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Benthic ecosystem functioning in hydrocarbon and heavy-metal contaminated sediments of an Adriatic lagoon

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ABSTRACT: Sediment samples were collected in 4 sites along a salinity gradient (between 0.2 and 27.3) of the Aussa River (Marano Lagoon, northern Adriatic Sea, Italy) and a decreasing gradient of industrial contamination downstream (from Stn A1 to Stn A4) to assess how a riverine-lagoonal system responds to chemical contamination by applying an integrative measure of its benthic ecological properties. Benthic biodiversity (primary producers, meiofauna, macrofauna) along with other structural and functional parameters were related to contamination. Meiofaunal abundance varied between 176 ± 65 and ~4000 ind. 10 cm^{-2} downstream. The macrofaunal composition shifted from one composed exclusively of chironomid larvae to a typically lagoonal one. At Stn A1, the benthic microalgal abundance exceeded 500 000 cells cm^{-3} and seemed unaffected by contamination. In the freshwater-impacted site, primary production was one order of magnitude higher than in the more saline one (7.20 and 0.60 µg C cm^{-3} h^{-1}, respectively). High organic carbon enrichment (65 mg C g_{dry}^{-1}) at Stn A2 probably caused the highest community respiration, estimated by O_2 microprofiles, along with the highest exoenzymatic activities (β-glucosidase, leucine aminopeptidase and lipase). The 4 investigated sites were well separated by principal component analysis along a decreasing gradient of organic load. An extremely active microbial community in the contaminated site ensured high production and degradation rates. Correct assessment of the benthic ecosystem functioning in polluted areas may represent a useful tool for transitional system management in order to plan environmental interventions in a rational way.

KEY WORDS: Lagoonal sediments · Biodiversity · Benthic community · Primary and secondary production · Exoenzymatic activities · O_2 microprofiles · Contamination

INTRODUCTION

Most of the available information on the relationship between biodiversity and benthic ecosystem functioning in coastal lagoons has been historically obtained from studies on macroscopic components. In the last few years there has been a growing interest in the microscopic components of coastal lagoons and their role in the energy transfer to higher trophic levels (Danovaro & Pusceddu 2007, Pusceddu & Danovaro 2009). Microbenthic organisms (microalgae, cyanobacteria and heterotrophic prokaryotes) colonise soft bottom sediments in shallow-water areas. These organisms play a major role in biogeochemical processes and drive central ecosystem functions, such as primary production (PP), decomposition and nutrient recycling (Larson & Sundbäck 2008).

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Despite their biological sensitivity, the north Adriatic coastal lagoons host vast human populations with their associated anthropogenic impacts. The human-derived pressures are diverse and result from many activities such as agriculture, industry, urban development, nautical activities, and sediment dredging. Contaminated systems are in general exposed simultaneously to combined stress factors, including (1) a suite of chemical contaminants (e.g. metals, petroleum hydrocarbons, insecticides and/or herbicides), (2) organic enrichment (and oxygen depletion) and (3) elevated nutrient levels. Each stressor can uniquely affect species within a community and may produce synergistic effects when combined. Multiple-stressor impacts may be relatively unpredictable at a community level (Fleeger et al. 2003). Laboratory studies are seldom able to predict long-term ecological effects of severe contamination by different chemical compounds, especially with regard to functional interactions among organisms and between trophic levels (Carman et al. 1995). Predators and prey are exposed simultaneously to toxicants in the field. The direct influence of contaminants on predators can lead to cascading indirect effects on resistant species in other trophic levels. Resource availability may be likewise influenced by contaminants, which may in turn modify important ecosystem functions (e.g. decomposition rates, oxygen dynamics and nutrient recycling) (Fleeger et al. 2003).

Aquatic ecosystems adapt and respond to pollution at several different levels (system, community and organism). On heavily polluted sediments, microbial communities resistant to specific pollutants or to a synergistic effect of several contaminants may frequently develop (Nogales et al. 2011). The benthic ecosystem adapts to human pressure, minimising its impact through the development of these stress-resistant communities that occupy new ecological niches. Therefore, a highly specialised food web that allows the benthic ecosystem to reach a state of equilibrium can thus be established.

Many studies report changes in abundance or diversity of microbial communities (in the broad sense) in chronically polluted marine environments. Other fundamental aspects, such as how pollution affects primary and secondary production, respiration rates or other microbial processes have not yet been analysed in chemically polluted environments. There is also a need for relating changes in diversity to metabolic processes, which are important for the functioning of the ecosystem (Nogales et al. 2011).

Few surveys have been carried out in the Grado-Marano lagoonal system (northern Adriatic Sea, Italy). They focused mainly on recent mercury contamination in the bottom sediments (Piani et al. 2005, Covelli et al. 2009). Biological studies have encompassed the distribution of picoplanktonic cyanobacteria (Paoli et al. 2007), phytoplankton (Facca & Sfriso 2009), microphytobenthos (MPB) (Sdrigotti & Welker 2002, Blasutto et al. 2005) and macroalgae (Falace et al. 2009). Higher heterotrophic levels have also been considered (Sconfietti & Marchini 2001, Dondi et al. 2003). However, with the exception of a few PP measurements (Blasutto et al. 2005), no other functional parameters (respiration, secondary production, degradation) have been estimated in this lagoonal system.

Despite a growing awareness and an increasing number of laboratory studies considering multiple stressors, the cumulative effects of co-occurring stressors in natural lagoonal communities remain largely unknown. The aim of the present study was to assess how a riverine-lagoonal system responds to chemical contamination by applying an integrative measure of its benthic ecological properties. We sought to answer the following questions in our study: (1) How do the biodiversity and the functioning of a riverine-lagoonal benthic ecosystem vary along a contamination gradient? (2) Is a contaminated site necessarily a low-diversity benthic system at all trophic levels? (3) What role do microbial communities play in the functioning of a heavily contaminated benthic ecosystem?

MATERIALS AND METHODS

Study site

With an average width of 5 km and extending for about 35 km, the Grado-Marano wetland system covers an area of 160 km^2 (Fig. 1). It is located along the northern Adriatic coast between the Tagliamento and Isonzo river deltas. The lagoon is characterised by semi-diurnal tidal fluxes (65 cm and 105 cm mean and spring tidal range, respectively). Salinity values are very low (2 to 7) in the areas close to the river mouths. They increase towards the tidal inlets, where values between 24 and 36 have been recorded (Brambati 2001). Freshwater inputs are very scarce and mostly limited to the western sector (Marano Lagoon). The overall amount of average freshwater discharge has been estimated to be about 70 to 80 $m^3 s^{-1}$, with a maximum peak of 100 $m^3 s^{-1}$ (Mosetti

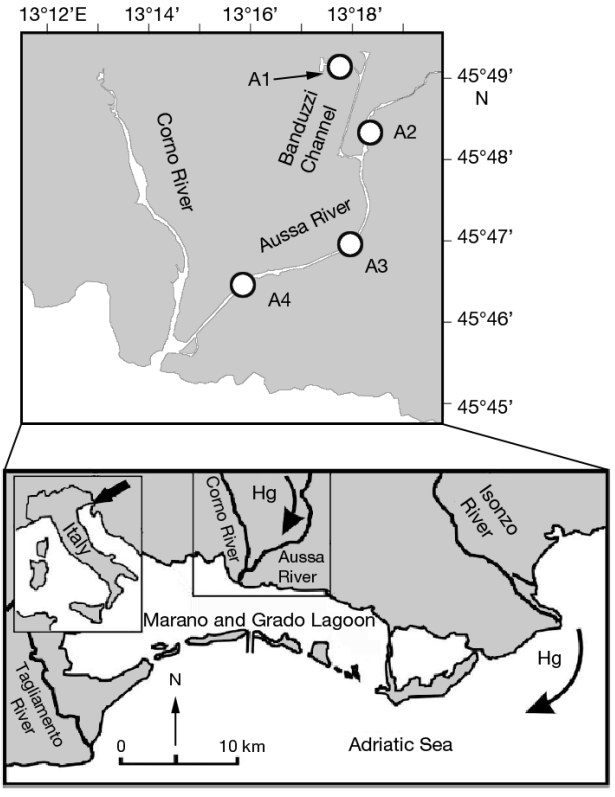

Fig. 1. Study sites in the Banduzzi Channel (Stn A1) and along the Aussa River (Stns A2 to A4) in the Grado-Marano lagoonal system. Modified after Covelli et al. (2009)

1983). The Aussa and Corno rivers originate from the spring line area located in the southern part of the coastal plain, and flow into the central sector of the lagoon (45 km^2). The Corno River, which has a total length of 17 km, drains a basin of about 5000 ha and receives few tributaries, hence its limited degree of discharge (5 to 8 $m^3 s^{-1}$). In contrast, the Aussa River has several tributaries, drainage channels and irrigation ditches and its total discharge reaches 8 to 20 $m^3 s^{-1}$. Maximum/minimum discharges occur in October/February and December/September for the Corno and Aussa rivers, respectively. During dry periods, thermo-haline stratification occurs and the presence of a net salt-wedge tip can be detected. In addition, downflowing is often hindered when particular weather conditions or high tides occur (Covelli et al. 2009). The highest contents of fine silt occur where the Zellina and Aussa-Corno rivers enter the lagoon. The ratio of fine silt (2 to 16 µm) to clay (<2 µm) identifies areas affected by freshwater inputs into the lagoon: a high ratio indicates an area of fresh water input and vice versa (Brambati 1997). Due to the substantial human impact caused by urban settlements and industrial activities, this area of the lagoon is highly vulnerable. Porto Nogaro, located 1 km north of the Aussa-Corno mouth, is the most important industrial site in the region. In addition, the Torviscosa industrial complex produced cellulose from cane (*Arundo donax*) from the 1940s to 1992. It is estimated that about 20 kg d^{-1} of Hg was discharged into the Aussa River since 1949 (when the chlor-alkali plant became operative). Soda production was increased from 4500 tons in 1950 to 20 000 tons during the following 10 yr. In 1970 the amount of discharged Hg decreased to 6–7 kg d^{-1}. Then it presumably stopped in 1984 when wastewater treatment systems were installed. At present, industrial effluents flow through submarine pipes that discharge processing effluent into the open sea (about 6.5 km offshore from the main tidal inlet). An estimated total amount of 186 000 kg of Hg was released into the river and a significant volume of this Hg probably reached the lagoon environment. This site is currently under intense investigation, and some corrective actions (e.g. soil removal and groundwater treatment) are in progress (Piani et al. 2005 and references therein). In August 2009 the Special Commissioner for the socio-economic and environmental emergency in the Lagoon of Marano Lagunare and Grado ordered measurements of several contaminants. Up to 75 different points were sampled within the Banduzzi Channel. Concentrations are summarised in Table 1.

Sampling

Samples were collected in December 2010 and January 2011. Sampling was performed by boat at 3 different stations along the main axis of the Aussa-Corno River system (Fig. 1) and one located within the Banduzzi Channel during spring ebb-tide conditions (tidal range: 55 to 56 cm). Bottom water samples were collected by means of a 2 l horizontal Niskin bottle. At the moment of sampling, photosynthetically active radiation (PAR) and water temperature were recorded *in situ* by a Profiling Natural Fluorometer PNF-300 (Biospherical Instruments). Salinity was measured from bottle-collected water by means of a conductivity meter (WTV LF196). At each station, 5 virtually undisturbed sediment cores were taken by SCUBA divers using polycarbonate sample tubes (12.7 cm inner diameter [i.d.] with a sample area of 127 cm^2); 1 sediment core was used for meiofauna sampling, and 1 for oxygen microprofiling. From the 3 remaining cores, 3 sediment layers

Table 1. Contaminants measured in surface sediments within the Banduzzi Channel. Concentrations of heavy metals are expressed in mg kg^{-1}; methylmercury and all other contaminants in µg kg^{-1} (sediment dry mass). LD: detection limit; TP: total phosphorus; PCBs: polychlorobiphenyls; PAHs: polycyclic aromatic hydrocarbons; DDTs: dichlorodiphenyltrichloroethanes; PCDFs: polychlorodibenzofurans; PCDDs: polychlorodibenzo-p-dioxins

Contaminant	Min.	Max.	No. of samples
As	<0.1	9.2	75
Cd	<0.1	1.2	75
Total Cr	7.5	110	75
Cr (VI)	<0.5	<0.5	10
Cu	3.6	150	75
Hg	<0.06	1529	75
Methylmercury	0.02	59.5	10
Ni	3.2	110	75
Pb	1.7	100	75
Se	<0.1	3.7	39
Va	11	94	39
Zn	10	150	75
TP	93	2000	75
Heavy hydrocarbons (C > 12)	10	1220	39
Hexachlorobenzene	<LD	820	39
Total PCBs	<LD	5500	29
Total PAHs	2.7	37000	39
Total DDTs	<LD	95	39
Monobutyltin	<0.1	4.6	10
Dibutyltin	<0.1	0.1	10
Tributyltin	<0.1	0.1	10
PCDFs	0.004	108.5	10
PCDDs	0.02	11	10

(ca. 0–2, 2–4 and 4–6 cm) were sampled and each layer homogenised. Additional sediment samples were collected using a stainless steel Van Veen grab (sampling area: 0.06 m^2).

Granulometry, total organic carbon and chl *a*

For each layer of each station, aliquots (10 to 15 g) of homogenised sediment were collected for grain-size analysis and processed as described by Cibic et al. (2007a). The analyses were performed using a Malvern Multisizer 2000S. Data are expressed as percentage of sand, silt and clay. For total organic carbon (TOC) analyses, triplicate subsamples of homogenised sediment (<250 µm) were weighed directly in a capsule (5 × 9 mm), treated with increasing concentrations of HCl (0.1 N and 1 N) to remove carbonates (Nieuwenhuize et al. 1994) and assessed according to the methods of Pella & Colombo (1973) and Sharp (1974). Pigments were extracted overnight (4°C, 90% acetone) from 0.7 to 0.9 g of wet sediment and spectrofluorometrically analysed following the procedures described by Lorenzen & Jeffrey (1980).

Labile organic matter

Subsamples of homogenised sediment were freeze-dried and processed for the determination of carbohydrates, lipids and proteins. Colloidal and EDTA-extractable carbohydrates were analysed following the method described by Blasutto et al. (2005). Lipids were analysed following the method proposed by Bligh & Dyer (1959) and modified for sediments. Proteins were extracted in NaOH (0.5 M) for 4 h and determined according to the methods in Hartree (1972). All analyses were carried out in 4 replicates. Carbohydrate, lipid and protein concentrations were converted to carbon equivalents (Fichez 1991). The sum of carbohydrates, lipids and proteins was referred to as labile organic matter (LOM).

Benthic prokaryotes

Three aliquots of sediment (0.5 g_{wet}) were withdrawn from each sediment sample, transferred into sterile test tubes and fixed with 3 ml of pre-filtered (0.2 µm pore size) and buffered formaldehyde solution (3% v/v final concentration in autoclaved seawater). The sediment slurry was kept at 4°C for 24 h, then washed twice with 3 ml of 1× phosphate-buffered saline (PBS) (pH: 7.2) by centrifuging at 500 × *g* to remove the supernatant, and stored in 1 × PBS-ethanol (1:1) at –20°C (Ravenschlag et al. 2000). For the subsequent sample processing, a modified protocol by Lunau et al. (2005) was followed: 100 µl of sediment slurry was diluted with 900 µl sterilised Milli-Q water and 100 µl methanol. The diluted samples were placed in a water bath at 35°C for 15 min, sonicated in ice (3 times for 1 min with 2 breaks of 30 s in between), diluted again with sterile Milli-Q water to a final concentration of 1:110 and filtered on black Nuclepore polycarbonate 0.2 µm pore-size filters. Filters were mounted on microscope slides, stained with a SYBR Green I-mounting medium Mowiol solution (1:15) and counted by epifluorescence microscopy at 1000× magnification (Leica DM2500). A minimum of 300 cells were counted for each filter in at least 20 randomly selected fields under a blue filter set (excitation: 450 to 490 nm; barrier: 515 nm).

Enumeration of functional bacterial groups

The standard 5-tube most-probable-number (MPN) method (Alexander 1982) was adopted to estimate the abundance of functional bacterial groups. Subsamples of homogenised sediment (~5 g_{wet}) were placed in sterile tubes with 45 ml of pre-filtered bottom water (0.2 µm filters), stirred, sonicated and centrifuged at 956 × *g* for 1 min to remove sediment particles. Supernatant and 5 serial 10-fold diluted inoculums were added to the wells containing specific medium (10% v/v) and incubated at 20°C. Cellulose-degrading bacteria were estimated using a medium enriched with nitrates and calcium carbonate in which pure cellulose strips were the only source of carbon; tubes in which the cellulose strip decomposed after 1 mo of incubation were considered as positives (MPAAF 2002). Alkane- and petroleum-degrading bacteria were enumerated by adding n-hexadecane (C_{16}) (3.4% v/v) and unleaded petrol (3.4% v/v) to BH medium (Bushnell & Haas 1941). Hydrocarbon degradation was detected after 15 d by adding a solution of 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyl-2H-tetrazolium (0.6 g l^{-1} final concentration) and the appearance of a red-violet colour after 24 h incubation was considered as a positive reaction (Johnsen et al. 2002).

MPB

For MPB analyses, aliquots of 2 cm^3 of each homogenised sediment layer were processed and counted following the protocol described by Cibic et al. (2007a,b); qualitative identification was carried out using the flora cited therein as well as the identification keys of freshwater microalgae by Lund & Lund (1995). The microalgal taxonomy was based on Round et al. (1992). Three replicates were counted for the uppermost layer at each station and only one replicate was counted for the subsurface layers.

Meiofauna

From a virtually undisturbed sediment core, 3 replicates were gently taken using cut-off plastic syringes (2.7 cm i.d., length: 11.4 cm) and immediately frozen at –20°C (Higgins & Thiel 1988). While sediment cores for the other parameters were sectioned in 3 layers (ca. 0–2, 2–4 and 4–6 cm), the top 10 cm of the sediment core was considered for meiofauna. Subsamples were preserved in buffered 4% formaldehyde solution using pre-filtered seawater, and stained with Rose Bengal (0.5 g l^{-1}). Sediment samples were sieved through 1000 and 38 µm mesh nets, and organisms (from the sediment retained on the 38 µm sieve) were extracted by centrifuging the samples 3 times (1932 × *g*, 10 min) with Ludox AM-30 (density: 1.15 to 1.18 g cm^{-3}) as described by Danovaro et al. (2004). All meiobenthic animals were counted and taxonomically classified into the main groups according to Higgins & Thiel (1988) under a stereomicroscope (Olympus SZX12; final magnification of 40 or 80×). The abundance was expressed as number of individuals per 10 cm^2.

Macrofauna

Macrobenthos were sampled with a Van Veen grab; 3 replicates per station were taken, sieved through a 1000 µm sieve and immediately frozen at –20°C. After defrosting and sorting, animals were counted and identified to the lowest possible taxonomical level using a stereomicroscope at 7 to 40× magnification (Rees et al. 1990). For the identification of organisms, the taxonomical keys listed in Morri et al. (2004) were used. The abundance was expressed as number of individuals per m^2.

PP of MPB

PP was estimated in the laboratory from ^{14}C incubation of slurries. Ten cm^3 of homogenised surface sediment were drawn off from the slurries, resuspended in 190 ml of overlying filtered seawater (0.2 µm filter) and inoculated with 20 µCi (0.74 MBq) of $NaH^{14}CO_3$ (DHI, Denmark) (Steemann-Nielsen 1952). After stirring, the slurry was transferred into 21 glass vials containing 9 ml which were divided as follows: 3 replicates to assess the sediment matrix effect, 3 dark replicates, and 3 replicates for each of 5 light intensities. Samples were incubated in a thermostatic chamber at *in situ* temperature under a gradient of light intensities (20, 30, 50, 100, 200 µmol photons m^{-2} s^{-1}) and after 45 min, carbon incorporation was stopped by adding 200 µl of 5 N HCl (final HCl concentration: 0.11 N) (Cibic & Virgilio 2010). Subsequently, samples were treated as described in detail by Cibic et al. (2008). PP_s refers to data obtained at *in situ* light conditions, whereas PP_p refers to the potential PP, i.e. the highest value obtained under a gradient of light intensities.

O_2 consumption rates

Intact sediment cores for O_2 consumption estimates in darkness were sampled and transferred to a cryostatic system to maintain the *in situ* temperature. A stable water flow above the sediment surface was established by a pressure-regulating air pump that maintained a diffusive boundary layer of 300 to 500 µm and kept the overlying water at atmospheric oxygen saturation at all times. Steady-state O_2 microprofiles were measured using Clark-type O_2 microelectrodes with a guard cathode (Revsbech 1989) with external tip diameter <100 µm, stirring sensitivity <2%, and a 90% response time <8 s. The sensor current was measured using a Unisense PA2000 picoammeter. Data were recorded with Unisense Profix software version 3.10. A step size of 100 µm was used. For the interpretation of the measured O_2 concentration profiles, the software PROFILE version 1.0 (Berg et al. 1998) was used. Areal rates of oxygen respiration were calculated as described by Cibic et al. (2007b).

Degradative activities

Extracellular enzymatic activities were assayed using fluorogenic substrata analogues (Hoppe 1993) derived from 7-amino-4-methyl-coumarin (AMC) and 4-methyl-umbelliferone (MUF). Leucine aminopeptidase activity was assayed as the hydrolysis rate of leucine-AMC. α-glucosidase, β-glucosidase, lipase and alkaline phosphatase were assayed using MUF-α-D-glucoside, MUF-β-D-glucoside, MUF-oleate, and MUF-phosphate, respectively. Enzyme activities were expressed in terms of the rate of MUF or AMC production. Sediment slurries were prepared by adding 6 ml of 0.2 µm-filtered bottom water to 0.5 g of wet sediment. After evaluating the saturating concentrations, hydrolysis rates were measured by incubating slurries with (final concentrations): 800 µM leucine-AMC, 400 µM MUF-α-D-glucoside, MUF-β-D-glucoside, MUF-oleate and 50 µM MUF-phosphate for 1 h in the dark at *in situ* temperature. Before spectrofluorometric measurements, each sample was centrifuged for 2 min at 956 × *g*. Fluorescence increase due to MUF and AMC hydrolysed from the model substrates was measured using a Shimadtzu RF-1501 spectrofluorometer (MUF: 365 nm excitation and 455 nm emission; AMC: 380 nm excitation and 440 nm emission). Standard solutions of MUF and AMC were used to produce calibration curves with 0.2 µm-filtered bottom water. Triplicate blanks without fluorogenic substrata were used to determine the natural fluorescence increase in the samples that was not attributable to the tested enzymes.

Prokaryotic carbon production

Prokaryotic carbon production (PCP) estimates in sediment samples were carried out by the method of Van Duyl & Kop (1994), as detailed by Manini et al. (2004). Each sediment sample (0.2 ml of 1:1 v/v slurry) was added to 6 µCi of ^{3}H-leucine and incubated in the dark for 1 h at *in situ* temperature. After incubation, radiotracer incorporation was stopped by adding 80% ethanol (1.7 ml). After 2 washes of the samples with ethanol (80%) by mixing, centrifuging and decanting the supernatant, the sediment was transferred with ethanol (80%) onto a polycarbonate filter (0.2 µm mesh size). Subsequently, the filters were washed twice with 5% trichloroacetic acid. Samples were heated in 2 M NaOH for 2 h in a water bath at 100°C. One ml of supernatant was transferred to scintillation vials and 10 ml of Hionic Fluor scintillation fluid was added. For each sample, 3 replicates and 2 ethanol-treated blanks were analysed. Activity in the samples was determined by a β-counter (TRI-CARB 2900 TR Liquid Scintillation Analyzer).

Statistical analyses

Differences in chemical and biological parameters among stations were assessed with 1-way ANOVA using STATISTICA. When significant differences were observed ($p < 0.05$), Tukey's pairwise comparison test was also performed. Only statistically significant data are presented. To highlight interactions between structural and functional variables, the non-parametric Spearman's rank correlation analysis (R) was applied.

Univariate diversity analysis (PRIMER software v.5) was applied to benthic diatom abundances of the surface layer considering richness (*d*; Margalef 1986), equitability (*J'*, Pielou 1966), diversity (*H'*; Shannon & Weaver 1949) and dominance (λ; Simpson 1949).

Principal component analysis (PCA) based on r algorithm (correlation coefficient) was carried out using MATEDIT software (Burba et al. 2008). Simultaneous ordination of biotic and abiotic variables was obtained using first and second autovectors and first and second principal components. PCA was performed considering the following variables, mea-

sured in the 3 layers of each station: sand, TOC, LOM, chl *a*, MPB, all tested enzymes and PCP. A 5% significance level was used for the correlation coefficients.

RESULTS

Physical-chemical data

Temperature ranged from 6.8°C at Stn A4 to 9.8°C at Stn A2. A salinity gradient was observed from Stns A1 to A4, indicating that the salt wedge tends to intrude for rather long distances upstream. The lowest PAR at the bottom was measured at Stn A3 due to cloudy weather conditions, although light penetration (%PAR) was inferior at Stn A2 (Table 2).

Table 2. Physical data measured at the 4 stations. PAR: photosynthetically active radiation. %PAR: percentage of irradiance measured at the bottom with respect to surface irradiance

Stn	Depth (m)	Temp. (°C)	Salinity	PAR (µmol photons m^{-2} s^{-1}) Bottom	Surface	%PAR
A1	2.6	8.6	0.2	35	165	21.2
A2	2.9	9.8	8.5	27	287	9.4
A3	3.4	7.4	21.1	12	113	10.6
A4	3.6	6.8	27.3	39	339	11.5

According to Shepard's (1954) classification, the sediment varied from sandy silt at Stns A1 and A2 to clayey silt at Stns A3 and A4 (Table 3). The percentage of sand at Stn A2 was significantly higher when compared to the other 3 stations ($p < 0.001$). Mean TOC content reached 59.08 mg g^{-1} at Stn A2 and was twice the mean value recorded at Stn A4 (27.22 mg g^{-1}). At the lagoonal Stn A4, TOC values were significantly lower than those measured upstream ($p < 0.001$). Similarly, the mean LOM value at Stn A2 was ca. 40% higher than that at Stn A4. LOM accumulation was noticed in the deepest layer at Stns A2 and A3. Except for the surface and subsurface layers at Stn A4, the protein content (50.3 to 72.8%) of the sediment was always superior to the lipid one (16.1 to 42.7%), whereas total carbohydrates did not exceed 11% of LOM (Table 3). The highest chl *a* values were measured in the uppermost sediment layers at Stns A2 and A3 (14.85 and 14.58 µg g^{-1}, respectively); chl *a* content was significantly lower at Stn A4 compared to the values measured upstream ($p < 0.01$).

Benthic communities

The abundance of benthic prokaryotes ranged between 1.26×10^9 and 4.32×10^9 cells g_{dry}^{-1}. In the surface layer, a slightly higher abundance was measured at Stn A4 than at the other sites, whereas major densities were observed in the 2–4 cm layer of Stns A1 and A2. However, no consistent differences

Table 3. Sand content and structural chemical data measured in the 3 sediment layers of the 4 stations. Values are average of 3 replicates ± SD. TOC: total organic carbon; CHO_{EDTA}: EDTA-extractable carbohydrates; CHO_{H2O}: colloidal carbohydrates extracted in water; LOM: labile organic matter

Layer (cm)	Sand (%)	TOC (mg C g^{-1})	Proteins (µg C g^{-1})	Lipids (µg C g^{-1})	CHO_{EDTA} (µg C g^{-1})	CHO_{H2O} (µg C g^{-1})	LOM (µg C g^{-1})	Chl *a* (µg g^{-1})
Stn A1								
0–2	19.0	56.38 ± 0.14	5032 ± 149	1766 ± 134	256 ± 26	148 ± 3.8	7201	12.12 ± 1.82
2–4	13.4	51.92 ± 0.27	5639 ± 174	1644 ± 75	506 ± 40	78 ± 1.8	7867	9.53 ± 1.43
4–6	18.4	46.16 ± 0.08	4976 ± 181	2165 ± 157	349 ± 7	112 ± 3.7	7601	7.77 ± 0.93
Stn A2								
0–2	34.2	64.94 ± 0.20	5928 ± 108	2436 ± 214	497 ± 67	110 ± 6.1	8971	14.85 ± 2.23
2–4	33.7	57.07 ± 0.12	3723 ± 134	3161 ± 24	419 ± 49	101 ± 1.1	7404	9.27 ± 1.39
4–6	28.7	55.23 ± 1.21	6850 ± 36	4083 ± 97	436 ± 11	84 ± 8.3	11454	8.10 ± 1.13
Stn A3								
0–2	9.5	48.83 ± 0.28	3262 ± 136	2673 ± 135	233 ± 8	103 ± 10.3	6271	14.58 ± 2.19
2–4	20.6	44.55 ± 1.98	3732 ± 1	2539 ± 88	295 ± 14	111 ± 7.8	6676	9.94 ± 1.49
4–6	13.4	45.13 ± 0.21	6897 ± 2	3296 ± 156	428 ± 10	113 ± 7.0	10734	11.51 ± 2.07
Stn A4								
0–2	4.9	29.57 ± 0.60	1827 ± 20	2768 ± 204	190 ± 14	73 ± 6.2	4858	4.24 ± 0.64
2–4	8.4	27.38 ± 0.34	1623 ± 84	4338 ± 88	401 ± 54	90 ± 5.9	6452	2.75 ± 0.41
4–6	4.9	24.72 ± 0.57	4129 ± 118	911 ± 38	512 ± 24	117 ± 2.0	5669	1.39 ± 0.21

Table 4. Biological structural parameters measured in the 3 sediment layers at the 4 stations. For microphytobenthos (MPB), values are average of 3 replicates ± SD for the 0–2 cm layer; the other depth classes had only 1 replicate. C_{16}: n-hexadecane; MPN: most probable number

Layer (cm)	Prokaryotes ($\times 10^9$ cells g_{dry}^{-1})	Bacteria (MPN g_{dry}^{-1})			MPB (cells cm^{-3})	Meiofauna (ind. 10 cm^{-2})	Macrofauna (ind. m^{-2})
		Cellulose-degrading	Petroleum-degrading	C_{16}-degrading			
Stn A1							
0–2	2.64 ± 0.10	488	92	18376	514500 ± 18243	1005.4 ± 152.0	638.9 ± 250.2
2–4	4.17 ± 0.57	131	112	2671	216600		
4–6	2.04 ± 0.19	588	6	1526	77400		
Stn A2							
0–2	2.54 ± 0.36	19	39	28200	247800 ± 25456	176.0 ± 64.8	44.4 ± 9.6
2–4	4.32 ± 0.58	37	6	21406	96000		
4–6	1.26 ± 0.16	10	6	7439	57000		
Stn A3							
0–2	2.39 ± 0.06	12	106	3548	177300 ± 8910	1639.5 ± 162.1	61.1 ± 9.6
2–4	3.28 ± 0.33	4	48	759	71400		
4–6	4.08 ± 0.17	1	12	269	70200		
Stn A4							
0–2	3.60 ± 0.32	1	33	573	59400 ± 1697	3986.2 ± 1124.1	533.3 ± 60.1
2–4	2.47 ± 0.27	0	41	291	16800		
4–6	2.16 ± 0.00	0	5	102	3600		

or dynamics were detected either among stations or among layers.

Cellulose-degrading bacteria enumerated in samples from Stn A1 (up to 588 MPN g^{-1}) were one order of magnitude higher than at Stn A2 and presumably related to the past pulp mill activity in the Banduzzi Channel (Stn A1). The ability to degrade cellulose lessened downstream along the Aussa River (Stns A2 to A4) (Table 4). Petroleum-degrading bacteria were more numerous in the surface and subsurface sediments of Stn A1 (92 and 112 MPN g^{-1}, respectively) and in the top layer of Stn A3, whereas at Stn A4 and especially at Stn A2, lower MPN were observed. In contrast, the ability to degrade n-hexadecane was superior at Stn A2 (28 200 MPN g^{-1}) than at Stn A1 (18 376 MPN g^{-1}), which was contaminated by hydrocarbons. Finally, lower n-hexadecane-degrading bacteria numbers were obtained downstream at Stns A3 and A4.

Microalgal abundance decreased downstream (Table 4). At Stn A1, MPB exceeded 500 000 cells cm^{-3} in the uppermost sediment layer, and cell numbers remained extremely high up to a depth of 6 cm. Although the microalgal density at Stn A2 was approximately half of that observed at Stn A1, it was still very high (247 800 cells cm^{-3}). Only at Stn A4, where the lagoonal features were more pronounced, was the MPB abundance of the same order of magnitude as that estimated in marine muddy sediments (Cibic et al. 2007a). The qualitative composition of the MPB community was quite diverse in the 4 investigated sites. Focusing on the surface layer, diatoms dominated at Stn A1, while their percentage decreased downstream (Table 5). In contrast, the contribution of Chlorophyta and Cyanobacteria increased downstream; Chlorophyta were more abundant at Stn A2 and Cyanobacteria at Stn A3. Undetermined phytoflagellates reached a higher relative abundance (RA) at the lagoonal Stn A4. Among diatoms, the most abundant genus at Stn A1 was *Craticula*, with an RA of >30%. (Table 5).

Diatoma was also abundant, reaching 15.2% of the total diatom community. At Stn A2, *Craticula* was still the most abundant diatom genus, followed by *Cocconeis* (18.6%) and *Melosira*. Increasing salinity (21.1) at Stn A3 enhanced the abundance of brackish and marine diatom taxa, such as several species of the genus *Gyrosigma*. Concomitantly, the RA of predominantly freshwater taxa (*Diatoma* and several *Fragilaria* spp.) decreased. At Stn A4, *Melosira varians* reached the highest RA, followed by *Cocconeis placentula*.

Univariate diversity indices revealed that the highest richness was observed at Stn A1 (d = 2.93), whereas the highest diversity was found at Stn A3 (H' = 2.63). The discrepancy between the 2 indices was due to the dominance of *Craticula*, which reached an RA of 30% at Stn A1. The diatom community was rather evenly distributed at Stn A3 (J' = 0.80 at Stn A3 and 0.66 at Stn A1). At this site, the

most abundant genus, *Gyrosigma*, did not exceed 15.6%.

Meiobenthic abundances ranged from 176.5 ± 64.8 to 3986.2 ± 1124.1 ind. 10 cm^{-2} at Stns A2 and A4, respectively (Table 4). A total of 11 taxa were detected: 7 belonged to permanent meiofauna (Nematoda; Copepoda: Harpacticoida and their nauplius stages; Kinorhyncha; Ostracoda; Rotifera; Tardigrada; Turbellaria). The remaining 4 taxa belonged to temporary meiofauna (juvenile macrofauna: Polychaeta, Oligochaeta, Amphipoda, Nemertina). Nematoda constituted >90% of the total meiofaunal community, except for Stn A2, where the RA was 87.1% (Table 5). Copepoda: Harpacticoida together with their nauplius stages were the second most abundant group, with RA ranging from 0.2% to 7.7% at Stns A1 and A3, respectively.

Table 5. Relative abundance (%) of the major groups constituting the 3 benthic communities and the major benthic diatom genera at the 4 stations. Microphytobenthos were analysed in the surface layer (0–2 cm) and meiofauna in the top 10 cm

Taxon	A1	A2	A3	A4
Microphytobenthos				
Chlorophyta	2.2	11.0	3.7	6.6
Cyanobacteria	1.7	9.6	17.8	3.5
Undet. Phytoflagellates	0.0	1.0	1.7	15.2
Spores and cysts	0.3	0.5	0.5	5.6
Bacillariophyta:	95.7	78.0	76.3	69.2
Amphora	2.7	0.9	0.7	2.9
Cocconeis	3.8	18.6	5.3	16.9
Craticula	31.2	21.7	11.3	1.5
Diatoma	15.2	9.5	1.6	6.6
Fragilaria	11.2	5.0	2.7	5.9
Gyrosigma	1.3	0.6	20.4	4.4
Melosira	10.1	9.8	14.0	18.4
Navicula	2.5	0.2	2.2	0.0
Nitzschia	1.5	4.8	4.0	2.2
Surirella	0.2	3.3	3.3	0.7
Other diatom genera	20.4	25.6	34.6	40.4
Meiofauna				
Copepoda	0.2	0.9	3.0	1.4
Nauplii	0.0	0.9	4.7	2.7
Nematoda	93.4	87.1	91.2	93.5
Polychaeta	0.0	0.9	0.0	0.9
Rotifera	1.9	3.1	0.0	0.0
Tardigrada	2.3	0.3	0.0	0.0
Others	0.2	2.5	0.2	1.1
Incertae sedis	2.0	4.1	0.8	0.4
Macrofauna				
Chironomus	99.1	37.5	0	0
Gammaridae	0	0	0	1.0
Incertae sedis	0	0	0	2.1
Muricidae	0	0	0	2.1
Polychaeta cf. Nereidae	0	50	100	39.6
Sadleriana fluminensis	0.9	0	0	0
Sternaspis scutata	0	12.5	0	55.2

Macrobenthic organisms were more abundant at Stns A1 (638.9 ± 250.2 ind. m^{-2}) and A4 (533.3 ± 60.1 ind. m^{-2}) than at the other 2 sites (Table 4). At Stn A1, larvae of *Chironomus* dominated, with an RA of 99.1% (Table 5). These organisms were also observed at Stn A2, constituting 37.5% of the total macrozoobenthic community. The Polychaeta cf. Nereidae was seen along the Aussa River (Stns A2 to A4) but not in the Banduzzi Channel (Stn A1). Its RA varied considerably, ranging from 39.6% at Stn A4 to 100% at Stn A3. However, this latter value corresponded to only 61.1 ± 9.6 ind. m^{-2}, compared to 211.1 ± 9.6 ind. m^{-2} at Stn A4. Another polychaete, *Sternaspis scutata* (RA: 55.2%), characterised the macrozoobenthic community in this lagoonal area.

Functional parameters

PP_s decreased from Stn A1 to Stn A4, following MPB abundance dynamics (Fig. 2). Although estimates were carried out at the end of December in low light conditions (35 µmol photons m^{-2} s^{-1}), a very high PP_s value was obtained at Stn A1 (7.2 µg C cm^{-3} h^{-1},

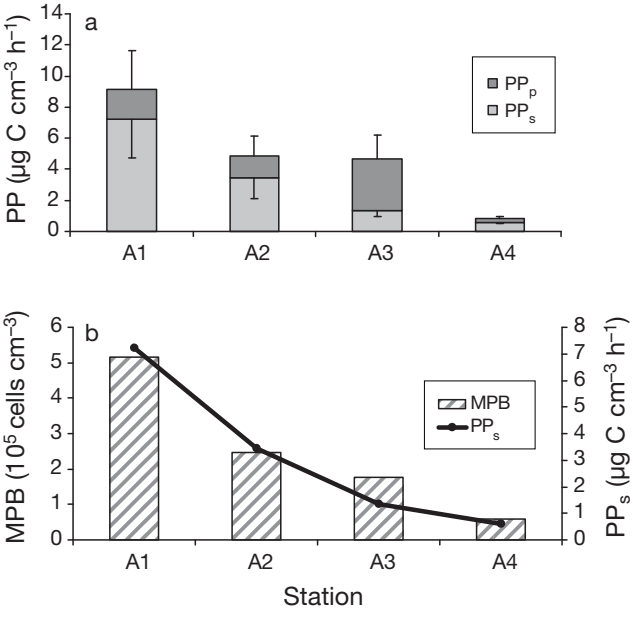

Fig. 2. (a) Microphytobenthic primary production (PP) as estimated at *in situ* conditions (PP_s) and the potential PP (PP_p) corresponding to the highest value obtained when exposing the community to a light gradient (from 20 to 200 µmol photons m^{-2} s^{-1}). (b) PP_s and microphytobenthic abundance (MPB)

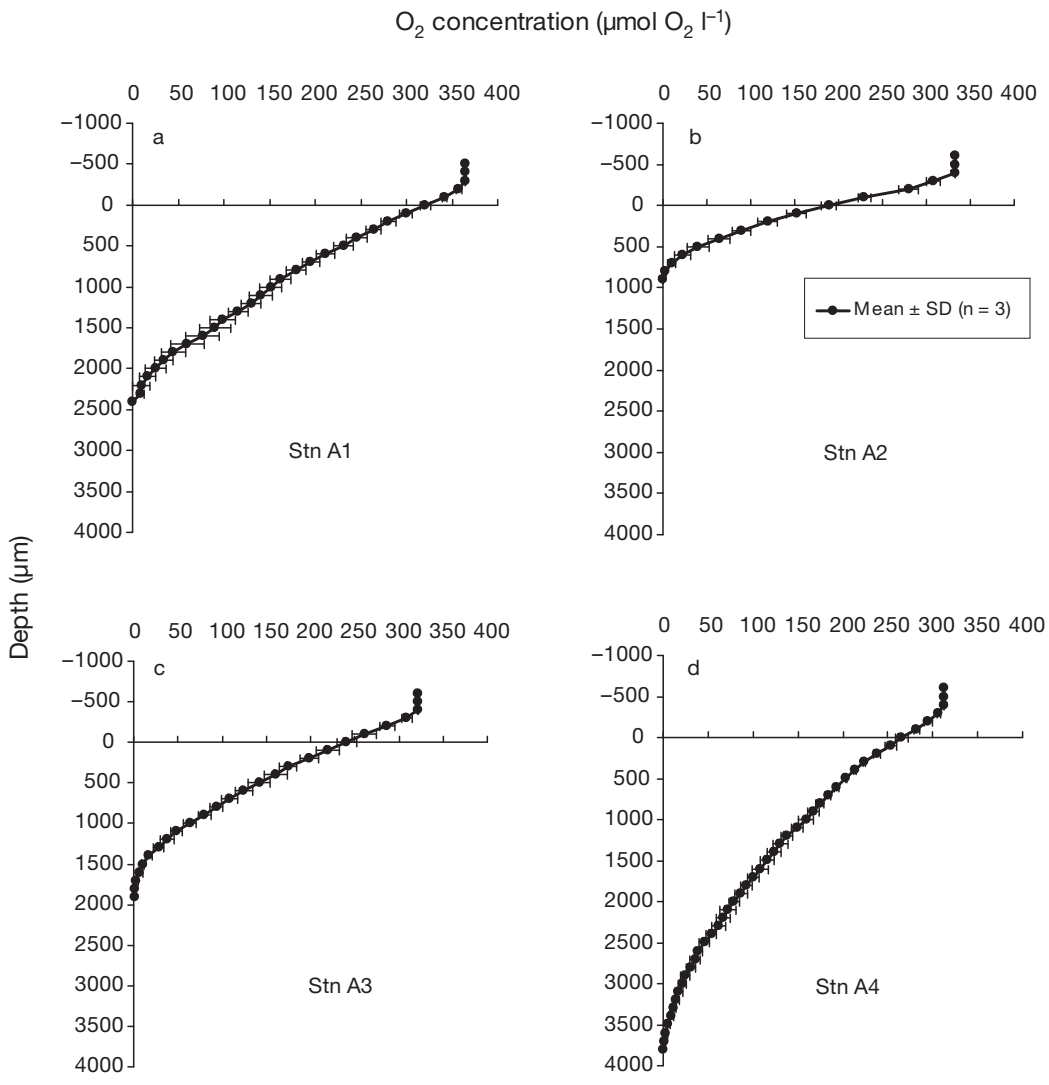

Fig. 3. O_2 microprofiles estimated in sediment core samples from (a) the Banduzzi Channel (Stn A1) and (b–d) along the Aussa River (Stns A2 to A4)

corresponding to 20.6 mg C m^{-2} h^{-1}). Without taking into account the contribution of macrophytes or aquatic phanerogams, this value was entirely due to the abundant MPB community. Not only was the microalgal community very abundant, but it was also photosynthetically active. In particular, *Craticula* and *Melosira* cells appeared viable, with a high pigment content. Overall, MPB seemed to be low-light adapted. In fact, after exposing the microalgae to increasing light intensities, the maximum PP_p (9.1 µg C cm^{-3} h^{-1}) was measured at 50 µmol photons m^{-2} s^{-1}, whereas at higher light intensities they showed photoinhibition. Although PP_s at Stn A2 was higher than that at Stn A3, comparable PP_p values were estimated in the 2 sites. This is probably because sampling at Stn A3 was performed in cloudy conditions, which cause low surface PAR (113 µmol photons m^{-2} s^{-1}).

Oxygen microprofiles revealed that oxygen penetration depth was quite diverse among the 4 sites (Fig. 3). In particular the thickness of the oxic layer at Stn A2 was exceedingly reduced and the oxygen was completely consumed within the top 0.8 mm. In contrast, oxygen penetration at the lagoonal Stn A4 reached a depth of 3.7 mm. At both Stns A1 and A3, the microprofiles showed intermediate dynamics between Stns A2 and A4. Oxygen consumption rates were calculated from the microprofiles performed in darkness.

The maximum oxygen consumption was observed at Stn A2 (Fig. 4a). The value at Stn A3 was 5 times lower at Stn A2, whereas Stns A1 and A4 showed comparable and modest rates. Data were converted to mg C m^{-2} h^{-1} to allow an estimation of the trophic status (autotrophy or heterotrophy) of the investigated sites. Gross primary production (GPP) represents the sum of net primary production (NPP) and community respiration (CR). The ^{14}C technique measures something between GPP and NPP, depending on the incubation time: shorter incubation times are closer to GPP whereas incubation times ≥6 h are closer to NPP (Gazeau et al. 2004). In our case, the incubation time was about 45 min, and therefore a GPP rate was measured. We attempted to estimate NPP by subtracting CR, assessed as oxygen consumption in the dark. In the Banduzzi Channel (Stn A1), only the benthic system was net-autotrophic, whereas along the Aussa River (Stns A2 to A4), the benthic community was net-heterotrophic (Fig. 4b).

The highest enzymatic activities were directed to the degradation of proteins. In all 4 sites, the leucine-aminopeptidase activity was more pronounced in the surface layer, with decreasing values towards the deeper layers (Fig. 5a). As in the subsurface sediment of all 4 sites, comparable values were obtained, but a remarkable difference was observed between the top layers of the Banduzzi Channel (Stn A1) and the Aussa River (Stns A2 to A4). At Stn A1, a lower hydrolysis was estimated (142.2 ± 4.8 nmol cm^{-3} h^{-1}), whereas the degradation of polypeptides displayed higher values at the other stations, with a decreasing gradient along the Aussa River (from 310.8 ± 30.9 nmol cm^{-3} h^{-1} at Stn A2 to 193.3 ± 17.7 nmol cm^{-3} h^{-1} at Stn A4). In contrast, alkaline phosphatase was more active at Stn A1, with decreasing values from the surface (67.0 ± 0.1 nmol cm^{-3} h^{-1}) to the deepest layer (41.6 ± 1.9 nmol cm^{-3} h^{-1}) (Fig. 5b). Significantly lower rates were found at Stns A2 and A3 ($p < 0.001$) compared to Stn A1, with no remarkable differences either among layers or between sites, while intermediate values were found at Stn A4 (mean of 3 layers: 27.4 ± 3.6 nmol cm^{-3} h^{-1}). Degradation of polysaccharides was evaluated via hydrolysis of α-glycosidic bonds, typical of starch, and β-glycosidic bonds, typical of linear molecules such as cellulose. In all 4 sites, the activity of α-glucosidase was lower than that of β-glucosidase (Fig. 5c,d). The highest rates of α-glucosidase were estimated in the Banduzzi Channel (Stn A1). Decreasing α-glucosidase rates were displayed both downstream and from the surface to the deeper layers. In contrast, β-glucosidase activity at Stn A1 was significantly lower ($p < 0.05$) compared to those along the Aussa River (Stns A2 to A3), where decreasing values were estimated downstream. The highest rate was reached in the surface layer of Stn A2 (22.6 ± 0.1 nmol cm^{-3} h^{-1}), whereas the minimum was obtained in the 4–6 cm layer at Stn A4 (3.9 ± 0.1 nmol cm^{-3} h^{-1}). Lipase was the least active among the tested enzymes. Similarly to protease, decreasing hydrolysis rates were observed from the top to the bottom layer (Fig. 5e). Slightly higher values were measured in the surface sediment of Stns A1 and A2 (5.30 and 5.67 nmol cm^{-3} h^{-1}, respectively) compared to Stns A3 and A4 (3.79 and 3.11 nmol cm^{-3} h^{-1}, respectively).

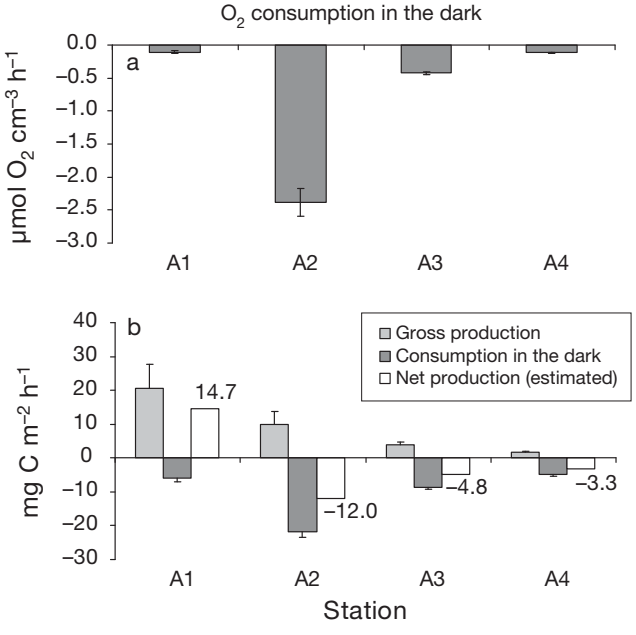

Fig. 4. (a) O_2 consumption rates estimated in darkness and at *in situ* temperature in the 4 studied areas; (b) rates of gross production (from ^{14}C data), consumption (converted from O_2 consumption data) and net production (estimated)

The microbial community was significantly more active in the Banduzzi Channel (Stn A1) than along the Aussa River (Stns A2 to A4) ($p < 0.01$) (Fig. 5f). At Stn A1, high and comparable PCP values were estimated in the surface and subsurface layers (0.77 ± 0.06 and 0.77 ± 0.07 µg C cm^{-3} h^{-1}, respectively), and even in the 4–6 cm layer a higher rate (0.63 ± 0.05 µg C cm^{-3} h^{-1}) was measured compared to the other 3 stations. Along the Aussa River, the maximum secondary production in the surface sediment was displayed at the lagoonal Stn A4 (0.53 ± 0.07 µg C cm^{-3} h^{-1}), with decreasing values upstream.

The highest prokaryotic C efficiency was calculated at Stn A1 (6.21%), while decreasing efficiencies

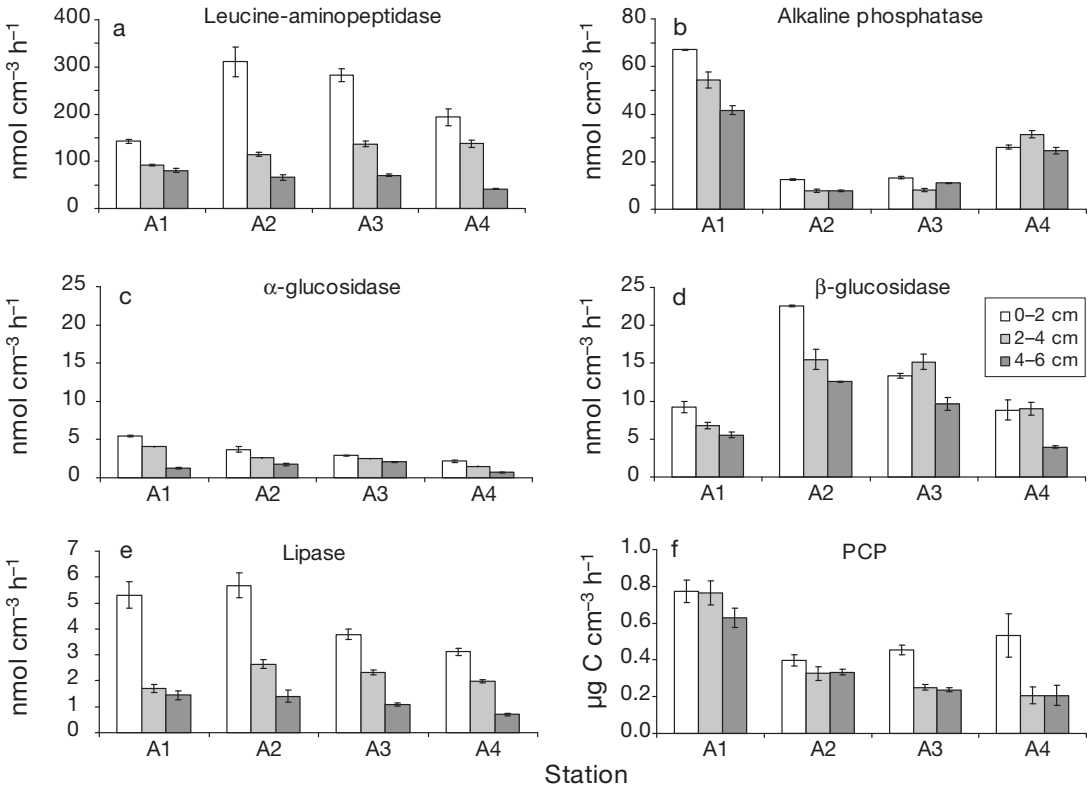

Fig. 5. Extracellular enzymatic activities and prokaryotic carbon production (PCP) in each sediment layer of each station: (a) leucine-aminopeptidase, (b) alkaline phosphatase, (c) α-glucosidase, (d) β-glucosidase, (e) lipase, (f) PCP

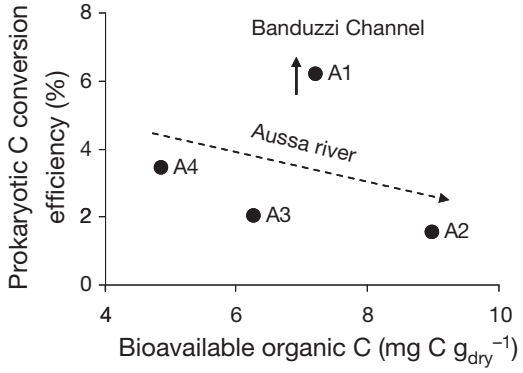

Fig. 6. Relationship between labile organic matter and prokaryotic C conversion efficiency determined as the ratio of prokaryotic C production and the amount of C degraded enzymatically and expressed as a percentage (as proposed by Danovaro & Pusceddu 2007). Data refer to the surface sediment layer

were observed upstream, from Stn A4 to Stn A2 (Fig. 6). Although no evident differences in the prokaryotic numbers were observed among the 4 studied areas, in the Banduzzi Channel (Stn A1) the microbial community seemed to be the most efficient in transforming the organic C pool into biomass. While degradation rates of lipids, polypeptides and carbohydrates at Stn A2 were higher than those estimated in the other 3 sites, the prokaryotic community was less efficient in transferring organic C to higher trophic levels.

PCA

The ordination plot of the considered structural and functional parameters accounted for 74.5% of the total variance (Fig. 7). Principal component axis 1 (PC1) explained 47.5% of the total variance and was correlated with TOC (0.88), chl *a* (0.87) and α-glucosidase (0.86). Principal component axis 2 (PC2) explained 27.0% of the remaining variance and was correlated with phosphatase (–0.90). The PCA separated well the 4 sites that were positioned along a decreasing gradient of organic load, from Stn A2, through Stns A3 and A1, to Stn A4. Stn A4 was characterised by more lagoonal features, minor organic content and lower enzymatic activities.

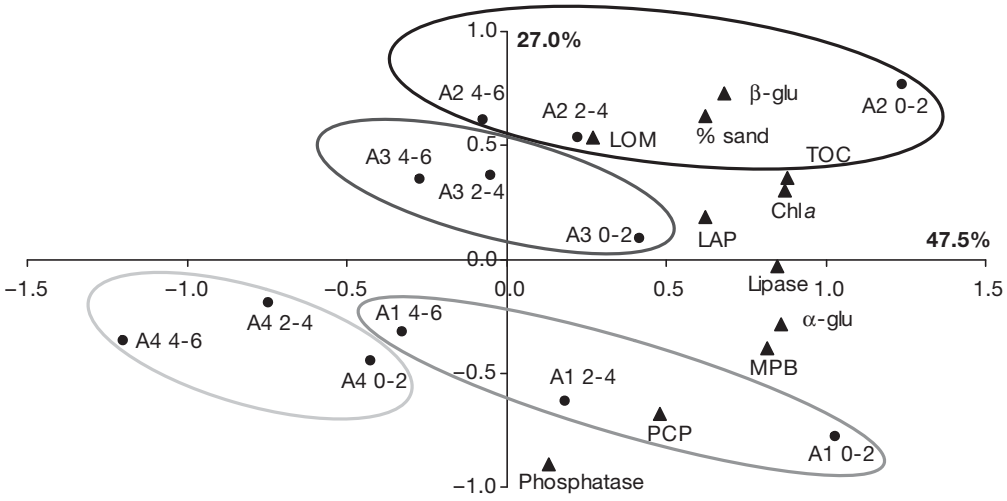

Fig. 7. Principal component analysis, based on r algorithm, of structural and functional parameters (▲) and samples (●) measured in the 3 sediment layers (0–2, 2–4 and 4–6 cm) of the 4 sites (Stns A1 to A4). LOM: labile organic matter; TOC: total organic carbon; MPB: microphytobenthos; PCP: prokaryotic carbon production; LAP: leucine-aminopeptidase; α-glu: α-glucosidase; β-glu: β-glucosidase

DISCUSSION

Synergistic effect of contaminants

The Banduzzi Channel is a nutrient-rich site and is severely polluted by several contaminants: heavy metals, hydrocarbons, dioxins and pesticides. Whilst some contaminants (e.g. heavy metals, pesticides) have an inhibitory effect, other compounds (e.g. nutrients, hydrocarbons) can have a stimulatory effect on the riverine food web. The resulting effect on the benthic communities is hardly predictable. For instance, high levels of N and P contained in pulp mill effluents discharged in the channel may stimulate primary producers (Culp et al. 2000). Phosphorus may also reduce the impact of elevated metals on biofilms by complexing metals (Fleeger et al. 2003 and references therein). Moreover, lignins in pulp mill effluent and other dissolved organic molecules are known to bind metals and thus affect their availability (Podemski & Culp 2001 and references therein). Petroleum hydrocarbons represent a significant source of organic matter that may stimulate bacterial productivity or biomass. These in turn support a bottom-up response of bacterivorous and/or deposit-feeding species. However, metals and insecticides do not appear to stimulate bacterial production and no evidence of bottom-up effects from these contaminants has been found (Fleeger et al. 2003 and references therein). Differences in chemical behaviour in marine and freshwater habitats may also contribute to different responses. For example, metal toxicity is in general negatively correlated with salinity due to effects on free metal ion concentration, while the toxicity of organo-phosphate insecticides increases with salinity.

Microbial response to contamination

Microbial communities in human-stressed environments are of paramount importance. Therefore the study of their composition and functioning is highly relevant in the mitigation of problems already present in our environment (Nogales et al. 2011). The total abundance of benthic prokaryotes in the Banduzzi Channel did not show significant differences compared to the other 3 sites. However, the enumeration of functional bacterial groups cultured on specific media indicated the presence of bacteria capable of degrading n-hexadecane and petroleum in this site. Typically, 16S rRNA gene sequences related to known hydrocarbon degraders are not (or seldom) detected in assemblages collected in chronically hydrocarbon-polluted areas. Nevertheless, they might become predominant when transferred to polycyclic aromatic hydrocarbon (PAH)-enriched culture media. This might indicate that those hydrocarbon degraders are active but constitute a minor component of these communities, and therefore are not evidenced by most fingerprinting techniques (Nogales et al. 2011). Our results are in accordance with those

of Carman et al. (1996), who reported that total bacterial abundance was not significantly affected by diesel-contaminated sediment. In contrast to bacterial abundance, those authors found that the bacterial degradation of ^{14}C-phenanthrene increased considerably in diesel-contaminated sediment. They suggested that the total number of PAH-degrading bacteria is insignificant in comparison with the total bacterial community and/or that resident assemblages have the capacity to switch to PAH utilisation. However, those authors also reported that ^{3}H-leucine incorporation was remarkably consistent among their treatments. This is in contrast with our data, since we found the highest secondary production at the contaminated Stn A1. In this site, together with a severe hydrocarbon contamination, bleached kraft mill effluents were spilled into the Banduzzi Channel from the 1940s until 1992; such effluents increase microbial production as a result of phosphorus enrichment (Culp et al. 2000). In fact, at Stn A1 a very high concentration of total phosphorus has been estimated (Table 1). Furthermore, the transformation of hydrocarbons into biomass by PAH degraders might increase the availability of utilisable organic C, ultimately stimulating PCP rates.

Among the functional microbial groups, we also tested cellulose-degrading organisms, which were highly abundant in the impacted site. Since cellulose is one of the waste products of pulp mill activity, the Banduzzi Channel was likely enriched in this organic compound. This allowed the development of microbial assemblages able to exploit the not-readily-available resource. The amount of cellulose degraders retrieved in the deeper sediment layers suggests that high cellulose loads are present not only in the upper 2 cm, but up to 6 cm depth. This has to imply its burial during the years, accompanied by a long-term evolution of this functional group at this site. The amount of cellulose-degrading microbes found along the Aussa River was much lower (10- to 100-fold), possibly reflecting the decreasing quantity of land plant-derived material while proceeding into the lagoon.

The extremely high abundance of benthic diatoms suggests that they are not influenced by heavy metals and hydrocarbon contamination in the Banduzzi Channel. Benthic diatom mats are rich in extracellular polymeric substances (EPS), which may function as a protective barrier against toxic compounds, as well as enhance the uptake of favourable ones. This feature could decrease the microbial mats' vulnerability to toxic compounds (Sundbäck et al. 2007 and references therein). Moreover, high organic C contents were measured at Stns A1 and A2 (Table 3). There is still little information about the combined effect of pollutants' organic load on diatoms. Overall, higher levels of contaminant are required to elicit toxic effects in sediments that are high in organic material (Carman et al. 1995 and references therein). The few existing studies on combined nutrient–stressor effects have generally found that nutrient-rich systems are less sensitive to stressors (Sundbäck et al. 2007 and references therein). We surmise that the toxic effect that might have been induced by heavy metals and organic pollutants on benthic diatoms in our study site was hidden by the stimulating effect of the organic load.

Benthic community structure at higher trophic levels

Total meiofaunal density was inversely related to microalgal abundance. The meiofauna was mainly composed of nematodes and harpacticoid copepods, which are the major consumers of benthic microalgae (Cibic et al. 2009). Under low grazing pressure, microalgae can develop relatively undisturbed (the impact of viral infection on benthic microalgae is scarcely known; Danovaro et al. 2008) and reach very high densities. Our results agree with Fleeger et al.'s (2003) review, which reported that primary producers, including microalgae, increased in numbers after contaminant-induced impacts on grazers in 60% of the reviewed studies. Strong top-down effects were found in studies of both freshwater and marine benthic systems: when grazers were selectively impacted by direct effects, metals, hydrocarbons and insecticides, they all elicited trophic cascades. However, there is no direct proof of these mechanisms other than the negative dynamics between the increase in microalgal biomass and decrease in meiofaunal abundance.

Surprisingly, meiobenthic abundance in the impacted Banduzzi Channel was higher than the one recorded at Stn A2. Not all studies reviewed by Carman et al. (1995) showed a negative impact of hydrocarbons on meiofaunal communities. Meiofauna in sediments of natural hydrocarbon seeps are either unaffected by oil or show rises in densities related to increased microbial and/or microalgal food resources. Diatoms exposed to bleached kraft mill effluents enhance their lipid content and may become a higher-quality food source for grazers. Increased dietary lipids have been proved to reduce toxicity (Podemski & Culp 2001). The lowest RA of

harpacticoids observed at Stn A1 confirmed that these organisms are more sensitive than nematodes to a variety of substances, including PAH (Carman et al. 1995).

The meiobenthic community seemed to be affected by the low oxygen availability detected at Stn A2 because the meiofaunal density was drastically lower than those recorded in the other areas. Most meiobenthic organisms are generally found in the top 2 cm of the sediment, which typically display oxic conditions. Copepods are one of the most sensitive taxa to oxygen limitation and therefore they are confined to the oxic sediment layer (Danovaro et al. 2004 and references therein). In fact, we found the absolute maximum of copepods at Stn A4 (55.33 ind. 10 cm^{-2}), in correspondence with the highest oxygen penetration in the sediment. At Stn A3, the intermediate oxygen availability could have led to a slightly reduced abundance (48.69 ind. 10 cm^{-2}), whereas at Stn A2, the thinnest oxic layer resulted in the lowest copepod density (1.66 ind. 10 cm^{-2}). Although nematodes are considered to be tolerant to hypoxia (Danovaro et al. 2004 and references therein), their abundance at Stn A2 did not exceed 153.3 ind. 10 cm^{-2}, suggesting that factors other than or in addition to oxygen influenced the faunal abundance in this site.

The macrozoobenthic community at Stn A1 was dominated by chironomid larvae, while the assemblage at Stn A4 was more diverse. Our data agree with the findings of Sconfietti & Marchini (2001), who reported that the macrobenthic communities in the innermost sites of the Stella and Aussa estuaries were biologically poorer than the lagoon one, characterised by the highest Shannon diversity index. The occurrence of *Chironomus* larvae at Stn A1 is also in accordance with the results of Sconfietti & Marchini (2001), who observed these organisms at the innermost station of the Stella River in the Marano Lagoon. Family Chironomidae is frequently the most abundant group of insects in freshwater environments, and its wide range of tolerance to environmental conditions, e.g. salinity and substrate, is well recognised (Pinder 1986). Many species of chironomid larvae are tolerant of poorly oxygenated conditions because they have haemoglobin. Heavy-metals pollution stress has been reported to virtually eliminate all invertebrates, except chironomids and oligochaetes (Pinder 1986 and references therein). Their dominance in the Banduzzi Channel could also be related to food availability. Diatoms are frequently a conspicuous component of the chironomid larvae diet in freshwater aquatic systems (Pinder 1986) as well as in coastal benthic ecosystems (Goldfinch & Carman 2000). Lipids are phagostimulants to some aquatic insects, thus, algae with a higher lipid content may become more palatable or attractive to grazers, leading to major growth and increased size of aquatic insects (Podemski & Culp 2001).

Benthic ecosystem functioning in contaminated sediments

PP decreased from Stn A1 to Stn A4. Especially at Stn A1 we estimated a very high PP_s rate, >20 mg C m^{-2} h^{-1}, indicating that a highly photosynthetically active microalgal community developed on and within these contaminated sediments. This high PP_s rate was probably due to a combination of different factors. Firstly, the microalgal community was well-adapted to low light conditions, typical of the winter months. Secondly, the community was not subjected to excessive grazing pressure. Thirdly, through the photosynthetic process, pennate diatoms extruded high amounts of EPS, creating a protective barrier against toxic compounds, which enabled them to be more resistant to contaminants. Finally, the community was not limited by nutrients, which were regenerated by an extremely active prokaryotic community. Our data are in accordance with those of Carman et al. (1995), who reported elevated incorporation of ^{14}C and high chl *a* values in their high-PAH treatments, suggesting that microalgal growth was stimulated in the high PAH treatments. Moreover, in our study the highest PP_s rate was recorded in conjunction with the highest prokaryotic C efficiency, thus suggesting that prokaryotic activity could enhance primary producers by providing nutrients. Bacteria in contaminated sediments metabolise N from an otherwise refractory pool of organic material. For instance, PAH degraders, which become enriched in diesel fuel-contaminated sediments because of their ability to co-metabolise complex ring structures, may also metabolise complex ring structures that contain N, such as detrital chlorophyll and phenolic and humic materials. Previous studies have shown that inputs of 'new' or 'labile' carbon to a system may enhance the decay of refractory carbon in sediments via the process of microbial stimulation or co-metabolism (Carman et al. 2000 and references therein).

At Stn A2, the oxygen consumption was much higher than in the 3 other sites. This is peculiar if we consider that at Stn A2 the sand percentage (34%) was higher than that measured for instance at Stn A4

(5%). Sandy sediments usually favour a deeper oxygen penetration compared to muddy sediments. Moreover, considering the particularly low meio- and macrobenthic abundances, the enhanced oxygen consumption rate may not be ascribable to respiration by larger benthic organisms either. The oxygen demand was likely due to degradation of the high organic load in this site (LOM: >11 mg C g^{-1}). Our results agree with those of Christensen et al. (2003), who found that oxygen consumption, measured by microprofiles, increased in the organic-rich sediments, thus resulting in a net-heterotrophic benthic community.

Prokaryotic activities, estimated both as enzymatic degradation and carbon production, are important factors affecting the transfer of detritus towards higher trophic levels. Alkaline phosphatase, essential for the mineralisation of PO_4^{3-} from organic molecules, is used by both prokaryotes and microalgae to meet their metabolic phosphorus requirements. The highest rate of alkaline phosphatase activity was measured at Stn A1, in correspondence with the maximum abundance of MPB. Due to this enzymatic activity, the increased availability of PO_4^{3-} could promote PP, thus contributing to support the overall benthic system functioning of the contaminated site where the highest PP rates were measured. High PP, together with fast P regeneration, could have fuelled secondary production rates (PCP), which were the highest in this site, too. Comparing our PCP data (converted in g dry sediment) with those of Manini et al. (2003) for the eutrophic-dystrophic Lesina Lagoon, the values obtained in the Banduzzi Channel were 3 times higher (3.1 µg C g^{-1} h^{-1}) than those reported by those authors in winter (0.7 to 1.1 µg C g^{-1} h^{-1}), whereas values obtained along the Aussa River were comparable. High PP_s and PCP rates, together with relatively low respiration rates (Fig. 4b), suggest an increased transformation of C (organic and inorganic) into biomass, possibly leading to an enhanced transfer of C to higher trophic levels. However, since predators' abundances were not particularly pronounced at this site, this scenario reflects into a solid benthic microbial loop (Caumette 1992, Deming & Barross 1993), with the possible intervention of functional microbial groups, capable of exploiting non-labile material such as hydrocarbons and cellulose of industrial origin.

Degradation of polysaccharides seemed to follow the dynamics of PP and microalgal abundance along the Aussa River with decreasing rates to the lagoonal station. It is noteworthy that leaf detritus was present in our samples, with decreasing content towards the lagoon (data not shown). Therefore glucosidase activity could also be linked to the presence of non-readily usable type substrate. In contrast, the intense photosynthetic activity in the Banduzzi Channel probably increased the amount of labile carbohydrates, which can be easily metabolised by prokaryotes without requiring further hydrolytic processes.

Unlike other Mediterranean lagoons (Manini et al. 2003), in our samples, proteins were always higher than carbohydrates, possibly being the cause for high polypeptide hydrolysis rates (with respect to other degradation processes). Comparing our leucine-aminopeptidase results with those reported by Manini et al. (2003), we obtained values ranging from 432 to 993 nmol g^{-1} h^{-1} in the top sediment layer at Stns A4 and A2, respectively, while rates reported by those authors in winter were much lower, varying from 9.6 to 304.7 nmol g^{-1} h^{-1}.

The relation between MPB and lipase ($R = 0.69$, $p < 0.05$) was likely ascribable to the high content of fatty acids in diatom cytoplasm. Microalgae react to toxicant stress with a change in cellular macromolecule content, generally increasing their lipid content (Podemski & Culp 2001) and ultimately stimulating their microbial degradation in the contaminated site (Stn A1).

We calculated the turnover rates of the main macromolecules as the ratio of enzymatically mobilised carbohydrates, lipids or proteins to the corresponding labile organic fraction. Values were expressed as days required for completing the turnover of each molecules' group. The Banduzzi Channel was characterised by the fastest turnover rates of both carbohydrates (3.9 d) and lipids (15.9 d), whereas an increasing gradient from the innermost station to the lagoon was observed for the Aussa River. These values further confirmed that the microbial benthic community at Stn A1 was more active than in the other studied areas. Protein turnover rates showed opposite dynamics, since the highest value was calculated in the Banduzzi Channel (5.1 d) and decreased along the Aussa River, indicating that the sediment of Stn A1 was characterised by a slower protein turnover, suggesting an accumulation of proteins. Nevertheless, the turnover of proteins was very fast compared with those calculated by Manini et al. (2003) in the Lesina and Marsala lagoons in winter (6 to 110 and 65 to 1464 d, respectively) suggesting that the accumulation of proteins in the Banduzzi Channel was negligible. Turnover data confirmed the higher efficiency of benthic prokaryotes at Stn A1 in mobilising the organic matter pool. Prokaryote C

conversion efficiency values provided more evidence of a well-developed microbial loop at Stn A1. At this site we detected the highest % efficiency (6.2%), whereas the other sites were characterised by decreasing values as proceeding upstream (at increasing LOM content). These results are in contrast to the findings of Danovaro & Pusceddu (2007), who described increasing efficiency along with increasing LOM load. It must be noted, however, that our LOM data are generally higher than those used by Danovaro & Pusceddu (2007) for their conceptual scheme and refer to lower efficiency. These results, coupled with previous findings, suggest that C conversion efficiency may be stimulated by higher LOM content up to a threshold value, after which the efficiency tends to decrease. An exception in this scheme would be provided by highly dynamic sites, such as those recovering from long-term pollution impacts (Stn A1).

Dissimilar environmental conditions such as grain-size and TOC content but especially increasing salinity from Stn A1 to Stn A4 selected different benthic assemblages and likely affected the benthic ecosystem functioning in the 4 studied sites. This was a descriptive study in which cause and effect relationships were not tested. Therefore, it was difficult to determine to what extent the differences among the benthic communities and their functioning were ascribable to synergistic effects of contaminants rather than to natural physical conditions occurring along a salinity gradient in a riverine-lagoonal system. However, at least in the severely impacted Stn A1, it is likely that a resistant though altered benthic community has been selected as a result of chronic perturbations that have occurred in the past few decades.

CONCLUSIONS

Looking at the macroscopic components of the benthic community, a low diversity system was observed in a contaminated site. However, when we focused on the microbial phototrophic community, in this site the highest richness was obtained. Primary producers sustained both higher trophic levels by direct input of biomass and microbial decomposers by input of labile carbon, which likely enhanced the degradation of refractory carbon via the process of microbial stimulation or co-metabolism. Even in a heavily contaminated site, high production and degradation rates were ensured by an extremely active microbial community.

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