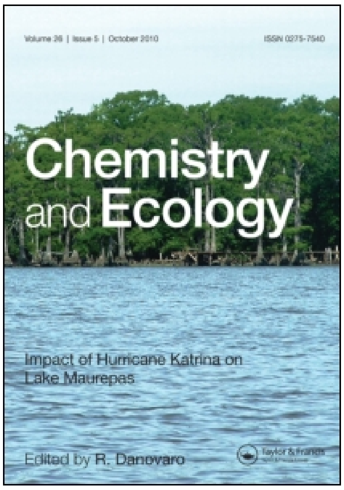

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Community Experiments Using Benthic Chambers: Microbial Significance in Highly Organic Enriched Sediments

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COMMUNITY EXPERIMENTS USING BENTHIC CHAMBERS: MICROBIAL SIGNIFICANCE IN HIGHLY ORGANIC ENRICHED SEDIMENTS

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The structure of the benthic microbial loop was studied in order to understand heterotrophic pathways in the suboxic sediments of the Rapallo Harbour in autumn, 1996. Sediments were characterized by the large accumulation of organic detritus (17.2 – 21.4 μg chloroplastic pigment equivalents (CPE)g^{-1}; carbohydrates and proteins: 7.8 – 16.7 and 6.7 – 7.5 mg g^{-1}). Due to the high organic load, benthic bacteria and protozoa displayed extremely high densities (1.4×10^9 cells g^{-1} and 26.9×10^5 cells g^{-1}). Meiofauna, protozoa and bacteria showed an approximate biomass ratio of 1:2:20. The presence of large amounts of organic matter appeared to determine a shift of the benthic size structure toward the increasing dominance of the smaller components of the benthic food webs. These data indicate that the sediments of the Rapallo Harbour were dominated by microbial biomasses to a larger extent than in non-food limited environments, characterized by a lower organic contamination. On the results presented in this study, the microbial dominance in highly organic enriched sediments can be explained with: (1) a reduction of the top-down control and grazing pressure of meio- and macrofauna on the microbial components; (2) the opportunistic composition and high metabolic activity of the microbial components.

Keywords: Benthic chambers; benthic microbial loop; microphytobenthos; bacteria

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INTRODUCTION

Azam *et al.* (1983) hypothesized that microbial components of the aquatic food web are likely to play a major role in the utilization of dissolved organic compounds (DOM) produced by phytoplankton (as exudates) or by higher trophic levels. Due to their high carbon conversion efficiencies, bacteria are able to convert DOM into microbial biomass. This fact might have a profound implication in the efficiency of material and energy transfer to higher trophic levels since bacteria are largely consumed (up to 90% per day) by protozoa (mainly heterotrophic nanoflagellates). These microbial components might be, in turn, utilized by mesozooplankton, therefore contributing to supply additional highly nutritional food sources to the "classical" pelagic food web. Such DOM channeling into a microbial pathway has been defined as the "microbial loop".

Although a large literature is available on the pelagic microbial loop (Sherr and Sherr, 1991), much less is known on the structure and role in benthic ecosystems (Kemp, 1988; Bak and Nieuwland, 1989; Hondeveld *et al.*, 1994). To date, it has been demonstrated that benthic bacteria play an essential role in recycling sedimentary organic detritus otherwise lost to marine food webs (Deming and Barross, 1993). The key steps of this trophic pathway are the production of nutritionally valuable bacterial biomass *via* consumption of detrital (particulate and/or dissolved) organic matter, largely composed of highly refractory compounds, and the transfer of this secondary production to higher trophic levels (protozoa, meio- and macrofauna). Degradation and remineralization rates of organic matter are generally much higher in shallow marine sediments and are constrained by the composition and metabolic activity of the benthic fauna. However, quantitative information on the main components of the benthic microbial loop (*i.e.*, organic detritus, bacteria, protozoa and meiofauna) is extremely limited (Alongi, 1987; Cunningham and Ustach, 1992; Kemp, 1994; Danovaro, 1996), especially in organic-enriched sediments.

In recent years, the high anthropogenic inputs of nutrients have enhanced the pelagic algal growth, followed by increasing organic particle flux towards the coastal sediments. Such particle flux has been demonstrated to enhance benthic oxygen demand (Jensen *et al.*, 1990;

Hansen and Blackburn, 1991), increasing the metabolic activity of benthic organisms. In highly eutrophic conditions, the increased metabolism may result in oxygen-depleted bottom waters and ultimately in the death of benthic infauna and fishes (Hansen and Blackburn, 1992).

Among the different sources of anthropogenic inputs of organic matter to the sediments, oil spills represent one of the major sources of food for the marine environment. However, our knowledge of the impact of oil on the structure and functioning of natural ecosystems is far from complete. In the Mediterranean Sea about $600-800 \times 10^6$ tons of hydrocarbons are transported each year, equivalent to about 30% of the world maritime transport of crude oil. Extraction activities further contribute to the release of hydrocarbons at sea at about 300.00 tons of crude oil are dispersed in the Mediterranean every year.

The rapid response of the benthic metabolism to organic input from the overlying water column has received considerable attention (Graf *et al.*, 1982, 1983; Van Duyl *et al.*, 1992; Fabiano *et al.*, in press). Several papers have demonstrated that both quality and quantity of organic matter reach the sediment surface in the form of sedimenting particles, and can be assumed to be one of the major factors influencing benthic community structure and metabolism (Graf *et al.*, 1983; Grant and Hargrave, 1987).

Previous studies to evaluate the effect of organic and/or hydrocarbon disturbance on the environment have considered benthos as a "retentive system" able to record the biological processes occurring in the entire ecosystem. In particular, the macrobenthic community structure was utilized in the assessment of the pollution of a certain area. On the light of the increasing importance of the microbial loop, both in terms of structure and functioning, recent studies have stressed the importance of considering, among the several compartments, the response of communities of size smaller than macrobenthos. Microbenthos (*i.e.*, microphytobenthos, bacteria and protozoa) and meiofauna appear adequate for environmental studies because of their high sensitivity, short generation time and, consequently, short response to disturbance events.

A general discussion of the effects of organic enrichment on microbenthic community structure and functioning is complex because organic impact can vary as a consequence of many factors. Among these quantity and quality of the organic input, the time of the year, the degree of exposure of the affected area, the hydrological features of the area and other environmental characteristics (*i.e.*, temperature, salinity) have been discussed extensively. All these factors, indeed, may affect the microbial loop functioning with important implications in terms of organic matter diagenesis, burial, and recycling. Moreover, the interpretation of the field results on the effects of organic enrichments is complicated by the limited knowledge on the whole structure of the benthic communities (from bacteria to macrofauna) and in most cases to the general lack of information on "pre-impact" conditions.

In the present paper, the ecological role of benthic microbial communities in an organic-enriched environment has been investigated. Benthic respiration was compared to the structure of benthic assemblages (bacteria, protozoa and meiofauna) and to the composition of the sediment organic matter. Results of this experimental design have been compared also with other studies carried out at different levels of organic matter load and composition.

MATERIAL AND METHODS

Study Area and Sampling

The study site was used from September 30 to October 4, 1996 in the Rapallo Harbour, Golfo Marconi, Ligurian Sea (Fig. 1). The Golfo Marconi area has been intensively investigated over the past 10 years for the dynamics of benthic bacteria, macrofauna, phytoplankton, particulate matter composition, seasonal cycles of sedimentation and primary production (Fabiano *et al.*, 1984; Albertelli and Fabiano, 1990; Danovaro, 1993). Silty sediments and large inputs of organic matter characterize the Rapallo Harbour.

A benthic chamber (~ 120 l, Ciceri *et al.*, 1992) was placed at 5 m depth by SCUBA divers for a period of 71 h to determine rates of oxygen consumption. Oxygen and pH levels were controlled during the chamber by injections of oxygen and sodium hydroxide. Water circulation inside the chamber was regulated and ensured using a magnetic stirrer.

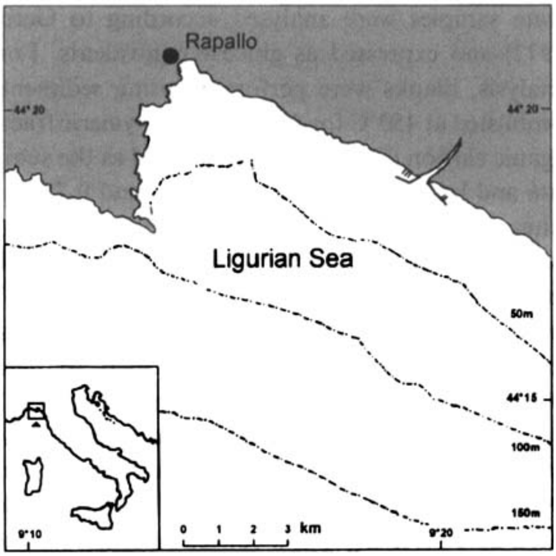

FIGURE 1 Sampling area in Italy and station location.

For bacterial and protozoan analyses, replicate cores ($n = 3$) were collected from the same surface sediments (0 – 1 cm), using sterilised 10 ml tubes and processed within two hours from collection.

An additional sediment core was collected, sliced into 5 horizons (0 – 2, 2 – 4, 4 – 6, 6 – 8 and 8 – 10 cm), and stored at – 20°C for the analysis of lipids, carbohydrates, proteins and chloroplastic pigments. All samples for biological and chemical analyses were collected at the beginning and at the end of the chamber use.

Phytopigments and Biochemical Composition of Sedimentary OM

After sonication in deionized water, total lipids were extracted from sediments by direct elution with chloroform and methanol according to Bligh and Dyer (1959) and Marsh and Weinstein (1966). Data are reported in tripalmitine equivalents. Protein (PRT) analyses of three replicates were conducted following extraction with sodium hydroxide (0.5M, 4h) and were determined according to Hartree (1972), modified by Rice (1982), to compensate for phenol interference and expressed as bovine serum albumin (BSA) equivalents. Carbohydrates (CHO) of three replicate samples were analysed according to Gerchacov and Hatcher (1972) and expressed as glucose equivalents. For each biochemical analysis, blanks were performed using sediments that had been pre-combusted at 450°C for 2 h. The biopolymeric fraction of sedimentary organic carbon (C-BPF) was calculated as the sum of protein, carbohydrate and lipid carbon using 0.49, 0.4 and 0.75 as conversion factors (Fichez, 1991).

Analyses of sedimentary chlorophyll-a (Chl-a) and phaeopigments (Phaeo) were carried out according to Lorenzen and Jeffrey (1980). Pigments were extracted with 90% acetone (24 h in the dark at 4°C). After centrifugation, the supernatant was used to determine the functional Chl-a and acidified with 0.1N hydrochloric acid to estimate the amounts of phaeopigments present (Plante-Cuny, 1974). Chloroplastic pigment equivalents (CPE) were calculated as the sum of chlorophyll-a and phaeopigments concentrations. Microphytobenthic carbon was calculated by converting Chl-a concentrations to carbon content (C-Chl-a) using a conversion factor of 40 (De Jonge, 1980). All data were normalised to dry weight after desiccation (60°C, 24 h).

Bacterial and Protozoan Analyses

Bacterial analyses were carried out as described by Danovaro and Fabiano (1995). Bacterial abundance and frequency of dividing cells were estimated. Bacterial biovolume was converted to carbon content assuming 310 fg C μm^{-3} (Fry, 1990). Microprotozoan density was estimated by a double-staining epifluorescence technique (Bak and Nieuwland, 1989) at $x = 1000$. All protozoa were measured with micrometric eye-pierce and converted to carbon content assuming $200 \times 10-9\,\mu g\,C\,\mu m^{-3}$ (Borsheim and Bratbak, 1987). All data were normalised to dry weight after desiccation (60°C, 24 h).

RESULTS AND DISCUSSION

Characterization of Sedimentary OM in the Rapallo Harbour

The concentrations of chlorophyll-a, phaeopigments, chloroplastic pigment equivalents, proteins, carbohydrates and lipids are reported in Table I.

TABLE I Biochemical composition of organic matter in the sediments of the Rapallo Harbour during the experiment. Chl-a = chlorophyll-a; Phaeo = phaeopigments; CPE = chloroplastic pigment equivalents (as sum of Chl-a and Phaeo); CHO = carbohydrates; PRT = proteins; LIP = lipids; C-BPF = biopolymeric carbon (as sum of carbohydrate, protein and lipid carbon)

Date	*Depth* (*cm*)	*Chl-a* $\mu g\,g^{-1}$	*std*	*Phaeo* $\mu g\,g^{-1}$	*std*	*CPE* $\mu g\,g^{-1}$	*std*	*CHO* $mg\,g^{-1}$	*std*	*PRT* $mg\,g^{-1}$	*std*	*LIP* $mg\,g^{-1}$	*std*	*CBPF* $mgC\,g^{-1}$
30 September	0–2	9.1	2.4	14.0	3.0	23.1	5.4	17.7	1.8	8.0	0.6	1.4	0.1	12.1
	2–4	10.1	1.0	7.8	2.4	17.9	3.4	17.1	6.7	6.2	0.9	1.1	0.2	10.7
	4–6	14.8	5.1	10.4	8.2	25.2	13.2	18.7	1.4	6.8	0.8	1.1	0.3	11.6
	6–8	12.9	5.4	10.7	7.8	23.6	13.2	19.0	4.1	7.3	1.1	0.6	0.2	11.6
	8–10	10.1	1.7	7.3	2.2	17.4	3.9	11.3	1.5	9.1	2.3	0.5	0.1	9.4
	Integrated (0–10)	11.4	0.9	10.0	1.1	21.4	7.8	16.7	1.3	7.5	0.5	0.9	0.1	11.1
4 October	0–2	7.1	0.6	11.1	1.3	18.2	2.0	9.4	0.6	5.9	0.9	1.1	0.2	7.5
	2–4	5.8	0.9	12.6	4.0	18.4	4.9	8.6	0.2	5.8	0.5	1.0	0.4	7.0
	4–6	9.3	1.8	7.7	1.1	17.0	2.8	8.2	2.9	8.3	2.1	1.1	0.2	8.2
	6–8	7.5	1.2	9.0	1.7	16.5	2.9	5.8	0.5	6.7	1.1	1.1	0.1	6.5
	8–10	6.5	0.7	9.5	0.2	16.0	0.9	7.1	0.5	6.9	2.2	1.7	0.3	7.5
	Integrated (0–10)	7.3	0.5	10.0	0.8	17.2	2.7	7.8	0.6	6.7	0.4	1.2	0.1	7.3

Chlorophyll-a concentrations in the Rapallo Harbour ranges from 5.8 to 14.8 $\mu g\,g^{-1}$. Chloroplastic pigment concentrations (CPE), ranged from 16.0 to 25.2 $\mu g\,g^{-1}$, indicating the presence of relatively large primary organic matter inputs. Chlorophyll-a concentrations reported for the Rapallo Harbour were about 3 to 4-fold higher than those reported for the coastal sediments of the Ligurian Sea (Danovaro *et al.*, 1996). Functional chlorophyll-a concentrations accounted for a significant fraction (about 48%) of CPE indicating that about half of the autotrophic biomass was active and probably of microphytobenthic origin.

The extremely high protein (range 5.8 – 9.1 $mg\,g^{-1}$) and carbohydrate (5.8 – 19.0 $mg\,g^{-1}$) concentrations and the fairly high lipid concentrations (0.5 – 1.7 $mg\,g^{-1}$) in the sediments of the Rapallo Harbour confirm the presence of large organic inputs. These values are about 10 – 20 times higher than those reported in other areas by highly productive systems such as seagrass beds and coastal lagoons (Danovaro, 1996; Pusceddu *et al.*, submitted).

Concentrations of the biopolymeric carbon in the sediments of the Rapallo Harbour (range from 6.5 to 12.1 mg $C\,g^{-1}$) were more than 100-fold higher than those reported in other adjacent coastal sediments (Fabiano *et al.*, 1995).

The relative significance of lipids, proteins and carbohydrates is shown in Figure 2. Carbohydrates were the dominant biochemical class of organic compounds, accounting on average for 58% of the biopolymeric matter (as the sum of lipids, proteins and carbohydrates), followed by proteins (37%) and lipids (5%). Such biochemical composition is very close to the one reported for most marine sediments, being similar to other highly productive-organic rich sediments (Pusceddu *et al.*, submitted), to marine cave sediments (Fichez, 1991) or to subtidal sandy sediments (Fabiano *et al.*, 1995). Therefore, the most striking difference between the study area and the other sites is not due to OM composition but to OM quantity.

In order to investigate the significance of the living component to the biopolymeric carbon pool, the contribution of the microphytobenthic biomass was estimated. Chlorophyll-a carbon (as microphytobenthic biomass) represented only a negligible fraction (on average 4%) of the total biopolymeric carbon. Therefore, more than 95% of the biopolymeric carbon in the Rapallo Harbour was of heterotrophic-

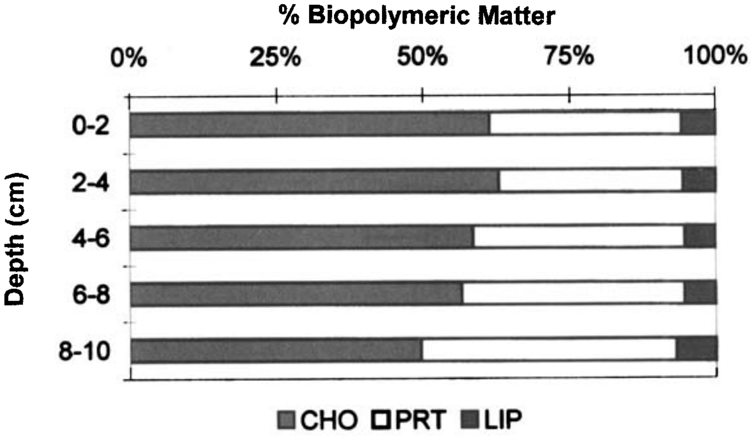

FIGURE 2 Biochemical composition of the biopolymeric matter: vertical contribution of carbohydrates, proteins and lipids (average of the two sampling periods).

detrital origin. However, the significant correlation found between CPE and C-BPF ($r = 0.821$, $p < 0.01$, $n = 9$) suggests that a coupling between primary production and the sediment organic matter concentration in the Rapallo Harbour. A significant correlation between CPE and carbohydrates was found ($r = 0.853$, $p < 0.01$, $n = 9$).

Vertical Distribution of Sedimentary OM

Vertical patterns of phytopigments investigated are illustrated in Figure 3. Chlorophyll-a concentrations displayed highest values in the 4–6 cm horizon, whereas phaeopigments were characterised by a slight decline with depth into the sediment core. These different patterns resulted in a quite constant concentration of CPE in the sediments.

Vertical patterns of carbohydrates, proteins and lipids are reported in Figure 4. According to CPE distribution, vertical patterns of carbohydrates displayed quite constant concentrations in the top 8-cm depth of the sediment core. This pattern was in contrast with that of proteins that increased in deeper sediment layers, and lipids displayed a peculiar vertical distribution, decreasing down to 8-cm depth and increased in the 8–10 cm horizon. The reasons for such different vertical patterns are largely unknown. From the available literature information, it

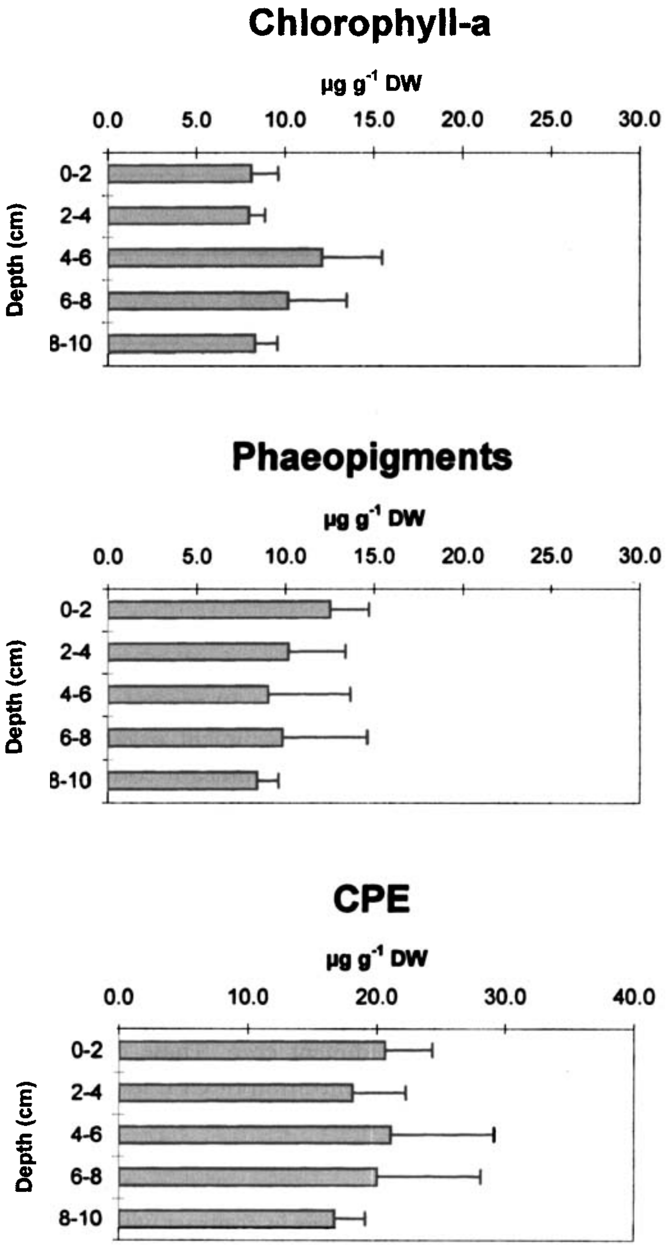

FIGURE 3 Vertical distribution of phytopigments (average of the two sampling periods). Reported are: (1) chlorophyll-a, (2) phaeopigments, (3) chloroplastic pigment equivalents (expressed as $\mu g\,g^{-1}$ sedimentary DW).

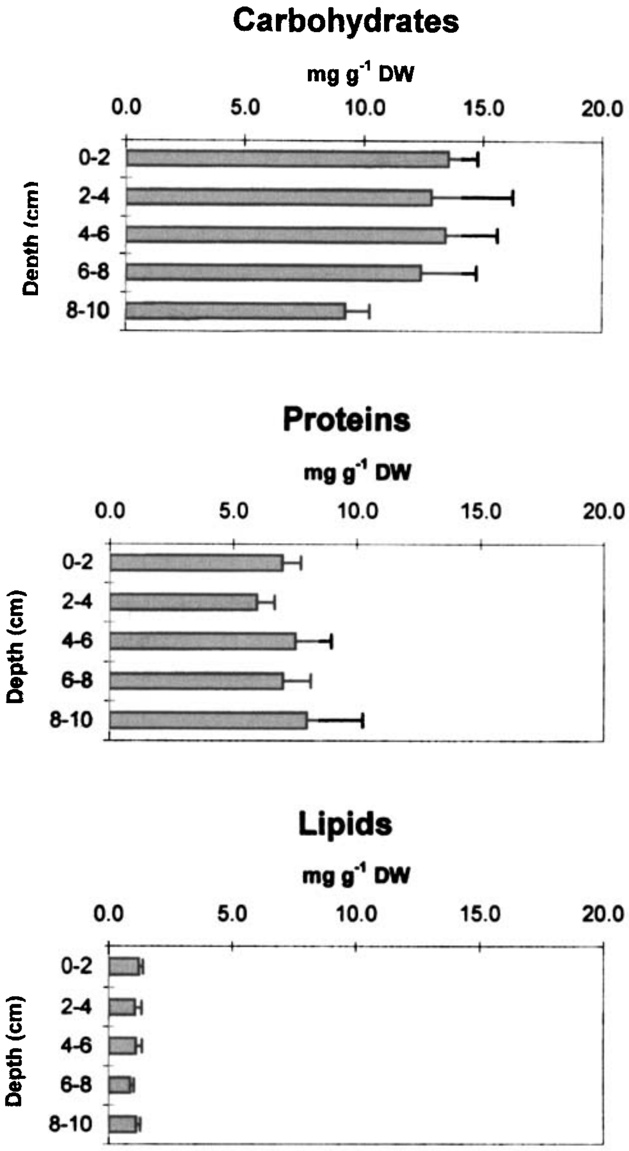

FIGURE 4 Vertical distribution of the main biochemical components of the sedimentary organic matter (average of the two sampling periods). Reported are: (1) carbohydrates, (2) proteins, (3) lipids (expressed as $\mathrm{mg\,g^{-1}}$ sedimentary DW).

would be expected that a high burial degree was found for the most refractory components of the sedimentary OM. Conversely, a clear accumulation in the deeper sediment layers characterized the two components (*i.e.*, proteins and lipids) are assumed to have a higher decomposition coefficient. Such different distribution of the three main biochemical classes of organic compounds has important consequences in terms of OM composition and quality. Vertical changes in the relative significance of proteins, lipids, and carbohydrates are reported in Figure 5. The most evident change with increasing depth into the sediments is the increasing significance of the protein contribution at the expense of the carbohydrate component, while lipid contribution, on average, remained quite constant. This indicates that the quality and bio-availability of organic matter in the sediments of the Rapallo Harbour increased in the deeper layers of the surface sediments.

Changes in OM Composition During the Experiment

Clear changes in all biochemical components of the sedimentary OM were observed in a short time scale (4 days between the beginning and the end of the experiment). Chl-a concentrations decreased significantly (t-Test, $p < 0.01$, $t = 3.41$) for about 36% during the duration of the experiment (Fig. 6). A similar decrease characterised chloroplastic pigment concentrations (CPE, 21.4–17.2 $\mu g\, g^{-1}$ at the beginning and at the end of the chamber use). Quantitative differences in the total amount of phytopigments were coupled with changes in the living autotrophic biomass contribution to CPE (chlorophyll-a accounted from 53.4 of CPE at the beginning and at 42.3% at the end of the chamber use). Such a decrease indicates a progressive conversion of living algal biomass into phytodetritus.

Sedimentary proteins (from 7.5 to 6.7 $mg\, g^{-1}$ at the beginning and at the end of the chamber use) and carbohydrates (from 16.7 to 7.8 $mg\, g^{-1}$ at the beginning and at the end of the chamber use), displayed similar short-term changes, with a decrease in concentration that was significant only for carbohydrates ($t = 5.80$, $P < 0.001$). By contrast, lipid concentrations (from 0.9 to 1.2 $mg\, g^{-1}$ at the beginning and at the end of the chamber use), although not significantly,

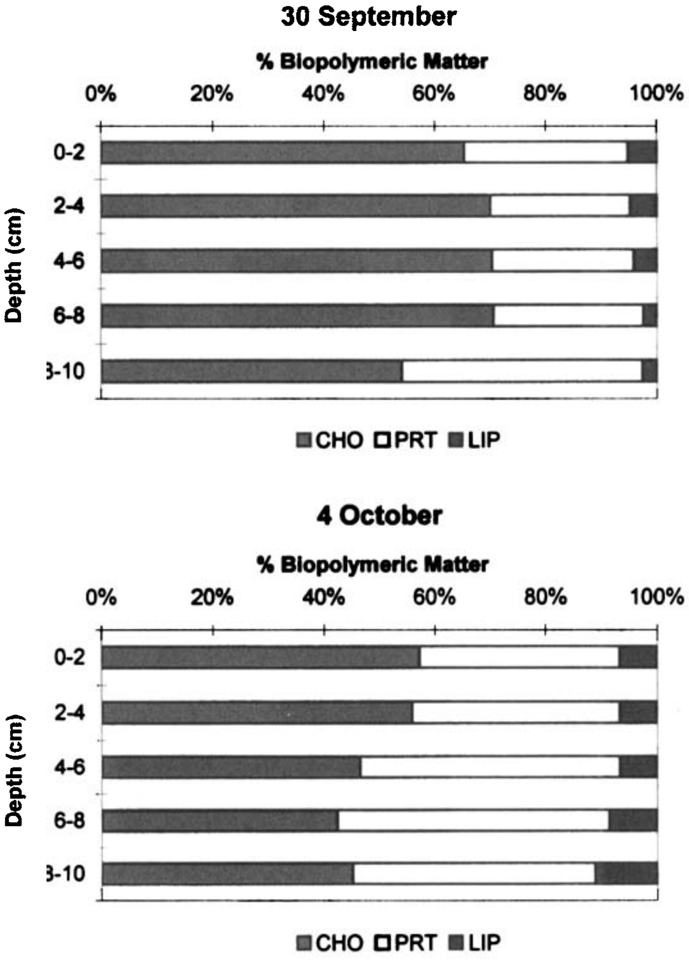

FIGURE 5 Biochemical composition of the biopolymeric matter: vertical contribution of carbohydrates, proteins and lipids (expressed as percentage, %) in September and October 1996.

increased. Such changes were responsible for the C-BPF pattern observed. C-BPF, indeed decreased significantly (for about 34%; $t = 6.65$; $p < 0.05$) during the experiment (Fig. 7).

Such different temporal pattern was reflected by a different OM composition. Carbohydrates, the dominant biochemical class of organic compounds, reduced significantly ($t = 3.83$, $P < 0.01$) their

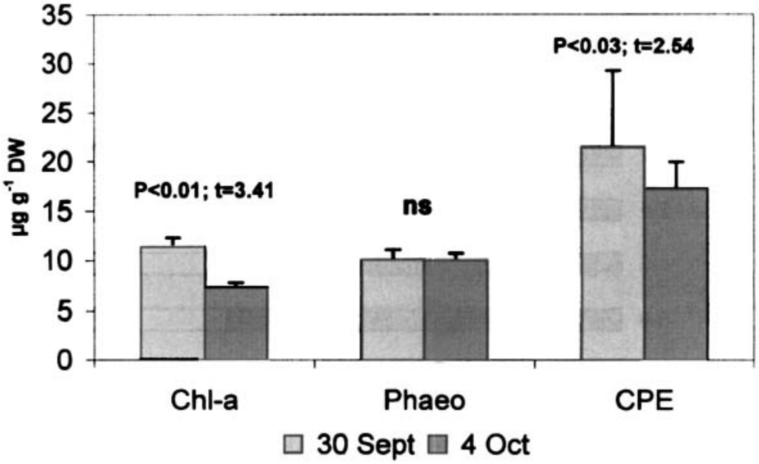

FIGURE 6 Comparison between chlorophyll-a, phaeopigment and CPE concentrations (data are integrated to 10 cm depth) in September and October 1996 (*t*-test values are reported).

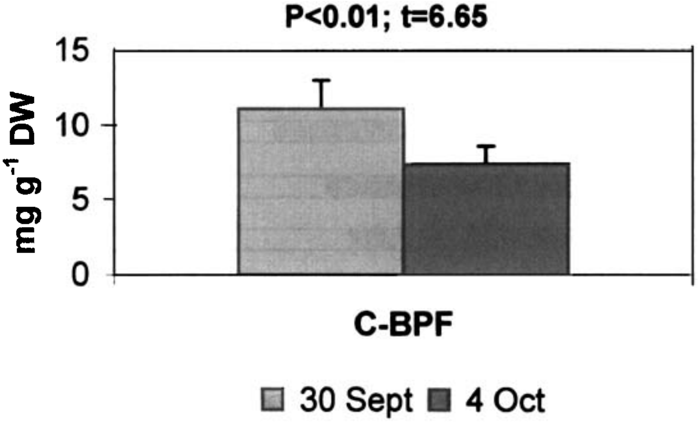

FIGURE 7 Comparison between biopolymeric carbon concentrations (C-BPF, data are integrated to 10 cm depth) in September and October 1996 (*t*-test values are reported).

relative significance from 66.1 to 49.5%. By contrast, proteins (from 30.2 to 42.7%) and lipids (from 3.7 to 7.8%) increased significantly ($t = -2.94$, $P < 0.05$; $t = -3.85$, $P < 0.01$) their contribution to the biopolymeric pool (Fig. 8).

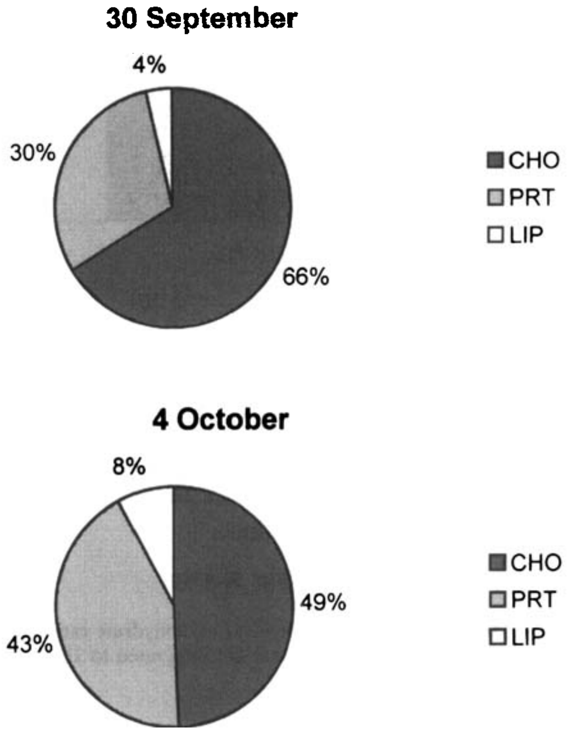

FIGURE 8 Relative contribution of the three main biochemical classes of organic compounds (reported are carbohydrates, proteins and lipids expressed in %). Data are integrated to 10 cm depth.

Such short-term changes suggest that OM pool in the sediments of the Rapallo Harbour, were characterized by a rapid conversion of the primary organic matter inputs (largely responsible for the decrease of the carbohydrate component) into proteins with a consequent strong increase of the protein to carbohydrate and protein to chlorophyll-a ratio (Figs. 9a and b).

Changes in Environmental Conditions due to the Organic Enrichment

The main effects of the organic matter accumulation into the sediments can be summarized in oxygen reduction and changes in sediment properties. Changes in Redox Potential Discontinuity (RPD)

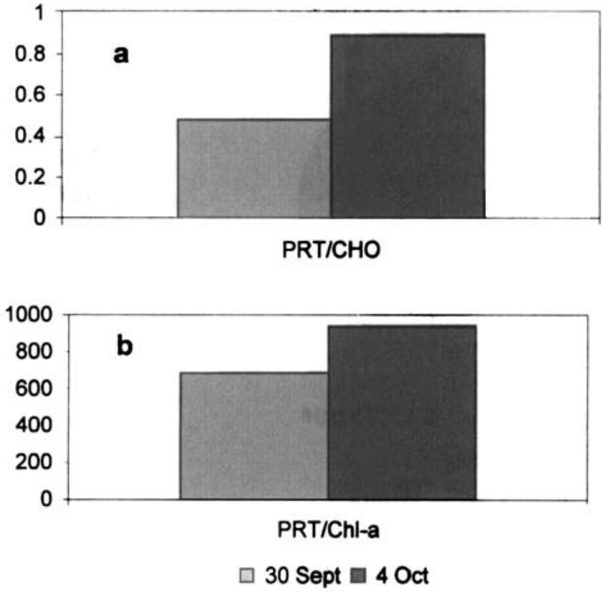

FIGURE 9 Comparison between: (a) protein to carbohydrate ratio (PRT:CHO) and (b) protein to chlorophyll-a (PRT:Chl-a). Data are integrated to 10 cm depth.

layer depths may indicate the effects of increased OM concentrations in the sediments. Generally, sediment hypoxia or anoxia are present only temporarily, but the very low depth of the RPD in the Rapallo Harbour (few mm), appears to be a quasi-permanent feature of this system. As a result of the high organic content, sediments were suboxic throughout the sediment core. This might have important consequences on both organism distribution, community composition (possible appearance of thiobios) and rates of organic matter degradation and diagenesis. In addition, organic particles may affect sediment structure and related sediment characteriztics. In fact, low energy sediments, such as those of the Rapallo Harbour, are a sink for suspended POM, so that benthic organisms are surrounded by muddy sediments coalesced by large slimes of organic matter. Organic contamination influences sediment particle aggregation with consequences in the porosity and interstitial sedimentary space and at the same time affect benthic fluxes reducing solute exchanges at water-sediment interface. Sedimentary organic matter, under normal conditions, is generally "removed" from the system by physical forces (tidal movements and currents, dispersion and/or adsorption on particles, chemical oxidation) that rapidly reduce the *in situ* concentrations so that sedimentary OM concentrations and composition might change within short-time scales. Benthic bacteria and protozoa are largely responsible for OM degradation processes that might be significantly reduced in anoxic conditions. To this regard, bioturbation by infauna (*e.g.*, *Capitella capitata*) can stimulate biodegradation to about 15%, increasing the oxygen concentration in deeper sediment layers (Zhang and Li, 1993). However, a large fraction of the OM might be buried in the sediments where microbial degradation plays an important role, unless OM reaches the deeper anaerobic layers where it might be undegraded for years.

Microphytobenthic Response to Sediment Organic Enrichment

The effects of organic enrichment on microphytobenthic communities in the field are difficult to identify. The significant decrease of sedimentary chlorophyll-a concentrations during the experiment suggests the presence of microphytobenthic mortality, not reflected by an increase in the phaeopigment concentration. These data do not seem to point out any evidence for a stress condition of the microphytobenthic community. OM enrichment might have positive effects on microphytobenthic communities. In fact, increased concentrations of dissolved organic material from both the OM input and the degradation of bottom fauna in the upper layers of the sediments contribute to the available nutrient supply of the benthic diatoms, thus increasing their biomass. Moreover, some benthic diatoms are known to grow heterotrophically (Elmgren *et al.*, 1980a, 1980b). This reason might explain why a large microphytobenthic biomass was observed in the Rapallo Harbour sediments, but do not justify the reasons for a short-term decrease. Two explanations might be hypothesized: (1) chamber deployment affected significantly the environmental conditions during the experiment; (2) the presence of constant suboxic conditions reduced significantly the autotrophic growth.

Bacterial Assemblages in OM Enriched Sediments

Bacterial density and biomass in the top 2 cm of the sediment slightly increased during the experiment (Tab. II), ranging from 13.7 to 14.1×10^8 cells g^{-1} and from 138.9 to 142.4 μg C g^{-1} sed. DW. Such densities appear to be very high when compared to those reported in other areas of the Mediterranean (Danovaro *et al.*, 1996) and, indeed, comparable only to those reported in eutrophic systems (such as the Northern Adriatic, Fabiano *et al.*, 1997). Although changes in bacterial parameters were limited, they would support the evidence for an increase of the bacterial component stimulated by the increased OM quality during the experiment. This was also confirmed by the increase in the frequency of dividing cells values (FDC, as a relative measure of the bacterial productivity) that ranged from 10.4 to 11.9%. The different bacterial size classes showed different responses to changes in OM composition. Small and large size bacteria decreased during the experiment, whereas medium size bacteria increased (Fig. 10). Consequently, OM quality rather than its quantity apparently had a stimulating effect on bacterial assemblages. These data indicate that bacterial response to increased OM nutritional value was rapid and that different size classes of bacteria respond differently to changes in OM composition. In particular, medium size bacteria revealed a higher opportunistic behaviour. As bacteria accounted on average for 1.5% to the C-BPF, it is evident that their contribution to the available organic carbon pool is negligible and indeed lower than the microphytobenthic contribution (estimated to about 4%). Such low bacterial contribution is likely to have negligible consequences also in OM composition. We estimated bacterial-nitrogen contribution to the ON pools in the sediments of the Rapallo Harbour, by converting

TABLE II Benthic microbial (bacteria and protozoa) parameters in the top 0–1 cm sediment layer of the Rapallo Harbour

BACTERIA	30 *September*	4 *tober*	*Average*
Density (n cell × $10^8 g^{-1}$)	13.71 ± 5.18	14.13 ± 3.6	13.92
Biomass (μgC g^{-1})	138.86 ± 48.89	142.40 ± 36.97	140.63
Frequency of dividing cells (%)	10.41 ± 1.95	11.89 ± 0.63	11.15
PROTOZOA			
Density (n cell × $10^5 g^{-1}$)	23.56 ± 9.52	30.21 ± 11.15	21.10
Biomass (μgC g^{-1})	16.97 ± 6.34	14.98 ± 3.69	12.76

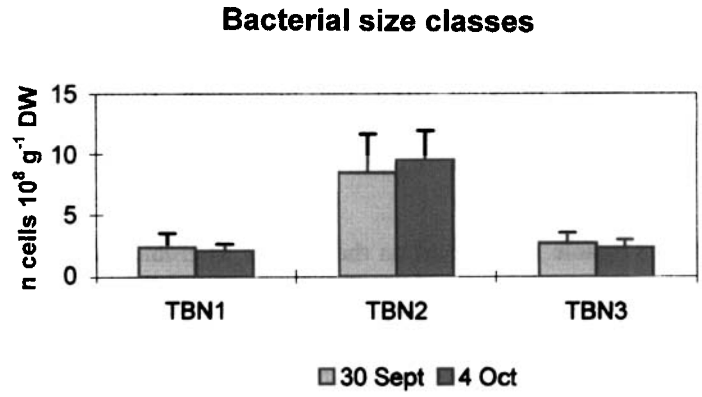

FIGURE 10 Comparison between bacterial density in September and October 1996 (data are relative to the top 0 – 1 cm layer): small (TBN1) medium (TBN2) and large size (TBN3) bacteria are reported.

bacterial biomass into nitrogen content (assuming a bacterial C/N = 4) and converting protein concentration in ON equivalents (assuming a conversion factor N-Proteins = Proteins/6.25). Our results point out that bacterial-N, accounting only for about 3% to the protein nitrogen pool, provided a negligible contribution to the increased relevance of the ON pool in these sediments.

Protozoan Response to Changes in OM Composition

As observed for bacteria, OM quality had important effects on protozoan assemblages. A stimulating effect was reported for microprotozoans that increased rapidly during the experiment. Protozoan density ranged from 23.6 to 30.2×10^5 cells g^{-1} at the beginning and at the end of the chamber use, an increase of 28% (Tab. II). Experimental studies carried out in enriched tanks (Elmgren *et al.*, 1980a, 1980b) confirmed these findings demonstrating that both ciliate and flagellate densities increased by about 3 times in 3 months. Similar experimental results were reported for foraminifer densities (Frithsen *et al.*, 1985) that were found to increase sharply and significantly with increasing OC concentrations and also to large, robust ciliates retained by a 40 μm sieve were significantly more abundant in organic enriched tanks than in controls. Therefore, it seems that high OM loads do not affect protozoa. Moreover, data from Albertelli *et al.* (present volume) indicate a parallel decrease of meiofauna during the experiment. Such increase in protozoan abundance is attributable to both predation and disturbance pressure from the metazoans (Elmgren *et al.*, 1980a, 1980b), the release of the protozoa from competition (Oviatt *et al.*, 1982), and overall increase in food at the sediment surface.

Effects of Organic Enrichment on the Benthic Microbial Loop

Bacterial and protozoan densities encountered in this study are among the highest reported in literature and are likely to be dependent upon the large availability of organic nutrients. It has been recently demonstrated that protozoan density is closely related to bacterial production (Danovaro *et al.*, 1998). The high protozoan density, carbon demand and bacterial frequency of dividing cells would indicate very high values of bacterial secondary production. This conclusion is also supported by the profiles of nitrite and nitrate (26.0 and $112.0\,\mu g\,N\,l^{-1}$) that indicate nitrification processes in the surface layer of the Rapallo sediments (Ceradini *et al.*, 1997).

The relative importance of the different benthic components is highlighted by the comparison in terms of biomass (data normalised to the top 1 cm); meiofauna, protozoa and bacteria showed an approximate biomass ratio of 1:2:20. Such biomass-ratio values are quite different from those reported in more oligotrophic areas. For instance, Dye (1981) reported values of the meiofauna to bacteria biomass ratio in exposed sandy beaches ranging from 12:1 to 20:1. Similarly, Albertelli *et al.* (submitted) reported for the oligotrophic Northwestern Mediterranean sediments a meiofauna to bacteria biomass ratio ranging from 3:1 to 11:1. Danovaro (1996), using sediments of a seagrass bed, reported a meiofauna to bacteria biomass ratio of about 5:1. By contrast, Koop and Griffiths (1982), in a non-food-limited environment, stressed the dominance of bacteria over meio- and macrofauna (1:1:3).

CONCLUSIONS

The increasing data base on the concept of a pelagic "microbial loop" (Azam *et al.*, 1983) has stimulated new thinking about the potential for similar microbial pathways and interactions to occur in the seabed. Detrital food webs in shallow marine sediments are dependent, indeed, on the catalytic activities of bacteria and on protozoan links between bacteria, meio- and macrofauna. Such a detrital benthic "microbial loop" can change in structure and function in response to environmental stress including organic carbon contamination. From data reported in field and experimental studies, it emerges clearly that the input of large amounts of organic matter to the sediments induces long-term changes in the structure of the benthic community increasing the relative importance of the smaller components of the benthic food web. Such a finding is consistent with studies carried out on benthic metabolism and community structures affected by oil contamination or in natural coastal petroleum seeps where microbial biomass and activity were significantly higher than in areas uninfluenced by oil contamination (Montagna *et al.*, 1986). Two main reasons might be invoked for explaining such benthic community structure shift towards smaller size organisms: (1) reduction of the top–down control and grazing pressure of meio- and macrofauna on the microbial components; (2) opportunistic behaviour and high metabolic activity of the microbial components.

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