-induced resuspension in a shallow tropical lagoon. Journal of Plankton Research 17, 557–574.

Bak, R.P.M., Nieuwland, G., 1989. Seasonal fluctuations in benthic protozoan populations at different depths in marine sediments. Netherlands Journal of Sea Research 24, 37–44.

Barnett, P.R.P., Watson, J., Connelly, D., 1984. A multiple corer for taking virtually undisturbed sediment samples from shelf bathyal and abyssal sediments. Oceanologica Acta 7, 399–408.

Bianchi, A., Tholosan, O., Garcin, J., Polychronaki, T., Tselepides, A., Buscail, R., Duineveld, G., 2003. Microbial activities at the benthic boundary layer in the Aegean Sea. Progress in Oceanography 57, 219–236.

Bligh, E.G., Dyer, W.J., 1959. A rapid method for total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37, 911–917.

Boetius, A., Scheibe, S., Tselepides, A., Thiel, H., 1996. Microbial biomass and activities in deep-sea sediments of the Eastern Mediterranean: trenches are benthic hotspots. Deep-Sea Research 43, 1439–1460.

Böttcher, M.E., Hespenheide, B., Llobet-Brossa, E., Beardsley, C., Larsen, O., Schramm, A., Wieland, A., Böttcher, G., Berninger, U.G., Amann, R., 2000. The biogeochemistry, stable isotope geochemistry, and microbial community structure of a temperate intertidal mudflat: an integrated study. Continental Shelf Research 20, 1749–1769.

Buscail, R., Pocklington, R., Germain, C., 1995. Seasonal variability of the organic matter in a sedimentary coastal environment: sources, degradation and accumulation (continental shelf of the Gulf of Lions–northwestern Mediterranean Sea). Continental Shelf Research 15, 843–869.

Carpenter, J.H., 1965. The Chesapeake Bay Institute technique for the Winkler dissolved oxygen method. Limnology and Oceanography 10, 141–143.

Christian, J.R., Karl, D.M., 1995. Bacterial ectoenzymes in marine waters: activity ratios and temperature responses in three oceanic provinces. Limnology and Oceanography 40, 1042–1049.

Cole, J.J., Pace, M.L., 1995. Why measure bacterial production? A reply to the comment by Jahnke and Craven. Limnology and Oceanography 40, 441–444.

Danovaro, R., Marrale, D., Dell'Anno, A., Della Croce, N., Tselepides, A., Fabiano, M., 2000. Bacterial response to seasonal changes in labile organic matter composition on the continental shelf and bathyal sediments of the Cretan Sea. Progress in Oceanography 46, 345–366.

Dauwe, B., Middelburg, J.J., Herman, P.M.J., 2001. Effect of oxygen on the degradability of organic matter in subtidal and intertidal sediments of the North Sea area. Marine Ecology Progress Series 215, 13–22.

Dayton, P.K., Thrush, S.F., Agardy, M.T., Hofman, R.J., 1995. Environmental effects of marine fishing. Aquatic Conservation: Marine Freshwater Ecosystem 5, 205–232.

Duineveld, G.C.A., Tselepides, A., Witbaard, R., Bak, R.P.M., Berghuis, E.M., Nieuwland, G., van der Weele, J., Kok, A., 2000. Benthic–pelagic coupling in the oligotrophic Cretan Sea. Progress in Oceanography 46, 457–480.

Epping, E., Kühl, M., 2000. The responses of photosynthesis and oxygen consumption to short-term changes in temperature and irradiance in a cyanobacterial mat (Ebro Delta, Spain). Environmental Microbiology 2, 465–474.

Epstein, S.S., 1995. Simultaneous enumeration of protozoa and micrometazoa from marine sandy sediments. Aquatic Microbial Ecology 9, 219–227.

Epstein, S.S., 1997a. Microbial food webs in marine sediments. I. Trophic interactions and grazing rates in two tidal flat communities. Microbial Ecology 34, 188–198.

Epstein, S.S., 1997b. Microbial food webs in marine sediments. II. Seasonal changes in trophic interactions in a sandy tidal flat community. Microbial Ecology 34, 199–209.

Epstein, S.S., Rossel, J., 1995. Enumeration of sandy sediment bacteria: search for optimal protocol. Marine Ecology Progress Series 117, 289–298.

Fabiano, M., Danovaro, R., 1998. Enzymatic activity, bacterial distribution, and organic matter composition in sediments of the Ross Sea (Antarctica). Applied and Environmental Microbiology 64, 3838–3845.

Fabiano, M., Marrale, D., Misic, C., 2003. Bacteria and organic matter dynamics during a bioremediation treatment of organic-rich harbour sediments. Marine Pollution Bulletin 46, 1164–1173.

Fiordelmondo, C., Pusceddu, A., 2004. Short-term response of benthic bacteria and nanoflagellates to sediment resuspension: an experimental study. Chemistry and Ecology 20, 107–121.

Fiordelmondo, C., Manini, E., Gambi, C., Pusceddu, A., 2003. Short-term impact of clam harvesting on sediment chemistry, benthic microbes and meiofauna in the Goro lagoon (Italy). Chemistry and Ecology 19, 173–187.

Garstecki, T., Wickham, S.A., Arndt, H., 2002. Effects of experimental sediment resuspension on a coastal planktonic microbial food web. Estuarine Coastal and Shelf Science 55, 751–762.

Gerchacov, S.M., Hatcher, P.G., 1972. Improved technique for analysis of carbohydrates in sediment. Limnology and Oceanography 17, 938–943.

Gislason, H., Sinclair, M., Sainsbury, K., O'Boyle, R., 2000. Symposium overview: incorporating ecosystem objectives within fisheries management. ICES Journal of Marine Science 57, 468–475.

de Groot, S.J., Lindeboom, H.J., 1994. Environmental impact of bottom gears on benthic fauna in relation to natural resources management and protection of the North Sea. Netherlands Institute for Sea Research, Texel.

Hamels, I., Sabbe, K., Muylaert, K., Vyverman, W., 2004. Quantitative importance, composition, and seasonal dynamics of protozoan communities in polyhaline versus freshwater intertidal sediments. Microbial Ecology 47, 18–29.

Hartree, E.F., 1972. Determination of proteins: a modification of the Lowry method that gives a linear photometric response. Analytical Biochemistry 48, 422–427.

Hedges, J.I., Stern, J.H., 1984. Carbon and nitrogen determinations of carbonate-containing solids. Limnology and Oceanography 29, 657–663.

Hondeveld, B.J.M., Nieuwland, G., van Duyl, F.C., Bak, R.P.M., 1995. Impact of nanoflagellate bacterivory on benthic bacterial production in the North Sea. Netherlands Journal of Sea Research 34, 275–287.

Hoppe, H.G., 1993. Use of fluorogenic model substrates for extracellular enzyme activity (EEA) of bacteria. In: Kemp, P.F., Sherr, B.F., Sherr, E.B., Cole, J.J. (Eds.), Handbook of Methods in Aquatic Microbial Ecology. Lewis Publishers, Boca Raton, FL, pp. 423–431.

Karageorgis, A.P., Anagnostou, C.L., 2001. Particulate matter spatial-temporal distribution and associated surface sediment properties: Thermaikos Gulf and Sporades Basin, NW Aegean Sea. Continental Shelf Research 21, 2141–2153.

Kemp, P.F., 1988. Bacterivory by benthic ciliates: significance as a carbon source and impact on sediment bacteria. Marine Ecology Progress Series 49, 163–169.

Kemp, P.F., 1990. The fate of benthic bacterial production. Reviews in Aquatic Sciences 2, 109–124.

Kim, S.J., 1990. Bacterial number, heterotrophy and extracellular enzyme activity in Bransfield Strait, Antarctica. Korean Journal of Polar Research 2, 9–16.

Kirchman, D.L., 1993. Leucine incorporation as a measure of biomass production by heterotrophic bacteria. In: Kemp, P.F., Sherr, B.F., Sherr, E.B., Cole, J.J. (Eds.), Handbook of Methods in Aquatic Microbial Ecology. Lewis Publishers, Boca Raton, FL, pp. 509–512.

Lampadariou, N., Hatzyianni, E., Tselepides, A. 2005. Meiofaunal community structure in Thermaikos Gulf: response to intensive trawling pressure. Continental Shelf Research, This issue.

Lindeboom, H.J., de Groot, S.J., 1998. The effects of different types of fisheries on the North Sea and Irish Sea benthic ecosystems. Netherlands Institute of Sea Research, Texel.

Lorenzen, C., Jeffrey, J., 1980. Determination of chlorophyll in seawater. UNESCO Technical Papers in Marine Sciences 35, 1–20.

Luna, G.M., Manini, E., Danovaro, R., 2002. Large fraction of dead and inactive bacteria in coastal marine sediments: comparison of protocols for determination and ecological significance. Applied and Environmental Microbiology 68, 3509–3513.

Lykousis, V., Chronis, G., 1989. Mechanisms of sediment transport and deposition: sediment sequences and accumulation during

the holocene on the Thermaikos Plateau, the continental slope, and basin (Sporadhes Basin), Northwestern Aegean Sea, Greece. In: Makris, J. (Ed.), Geological Aspects and Tectonic Evolution of Mediterranean Seas. Marine Geology 87, 15–26.

Manini, E., Fiordelmondo, C., Gambi, C., Pusceddu, A., Danovaro, R., 2003. Benthic microbial loop functioning in coastal lagoons: a comparative approach. Oceanologica Acta 26, 27–38.

Marsh, J.B., Weinstein, D.B., 1966. A simple charring method for determination of lipids. Journal of Lipids Research 7, 574–576.

Mayer, L.M., 1989. Extracellular proteolytic enzyme activity in sediments of an intertidal mudflat. Limnology and Oceanography 34, 973–981.

Meyer-Reil, L.A., Koester, M., 1992. Microbial life in pelagic sediments: the impact of environmental parameters on enzymatic degradation of organic material. Marine Ecology Progress Series 81, 65–72.

Misic, C., Povero, P., Fabiano, M., 2002. Ectoenzymatic ratios in relation to particulate organic matter distribution (Ross Sea, Antarctica). Microbial Ecology 44, 224–234.

Montagna, P.A., 1982. Sampling design and enumeration statistics for bacteria from marine sediments. Applied and Environmental Microbiology 43, 1366–1372.

Novitsky, J.A., 1987. Microbial growth rates and biomass production in a marine sediment: evidence for a very active but mostly nongrowing community. Applied and Environmental Microbiology 53, 2368–2372.

Pearson, T.H., Stanley, S.O., 1979. Comparative measurements of the redox potential of marine sediments as a rapid means of assessing the effect of organic pollution. Marine Biology 53, 371–379.

Pilskaln, C.H., Churchill, J.H., Mayer, L.M., 1998. Resuspension of sediment by bottom trawling in the Gulf of Maine and potential geochemical consequences. Conservation Biology 12, 1223–1229.

Poremba, K., 1995. Hydrolytic enzymatic activity in deep-sea sediments. FEMS Microbiology Ecology 16, 213–222.

Poremba, K., Hoppe, H.G., 1995. Spatial variation of benthic microbial production and hydrolytic enzymatic activity down the continental slope of the Celtic Sea. Marine Ecology Progress Series 118, 237–384.

Poulos, S.E., Chronis, G.Th., Collins, M.B., Lykousis, V., 2000. Thermaikos Gulf coastal system, NW Aegean Sea: an overview of water/sediment fluxes in relation to air–land–ocean interactions and human activities. Journal of Marine System 25, 47–76.

Pusceddu, A., Fiordelmondo, C., Polymenakou, P., Polychronaki, T., Tselepides, A., Danovaro, R., 2005. Effects of bottom trawling on the quantity and biochemical composition of organic matter in coastal marine sdiments (Thermaikos Gulf, northwestern Aegean Sea). Continental Shelf Research, this issue, doi:10.1016/j.csr.2005.08.013.

Rice, D.L., 1982. The detritus nitrogen problem: new observations and perspectives from organic geochemistry. Marine Ecology Progress Series 9, 153–162.

Ritzrau, W., Graf, G., 1992. Increase of microbial biomass in the benthic turbidity zone of Kiel Bight after resuspension by a storm event. Limnology and Oceanography 37, 1081–1086.

Sánchez, P., Demestre, M., Martin, P., 2004. Characterisation of the discards generated by bottom trawling in the northwestern Mediterranean. Fish Research 67, 71–80.

Schratzberger, M., Jennings, S., 2002. Impacts of chronic trawling disturbance on meiofaunal communities. Marine Biology 141, 991–1000.

Schratzberger, M., Dinmore, T.A., Jennings, S., 2002. Impacts of trawling on the diversity, biomass and structure of meiofauna assemblages. Marine Biology 141, 83–93.

Sherr, E.B., Caron, D.A., Sherr, B.F., 1993. Staining of heterotrophic protists for visualization via epifluorescence microscopy. In: Kemp, P.F., Sherr, B.F., Sherr, B.E., Cole, J.J. (Eds.), Aquatic Microbial Ecology. Lewis Publishers, Boca Raton, FL, pp. 213–227.

Smith, D.C., Azam, F., 1992. A simple, economical method for measuring bacterial protein synthesis rates in seawater using ^{3}H-leucine. Marine Microbial Food Webs 6, 107–114.

Sparks-McConkey, P.J., Watling, L., 2001. Effects on the ecological integrity of a soft-bottom habitat from a trawling disturbance. Hydrobiologia 456, 73–85.

Stergiou, K.I., Christou, E.D., Georgopoulos, D., Zenetos, A., Souvermezoglou, E., 1997. The Hellenic seas: physics, chemistry, biology and fisheries. Oceanology and Marine Biology: an Annual Review 35, 415–538.

Thrush, S.F., Dayton, P.K., 2002. Disturbance to marine benthic habitats by trawling and dredging: implications for marine biodiversity. Annual Review of Ecology and Systematics 33, 449–473.

Tselepides, A., Polychronaki, T., Marrale, D., Akoumianaki, I., Dell'Anno, A., Pusceddu, A., Danovaro, R., 2000. Organic matter composition of the continental shelf and bathyal sediments of the Cretan Sea (NE Mediterranean). Progress in Oceanography 46, 311–344.

Turley, C.M., 1999. The changing Mediterranean Sea—a sensitive ecosystem? Progress in Oceanography 44, 387–400.

van Duyl, F.C., Kop, A.J., 1994. Bacterial variation in North Sea sediments: clues to seasonal and spatial variations. Marine Biology 120, 323–337.

Wainright, S.C., 1990. Sediment-to-water fluxes of particulate material and microbes by resuspension and their contribution to the planktonic food web. Marine Ecology Progress Series 62, 271–281.

Warnken, K.W., Gill, G.A., Dellapenna, T.M., Lehman, R.D., Harper, D.E., Allison, M.A., 2003. The effects of shrimp trawling on sediment oxygen consumption and the fluxes of trace metals and nutrients from estuarine sediments. Estuarine Coastal and Shelf Sciences 57, 25–42.

Watling, L., Findlay, R.H., Mayer, L.M., Schick, D.F., 2001. Impact of a scallop drag on the sediment chemistry, microbiota, and faunal assemblages of a shallow subtidal marine benthic community. Journal of Sea Research 46, 309–324.

Wieltschning, C., Kirschner, A.K.T., Steitz, A., Velimirov, B., 2001. Weak coupling between heterotrophic nanoflagellates and bacteria in eutrophic freshwater environment. Microbial Ecology 42, 159–167.

Yentsch, C.S., Menzel, D.W., 1963. A method for the determination of phytoplankton chlorophyll and phaeophytin by fluorescence. Deep-Sea Research 10, 221–231.

Zervakis, V., Karageorgis, A., Kontoyiannis, H., Papadopoulos, V., Lykousis, V., 2005. Hydrology, circulation and distribution of particulate matter in the Thermaikos Gulf (NW Aegean Sea) during September–October 2001 and February 2002. Continental Shelf Research, This issue.