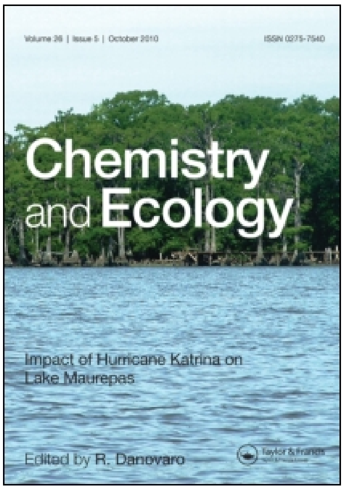

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ROLE OF MICROORGANISMS AND MACROFAUNA IN BENTHIC PHOSPHORUS DYNAMICS IN THE PO RIVER–ADRIATIC SEA FRONTAL SYSTEM: AN EXPERIMENTAL APPROACH

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Sediment biotic and abiotic attributes were determined during the PRISMA II oceanographic campaigns in the northern and central Adriatic Sea. The radiotracer orthophosphate ^{32}P was used under laboratory conditions to analyse the role of micro- and macrofauna in phosphorus dynamics at the water-sediment interface. Effects of infaunal suspensivores on microfungal growth were also investigated.

Our findings emphasised: 1) significant differences in sediment ^{32}P uptake as related to the sampling area, occurrence of the pelagic frontal system, sediment grain size, microbial activity, and anoxia; 2) the crucial role played in ^{32}P dynamics by different tropho-functional groups – *i.e.*, infaunal filter-feeders and epifaunal detritivore characterising the benthic community of the northern Adriatic basin.

The significant changes in ergosterol concentration (adopted as an index of microfungal growth) observed in sediments subjected to infaunal activity, eventually provided a comprehensive insight into macrofauna-microorganism interactions. The potential impact of macrofaunal activity in phosphorus dynamics in the Adriatic benthic system is discussed.

Keywords: Microorganisms; macrofauna; phosphorus dynamics; sediments

INTRODUCTION

In aquatic environments, a significant fraction of the phosphorus pool is sequestered within the sedimentary compartment (Boers *et al.*, 1993). In relation to abiotic (pH, redox potential) and biotic (organic matter content, micro- and macrofauna) factors, the sediment may act as a "sink" or as a "source" of dissolved phosphates (Holdren and Armstrong, 1980).

Invertebrate activity at the water-sediment interface can strongly affect phosphorus dynamics in benthic systems. In fact, they can disrupt and rework the substrate surficial layers, varying the redox profiles and affecting phosphorus adsorption (Kamp-Nielsen *et al.*, 1982); on the other hand, they can alter phosphorus mineralisation rates, ex-

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ploiting the organic matter interspersed in the sedimentary matrix (Gardner *et al.*, 1981; Nalepa *et al.*, 1991) and interfering with the heterotrophic microbial component colonising the water-sediment interface (Gätcher *et al.*, 1988).

In coastal benthic environments, the resolution of relationships occurring among macrofauna, micro-organisms, and phosphorus dynamics can provide crucial information on the functional links established between the benthic and pelagic systems and on food web dynamics (Carpenter *et al.*, 1992). This resolution is particularly needed for shallow marine environments, like the Northern and Central Adriatic Sea, where significant exchanges of dissolved and particulate matter occur commonly among the water column, the sedimentary compartment, and the macrobenthic assemblage (Ott, 1992).

The aims of the present study were:

1. to determine the abiotic and biotic characteristics of sediments (*e.g.*, grain size distribution, organic content, microfungal density, relative importance of macrofaunal trophic groups) in two areas sampled within the PRISMA II project in northern and central Adriatic Sea;
2. to investigate, in laboratory microcosms, sediment phosphorus uptake in relation to substrate physical attributes and the occurrence of the Po-Adriatic Sea pelagic front, focusing on the role played by micro-organisms under natural and anoxic conditions;
3. to analyse the specific effects of epifaunal and infaunal activity on phosphorus dynamics at the water-sediment interface.

Furthermore, we evaluated the effects of infaunal suspensivores on microfungal growth following short-term changes in ergosterol content of sediment surficial layers.

MATERIALS AND METHODS

Study Site – Figure 1 shows the sampling stations of the four PRISMA II campaigns. Oceanographic cruises were carried out every semester from June, 1996 until February, 1998 (they are indicated in the following text as 1st, 2nd, 3rd, and 4th campaign). Site locations in both northern and southern sampling areas were chosen as to cross the pelagic Po-Adriatic frontal system.

Sediment Attributes and Macrobenthic Community Analysis (1st and 2nd campaign) – Sediment grain size composition (expressed as Mdϕ) and organic matter content (percent OM in the text), together with attributes of the macrobenthic community expressed in terms of spatial density of dominant trophic groups (expressed as n. individuals $\cdot$ m^{-2}) were determined on sediment cores (in triplicate) retrieved by a ship-operated multicorer and processed according to laboratory methodologies described in Mancinelli *et al.* (1998). Microfungal density (δ: n. colonies $\cdot$ ml^{-1}) was determined by plating on solid culture medium (Malt-Extract Agar) known aliquots of sediment suspension, followed by colony counting after 5 and 10 days at 18°C (Park, 1972).

Experimental Design (1st, 2nd, and 3rd campaign) – The whole set of experimental activities was carried out under laboratory conditions using the radiotracer orthophosphate ^{32}P in simplified water-sediment microcosms.

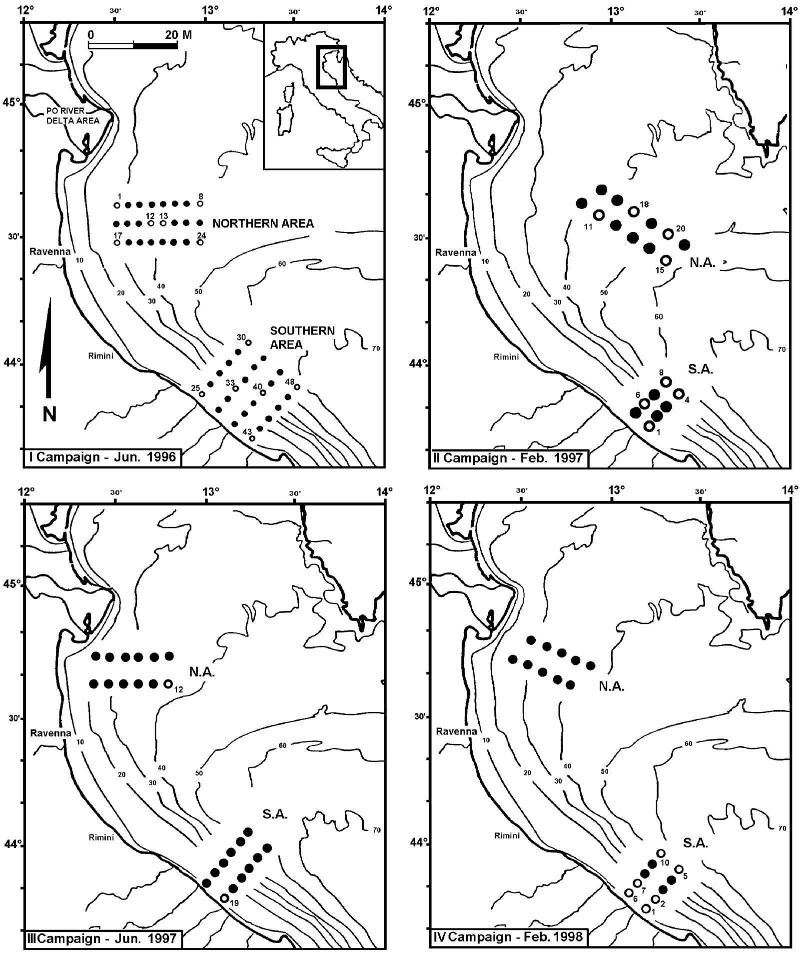

FIGURE 1 Sampling stations of the four PRISMA II oceanographic campaigns. Empty dots indicate sampling stations cited in the text.

At the start of every experimental phase, ^{32}P was introduced in dissolved form in the aqueous compartment; ^{32}P concentration in water was followed by means of repeated samplings (50 μl, 3 replicas) taken on a geometrical time scale (after 1, 2, 4, 8, 16, 32, 64 h if not otherwise specified); only the final distribution of the radiotracer among biotic (invertebrates) and abiotic (water, sediment) compartments was assessed in both solid and aqueous phases. ^{32}P content (expressed as dpm: n. disintegration $\cdot$ min^{-1}) in aqueous samples was determined by liquid scintillation counting in a Packard Tri-Carb 4000B counter after correction for counting efficiency and colour quenching, and expressed as dpm $\cdot$ ml^{-1} (experiment 2: see further in the text) or as per cent fraction of the initial radiotracer concentration in water (experiment 1 and 3). Sediment and animal samples were oven-dried at 60°C for at least 72 h and weighed. Samples were consequently digested in NCS tissue solubilizer, and counted after efficiency/colour quenching correction; specific activity (AD, activity density: dpm $\cdot$ mg^{-1} animal or sediment dry weight) and concentration factor (CF: $AD_{animals} \cdot AD^{-1}_{sediment}$) were adopted, according to Whittaker (1961), to express radiotracer concentration in solid phases.

Three experimental investigations were carried out:

1. analysis of sediment ^{32}P uptake under different conditions of anoxia and microbial colonisation (2nd campaign: northern area: sites 11, 15, 18, 20; southern area: sites 1, 4, 6, 8; Fig. 1). In water-sediment systems (15.0 ml of sterilised sea water; 4069 ± 97 mg (mean dry weight ± SE, DW in the following text) of sediment) orthophosphate ^{32}P was introduced to reach a final water concentration at $T = 0$ of $455{,}315.16 \pm 3500$ (southern area sites) and $343{,}188 \pm 4100$ (northern area sites) $dpm \cdot ml^{-1}$ (means ± SE). Three treatments were used: microbial suppression (autoclaving at 1.2 atm, 120°C for 20 min; ST in the following text); anoxia (induced by continuous nitrogen insufflation; AN in the text), and defaunated controls (N), consisting of intact sediment.

   Sediment radiotracer uptake was calculated as the loss of ^{32}P from the aqueous compartment at the end of the experiment; ^{32}P final fluxes across the water-sediment interface (F) were determined as the radiotracer loss from the aqueous compartment during the experiment per unit area ($dpm \cdot cm^{-2} \cdot h^{-1}$);
2. response of water-sediment systems to epifaunal activity (2nd campaign). In microcosms containing 200 ml of sterilised sea water and $4 \cdot 10^4$ mg (DW) of sediment, ^{32}P was introduced to reach a final water concentration at $T = 0$ of $455{,}315.16 \pm 3500$ (southern area sites) and $343{,}188 \pm 4100$ (northern area sites) $dpm \cdot ml^{-1}$. Radiotracer concentration was determined on an exponential time scale for the first 24 hours; consequently, samples were taken every 24 h. At the 96th h, ^{32}P concentration did not vary significantly in comparison to the 72th h sampling, indicating a negligible sediment ^{32}P net uptake flux. At that time, four similar specimens (3.99 ± 0.26 mg individual mean DW ± SE) of the amphipod *Gammarus* sp. were introduced in the systems. Consequently, radiotracer concentration in water was determined every 24 h until the 240th h.
3. Analysis of sediment ^{32}P uptake and release in relation to infaunal activity (3rd campaign). Two representative sites in terms of grain size distribution, organic content and suspensivores occurrence in the benthic assemblage were chosen in the southern (site 19) and northern (site 12) areas. For each site, in microcosms containing 14 ml of sterilised sea water and 243.3 ± 17.5 mg DW of sediment, ^{32}P was introduced to reach a final water concentration at $T = 0$ of $455{,}315 \pm 3500$ (southern area sites) and $343{,}188 \pm 4100$ (northern area sites) $dpm \cdot ml^{-1}$. Two treatments were used: 1) defaunated intact sediment (treatment N); 2) presence of three similar specimens (197.9 ± 13.7 mg DW) of the infaunal filter-feeder *Tapes* sp. (treatment $N + A$). Radiotracer concentration was determined on an exponential time scale until the 64th h; consequently, the whole aqueous compartment was replaced by an identical volume free of ^{32}P. The radiotracer release in water was followed for 64 h on an exponential time scale.

Variations in Microfungal Growth Induced by Infaunal Suspensivores (4th campaign) – In two experimental sets, sediment cores sampled in the southern area (sites 1, 2, 5, 6, 7, 10; Fig. 1) were acclimated in microcosms for 24 h, and initial ergosterol concentration (expressed as $\mu g \cdot g^{-1}$ sediment DW) in the surficial layer was determined (in triplicate) by HPLC analysis (Waters 600–996 HPLC system, μBondapak C18 reversed phase column, isocratic eluent methanol-water 95:5; 1 ml $\cdot$ min^{-1} flux; (see Newell *et al.*, 1988; Gessner *et al.*, 1991; Gessner and Chauvet, 1993) for extraction procedures and high-pressure chromatography methods). 10 similar specimens (1277.4 ± 40.9 mg mean DW ± SE) of *Tapes* sp. were introduced in every microcosms of the first set (AN treatment); the other one was used as a defaunated control (CON treatment). After 5 days, aliquots (3 replicas) of the sediment surficial layer were taken from every microcosm and consequently analysed for ergosterol determination.

Values are expressed in the following text as a mean ± SE if not stated otherwise; for parametric statistical analysis, data were tested for conformity to assumptions of variance homogeneity (Cochran's C-test) and transformed when required. When data transformation failed to meet the assumption of homogeneity of variance, nonparametric tests were performed.

RESULTS

Sediment Features and Benthic Community Structure

Sediment grain size (expressed as Mdϕ), per cent organic matter content (%OM), microfungal density (δ), and spatial densities of trophic groups dominating the macrobenthic assemblage are summarised in Table I. In summer (1st campaign), sediments from the northern area were characterised by a gradual shift from coastal clay-silt deposits (Mdϕ 4.25–3.95) to off-shore relict sands (Mdϕ 1.96–1.75); southern sediments varied from near-shore sandy deposits, low in the finest fractions (sites 25–43: Mdϕ 3.17–3.55), to clayish sediments in the central stations (sites 33–40: Mdϕ 4.25–4.23), and eventually showed an increase in granulometry in the off-shore stations (Mdϕ 2.27–3.17). Sediment total organic content resulted inversely related to grain size: in both sampling areas the finest sediments were also the richest in organic matter

TABLE I Sediment biotic and abiotic features determined in the 1st and 2nd sampling campaign: Mdϕ, organic content (%OM), microfungal density δ (n.colonies $\cdot$ gr^{-1} sediment DW $\cdot$ 10^3), and spatial density (n. individuals $\cdot$ m^{-2}) of dominant macrofaunal trophic groups. DF: infaunal deposit-feeders; SDF: epifaunal deposit-feeders (*Gammarus* sp.); SF: suspension-feeders. SE not shown

Southern Area							*Northern Area*						
Site	*Mdϕ*	*%OM*	*δ*	*DF*	*SDF*	*SF*	*Site*	*Mdϕ*	*%OM*	*δ*	*DF*	*SDF*	*SF*
1st Campaign – June 1996													
43	3.17	3.15	0.34	1201		424	1	4.25	7.93	2.32	259		731
25	3.55	3.40	0.17	870	942	2518	17	4.25	8.56	2.38	1270	47	47
33	4.25	6.99	0.46	47			12	3.95	5.99	1.24	194		
40	4.23	8.13	0.65	47			13	3.39	5.12	0.43			47
30	2.27	2.07	0.29				8	1.96	1.91	0.36	212		
48	3.17	3.20	0.23				24	1.79	1.92	0.14	377		
2nd Campaign – February 1997													
1	3.38	5.03	0.56	141	71	1199	11	2.16	6.33	0.17			
6	4.18	6.96	0.08		141	3880	18	1.96	3.36	0.04			
4	4.17	12.20	0.27				20	1.82	4.76	0.06	71		
8	4.18	11.46	0.39				15	1.95	4.94	0.29	71		

(4 d.f., $r = 0.91$, $p < 0.05$ northern and southern area). In particular, in the southern area the central sites showed organic contents two-fold higher than those characterising respectively the coastal and offshore stations; %OM in the northern area displayed a clear inshore-offshore gradient, shifting from values comparable to those determined for the southern central sites (7.93–8.56%), to remarkably low values for the most offshore sites (1.91–1.92%).

The microfungal density resulted correlated to sediment organic matter content in both southern and northern stations (4 d.f., $r = 0.87$, $p < 0.05$).

In the 2nd sampling campaign (February, 1997), sediments grain size of northern area gradually increased in relation to the distance from the coast; conversely, the southern area stations were characterised by deposits dominated by the finest dimensional fraction, with site 1 as the only exception. In comparison to the 1st campaign, significant differences were found between the two sampling areas in total organic content: southern sediments showed an average organic content *ca* two-fold higher than northern deposits (8.91 *vs.* 4.84%; 1st campaign: 5.24 *vs.* 4.49%). Mdϕ and organic content in southern sediments showed a non-significant positive relation (2 d.f., $r = 0.74$ NS); for northern sediment an even weaker relation was detected (2 d.f., $r = 0.61$, NS).

No significant relationships were determined between microfungal density and sediment organic content either in the northern (2 d.f., $r = 0.56$ NS) or in the southern area (2 d.f., $r = 0.21$ NS).

The benthic macrofauna was characterised in both the 1st and 2nd campaign by a clear inshore-offshore density gradient (Tab. I), unrelated to granulometry, %OM and microfungal density. During the 1st campaign (summer), in the northern area macrobenthos densities in the inshore sampling sites were higher than those determined for the offshore sites, characterised by a strong dominance of infaunal deposit-feeders (DF). In the southern area, in comparison to the high densities characterising the coastal stations, central and offshore stations showed significant density decreases (Tab. I). In both areas, coastal sites were characterised by the occurrence of epifaunal detritivores (mainly the amphipod *Gammarus* sp.; SDF in Tab. I); in particular, in the southern area macrobenthic assemblage (Site 25), *Gammarus* sp. accounted for *ca* 50% of the entire deposit-feeder group.

In winter, faunal density significantly decreased in both sampling areas (2nd campaign): in the southern area animal density changed from 9933 (summer) to 5432 (winter) individuals $\cdot$ m^{-2}, whereas the macrobenthos of the northern area showed a dramatic negative variation dropping from 4056 individuals $\cdot$ m^{-2} in summer to 142 individuals $\cdot$ m^{-2} in winter. The infaunal assemblage showed in the northern area an overall strong dominance of deposit-feeders and a low occurrence of infaunal filter-feeders; in the southern area, on the other hand, filter-feeders dominated the macrobenthic assemblage in sampling sites close to the coast (Sites 1 and 6), and decreased to negligible densities in the open sea stations.

The lack of correlation between sediment grain size and organic content determined in the 2nd campaign indicated that in winter the organic input to the benthic systems of both sampling areas resulted, independent from general sedimentation patterns of suspended material from the water column. In this perspective, the subsequent laboratory experimental phase on ^{32}P sediment uptake was carried out using winter sediment samples, as they actually provided the opportunity to discriminate between effects related to different sediment attributes – namely grain size and organic matter content – on ^{32}P uptake patterns.

Microorganisms and Sediment ^{32}P Uptake under Natural and Anoxic Conditions

Under natural conditions of oxygenation and microbial colonisation, ^{32}P uptake in the northern area stations resulted significantly lower than southern sites (Tab. II; Mann-Whitney U test, $Z = -2.3$, $p = 0.02$). The southern area sediments sequestered *ca* 90% of the ^{32}P originally present in the aqueous phase, whereas northern sediments sequestered *ca* 47% of the radiotracer. For both the sampling areas, it was possible to recognise a remarkable variation in sediment uptake patterns related to the occurrence of the pelagic frontal boundary: in fact, in the southern area, the central sites (sites 6–4) showed both maximum phosphorus uptake activities and fluxes; in the northern area, coastal stations were characterised by maximum ^{32}P uptake and fluxes (sites 11 and 18), with a strong decrease in coincidence with the frontal boundary. ^{32}P general uptake patterns for both sampling areas resulted non-significantly related either to grain size (2 d.f., $r = 0.42$ and $r = 0.35$), or to organic matter content (2 d.f., $r = 0.26$ and $r = 0.34$).

Microbial suppression resulted in an overall decrease in sediment ^{32}P uptake (Site 8, in the southern area, represented a remarkable exception), particularly evident in the northern area sites (Tab. II; Mann-Whitney U test, $P = 0.02$). Whereas non relationship was detected for the southern area, in the northern area sediment ^{32}P uptake resulted significantly related to organic matter content (2 d.f., $r = 0.95$, $p < 0.05$). CVs variation coefficient, determined as $\mathrm{CV} = (\mathrm{SD} \cdot \mathrm{AV}^{-1}) \cdot 100$ (where SD = standard deviation, AV = mean) calculated between ^{32}P uptakes under natural and anoxic conditions (Tab. II), indicated for northern sites variations ten-fold higher than southern sites (site 18 showed the maximum variation). Moreover, in both areas sampling sites located near the frontal boundary (site 6 and 18) showed the highest CVs.

TABLE II ^{32}P remaining in the aqueous compartment at the end of the experimental phase (% of initial concentration); uptake fluxes of the radiotracer across the water-sediment interface in natural conditions (F: $\mathrm{dpm} \cdot \mathrm{cm}^{-2} \cdot \mathrm{h}^{-1}$). N: natural conditions; ST: microbial suppression; AN: anoxia. CV: variation coefficients determined between natural/sterilised (ST) and natural/anoxic (AN) treatments. SE not shown

Condition	*Dissolved ^{32} P%*			*^{32}P Uptake flux F*	*Variation coefficient CV*	
Site	*N*	*ST*	*AN*	*N*	*ST*	*AN*
Southern area						
1	87.0	83.8	84.2	1120.6 (51.7)	2.6	2.3
6	96.1	81.2	96.0	1238.2 (6.9)	11.9	0.1
4	88.5	82.3	92.6	1140.1 (33.9)	5.1	3.2
8	83.5	87.2	91.3	1138.9 (73.8)	3.1	6.3
Northern area						
11	57.2	18.2	32.8	555.6 (40.0)	73.2	38.4
18	59.5	6.0	30.2	577.6 (11.7)	115.5	46.2
20	40.9	11.7	15.4	339.5 (2.3)	78.4	64.1
15	30.0	8.8	14.7	291.2 (4.1)	77.0	48.2

Negligible variations in ^{32}P uptake were detected in southern sediments under anoxic conditions (Tab. II, Mann-Whitney U test, $Z = -1.2$, NS), whereas northern sediments showed a stronger, even though not-significant (Mann-Whitney U test, $Z = -1.8$, $p = 0.08$), decrease in radiotracer sediment uptake from coastal to open sea stations, confirmed by the higher CVs determined between natural and anoxic conditions.

The Role of Detritivores and Suspensivores in Sediment ^{32}P Uptake

a) *Epifaunal Detritivores* (Experiment 2) – In microcosms containing northern area sediments, epifaunal activity caused strong increases in the sediment radiotracer uptake, indicated by the significant loss of ^{32}P from the aqueous compartment occurred after amphipod introduction. On the other hand, scarce variations in ^{32}P concentrations were detected in microcosms containing southern area sediments (Fig. 2). Final changes in ^{32}P concentration after animal introduction resulted significantly related to animal concentration factors in northern sediment microcosms (Fig. 2; Kendall Correlation, $Z = 2.1$, $p = 0.04$); thus suggesting that the observed variations might be related to amphipod trophic exploitation of sediment organic content or of the asso-

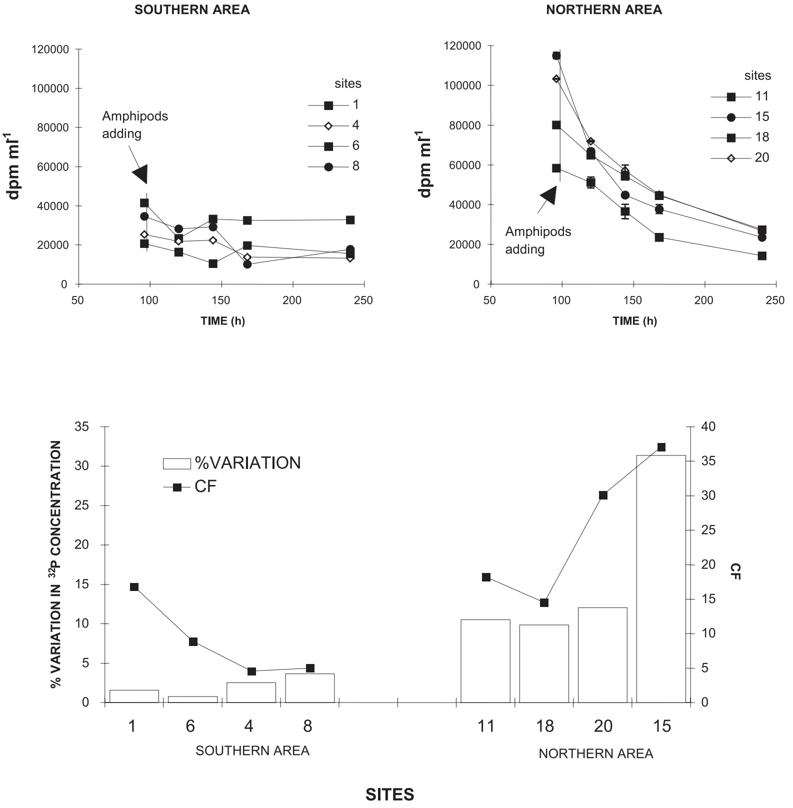

FIGURE 2 Variations in ^{32}P water concentration induced by *Gammarus* sp. Activity (top); bottom: percentage variations in ^{32}P water concentration after animal introduction and animal concentration factors ($AD_{animal} \cdot AD_{sediment}^{-1}$).

ciated microbial component. No significant relationship was detected for southern area sediments ($Z = -0.6$, $p = 0.49$).

b) *Infaunal Filter-Feeders* (Experiment 3) – In Figure 3 temporal patterns of sediment ^{32}P uptake and release are shown under defaunated conditions (N) and in the presence of infaunal suspensivores (N + A). Infaunal activity induced a generalised, remarkable loss of ^{32}P from the aqueous compartment (at the end of the uptake phase in both southern and northern areas animal activity caused similar – *ca* 30% – radiotracer losses), even though sediments from the two sampling areas were characterised by different uptake and release patterns. The southern area sediment showed a rapid ^{32}P uptake (more than 80% of the radiotracer was lost from the aqueous compartment within the first 10 h), that corresponding to a negligible release of the radiotracer in water during the subsequent release phase (*ca* 3% after 60 h). Where *Tapes* sp. speci-

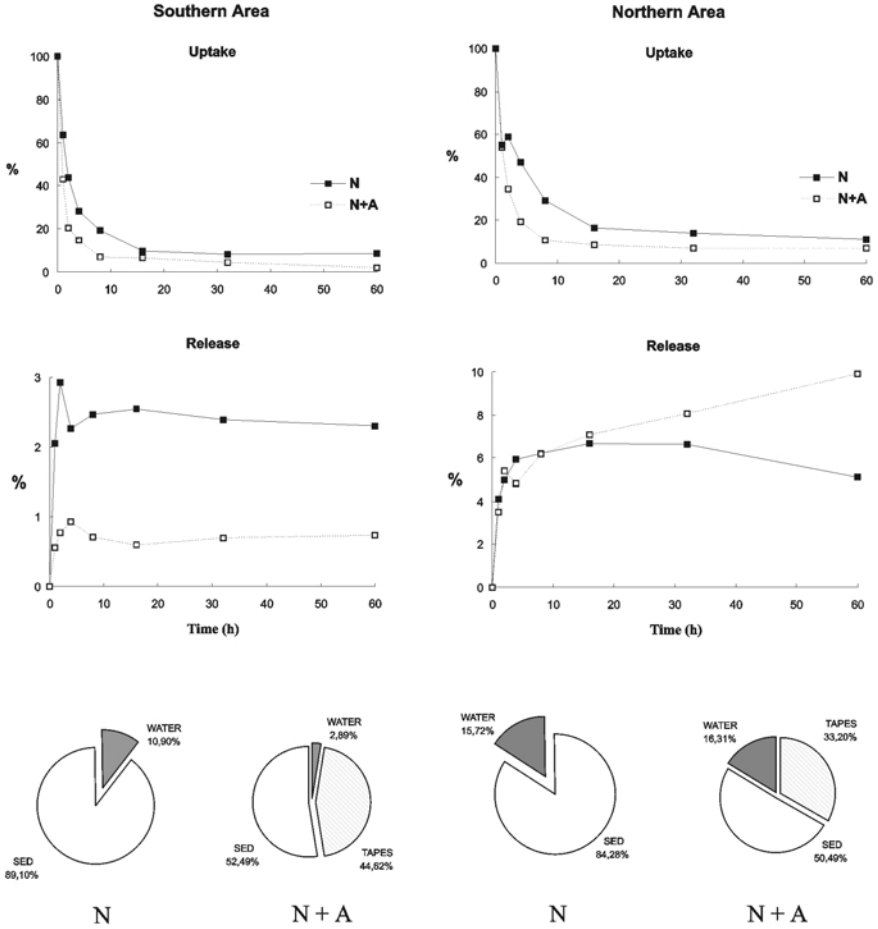

FIGURE 3 Temporal variations in ^{32}P water concentration during uptake (top; expressed as % of initial concentration) and release phases (centre; expressed as % of the radiotracer sequestered in sediment + sacrofauna compartment during the uptake phase); bottom: % distribution among compartments of the radiotracer initially introduced. SED: sediment pool; WATER: water pool; TAPES: animal pool.

mens were present, the radiotracer release resulted even lower (*ca* 1% after 60 h; Mann-Whitney U test, $Z = -2.32$, $p = 0.02$).

The northern area sediment was characterised by slower uptake patterns under both N and N + A conditions (80% of the radiotracer was lost from the aqueous compartment after 20 h).The ^{32}P release (Fig. 3; *ca* 6% after 60 h in defaunated controls), resulted significantly enhanced by the presence of *Tapes*: after 60 h more than 10% of the radiotracer sequestered during the uptake phase by the sedimentary and animal compartments was released into the aqueous compartment (Mann-Whitney U test, $Z = -2.12, p = 0.03$). Moreover, in comparison to the southern area, the northern area sedimentary and animal ^{32}P pools resulted to be, at the end of the release phase, far from equilibrium with the aqueous compartment. The differentiated response to animal activity of northern and southern sediments was confirmed by the analysis of final water-sediment CFs: in the southern area sediment CF resulted significantly increased by animal activity (3600 ± 342 and 5387.6 ± 489, respectively for N and N + A treatments; Mann-Whitney U test, $Z = -2.1$, $P = 0.04$), while northern sediments showed an inverse pattern (2910.1 ± 533 and 665.8 ± 257, respectively for N and N + A conditions; Mann-Whitney U test, $Z = -2.38$, $p = 0.02$).

Microfungal Growth and Infaunal Activity

Ergosterol concentration in sediments surficial layer was initially characterised by a remarkable homogeneity (Fig. 5; site 7 represented an exception), After 5 days, ergosterol concentration dropped to negligible values (site 2 as the only exception) in defaunated controls (CON treatment), whereas a significant increase occurred in animal treatments. In fact, where *Tapes* sp. specimens were present, ergosterol concentration in surficial layers showed a significant positive variation, differentiated among sites (Kruskal-Wallis 1-WAY ANOVA, $H = 12.89$, $p < 0.01$).

DISCUSSION

Sedimentary Features and Benthic Community Structure

Our results on sediment grain size and organic matter content are consistent with the literature available on the area (*e.g.*, Buljan and Zore-Armanda, 1976; Brambati *et al.*, 1984; Bortoluzzi *et al.*, 1984a, 1984b). The two sampling areas displayed significant differences: southern sediments showed heterogeneous features, mainly related to autochthonous (coastal hydrodynamism, occurrence of relict facies) and allochthonous factors (River Po plume); northern deposits were characterised by an offshore-inshore grain size gradient, shifting from relict facies to fine, clayish sediments characterising the most off-shore bottoms of the northern basin (Brambati *et al.*, 1984). Besides the actual difficulty of any direct comparison caused by the significant variation in site locations between the 1st and 2nd sampling campaign showed stronger fluctuations. The northern area demonstrated an effective seasonal stability in its sedimentary features, whereas the southern area benthic system showed stronger fluctuations between winter and summer. The high temporal variability of sediment features in the central Adriatic has been already described (Bortoluzzi *et al.*, 1984a, 1984b) as a result from

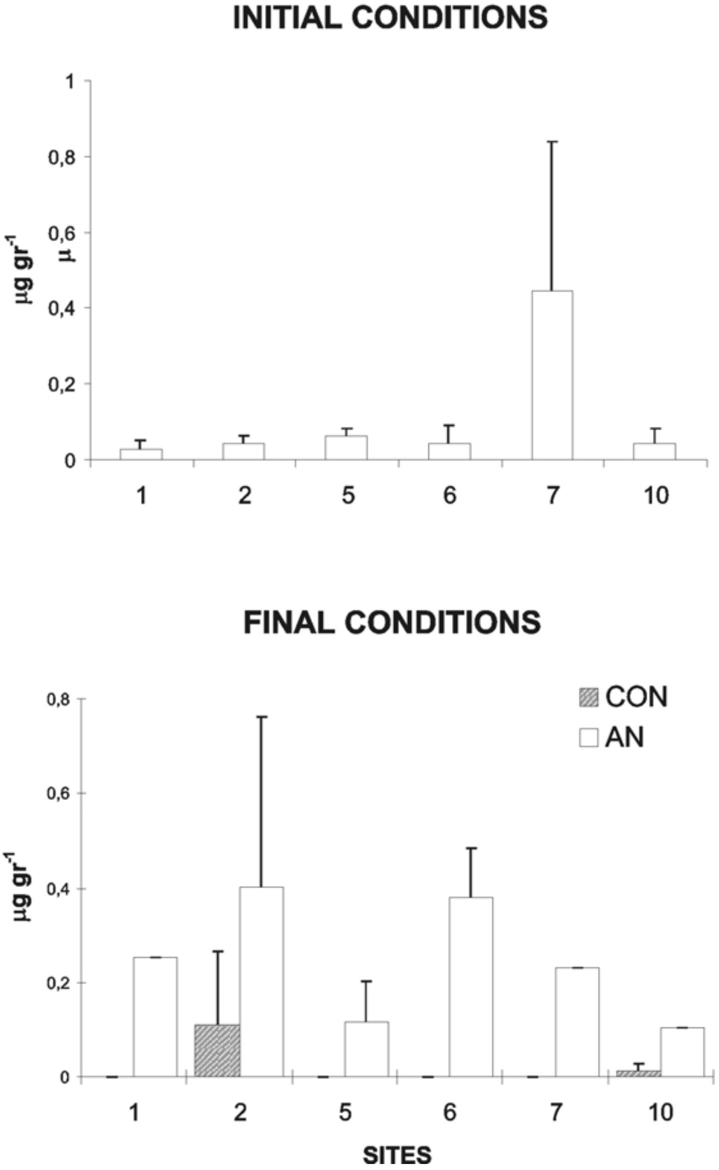

FIGURE 4 Variations in ergosterol concentration ($\mu g \cdot mg^{-1}$ sediment DW) in sediments of the different sites under analysis. Top: initial ergosterol concentration; bottom: final conditions, after 5 days; CON: defaunated control; AN: animal treatment.

localised hydrodynamism and occurrence of secondary facies. On the other hand, microfungi showed an inverse pattern: in comparison to the southern sites, northern stations were subjected in winter to a significant decrease in microfungal density, indicating a stronger dependence of the benthic microbial component upon variations in water column abiotic parameters (*e.g.*, salinity, temperature), mainly related to the seasonality of the river Po plume (Buljan and Zore-Armanda, 1976; Franco and Michelato, 1992).

Macrofaunal patterns resulted similarly affected by seasonality: the benthic community of the southern area showed low changes between summer and winter density values, and a generalised stability characterised the dominant tropho-functional groups. In particular, the constant presence of the macrophagous detritivore *Gammarus* sp. indicates that the southern area may be characterised by autochthonous amphipod populations, most probably supported by macrodetritus export from coastal sources. The northern area macrobenthic community, on the other hand, was characterised by strong, negative variations in winter, and an almost complete disappearance of both infaunal suspensivores and epifaunal detritivores.

The Role of Microorganisms and Anoxia on Sediment Phosphorus Uptake

Intact sediments of the northern stations showed lower ^{32}P uptake in comparison to the southern area (Tab. II, N treatment), and were characterised by an inshore-offshore pattern, corresponding to a stronger response to microbial suppression and anoxia, indicated by the lower variation coefficients. Sediment grain size and oxygen saturation of the overlying waters represent crucial factors in phosphorus dynamics across the water-sediment interface (Holdren and Armstrong, 1980), strongly interacting (according to the abiotic Einsele-Mortimer model: Einsele 1936, 1937, 1938; Mortimer 1941, 1942, 1971) in relation to phosphorus binding capacity of ferrous oxides characterising the sedimentary clayish fractions. Microbial activity may play a significant role in increasing sediment phosphorus uptake (Gätcher *et al.*, 1988; Sinke and Cappenberg, 1988; Hupfer and Uhlmann, 1991), competing successfully with metal oxides under oligotrophic-mesotrophic conditions (Fleischer, 1986). In this perspective, the analysis of variation coefficients in phosphorus uptake between intact and sterilised – anoxic sediments emphasised two opposite conditions: in the northern area, besides the low binding capacity of sediments probably related to the occurrence of sandy dimensional fractions, the high variation coefficients determined for ST and AN treatments in respect to the intact N control indicate both a significant microbial contribution to sediment phosphorus uptake and a high sensitivity to anoxic conditions, suggesting, in turn, the persistence in the northern Adriatic benthic system of oxidised conditions. On the other hand, the weak response to microbial suppression and anoxia determined for the southern area indicates both a secondary contribution of the microbial component in sediment phosphorus uptake and the significant occurrence of reduced metal oxides and/or of sulphur in sediment surficial layers, suggesting an anoxic, organically enriched benthic system.

The significant inversions in CVs pattern observed for the ST and N treatments across the frontal system indirectly suggests in both areas the occurrence of a pelagic-benthic coupling process. In the marine environments, the coupling between pelagic and benthic systems has represented a subject of increasing interest in recent years (Graf, 1992; Josefson and Conley, 1997). Fronts in estuarine and shallow marine environments represent a source of heterogeneity in pelagic productivity potentially influencing the benthic system; several investigations report in such areas increased pelagic primary production (Heilmann *et al.*, 1994; Josefson and Conley, 1997), strongly influencing the benthic system, *e.g.*, through anomalous organic matter loading (Josefson and Conley, 1997). Our laboratory investigations provide only partial supports to such a hypothesis. In fact, a significant coupling of microbial-related phosphorus uptake actually corresponded to the strong physico-chemical heterogeneity (Franco and Michelato, 1992) and high primary production (Gilmartin *et al.*, 1990) characterising the Po River-Adriatic Sea frontal system, but resulted unrelated to sediment organic matter content. The discrepancy can result from the heterotrophic decomposition of organic matter directly in the water column (Gilmartin *et al.*, 1990; Karner *et al.*, 1992) that may alter organic matter inputs to the northern Adriatic benthic system; the remarkable heterogeneity in sediment ^{32}P uptake across the frontal system showed by our laboratory investigations may be due to the prevalence of other physico-chemical factors (*e.g.*, temperature, salinity) upon sediment organic matter content in affecting benthic microbial activity.

The Role of Epifaunal Detritivores and Suspensivores in Sediment Phosphorus Uptake

Both epifaunal detritivores and infaunal suspensivores deeply affected phosphorus dynamics at the water-sediment interface, whereas their effects showed significant differences in relation to the sampling area and sediment grain size.

A strong impact on sediment phosphorus dynamics is widely recognised for suspensivores (*e.g.*, Nalepa *et al.*, 1991; Prins and Smaal, 1994) and for epifaunal detritivores (*e.g.*, Mulholland *et al.*, 1985). In particular, the removal of seston from the water bottom layers related to filtration activity together with sediment reworking and resuspension of the finest fractions may increase sedimentation rates and loss of total phosphorus from the water column (Nichols, 1985; Kautsky and Evans, 1987). A major proportion of the removed phosphorus is re-introduced in the sediment in particulate form (Asmus and Asmus, 1991; Prins and Smaal, 1994). Detritivores activity in sediment surficial layers may affect P dynamics through a complex spectrum of effects, both mechanical (*e.g.*, increase in the depth of the oxidised front caused by the surficial layer turbation: Kristensen and Blackburn, 1987; Clavero *et al.*, 1992) and trophic (*e.g.*, direct exploitation of organic matter and consequent increase in phosphorus mineralisation: Mulholland *et al.*, 1985; Qin and Threlkeld, 1990). In our investigations, both phosphorus uptake patterns in the presence of epifaunal detritivores and phosphorus uptake/release patterns in the presence of suspensivores (Fig. 2) showed a strong dependence of animal effects upon sediment characterisation. In fact, epifaunal detritivores exerted a negligible effect on ^{32}P uptake of the clayish southern sediments: after *Gammarus* sp. introduction in the bi-compartmented water-sediment systems, ^{32}P concentration in water remained almost unaltered. On the other hand, epifaunal detritivores strongly affected ^{32}P uptake of northern sediments both mechanically (a steep increase occurred in uptake patterns and in sediment phosphorus concentration, most probably related to an animal-related increase in the thickness and phosphorus-sequestering ability of sediment surficial layers, facilitated by the sediment structure) and trophically (indicated by the relationship occurring between animal concentration factors and variation in ^{32}P concentration after animal introduction in the systems).

The significant enhancement of ^{32}P uptake in both northern and southern sediments caused by the activity of infaunal suspensivores is consistent with available information on the tropho-functional group (Asmus and Asmus, 1991; Prins and Smaal, 1994), whereas a grain size-related decrease in radiotracer uptake most probably occurred for northern sediments in the defaunated control (N) and animal treatment (N + A). The ^{32}P release patterns were characterised by the most evident discrepancies. Where suspensivores were present in sediments from the northern area, the significant radiotracer release from the sedimentary compartment observed in the defaunated controls resulted enhanced by animal activity: ^{32}P was continuously released from the sedimentary compartment, and *ca* 10% of the radiotracer sequestered during the uptake phase was present in dissolved form at the end of the release phase. Northern sediments demonstrated to react rapidly to variations in the radiotracer concentration gradient across the water-sediment interface, and infaunal suspensivores contribute actively to the predominantly abiotic process (related to sediment porosity and grain size). Southern sediment demonstrated to be basically unaffected by changes in ^{32}P water concentration: no more than *ca* 3% of the radiotracer was released after 64 h, and ^{32}P concentration in water remained unvaried during the whole release phase. Differences in grain size, organic content and microbial activity may probably represent the clues to explain the observed differences in the response of sediment ^{32}P uptake between the northern and southern area. In particular, the prevalence in southern sediments of clayish, fine dimensional fractions may represent a strong causative factor for the high phosphate binding capacity and for the negligible response to gradient variations across the sediment interface. Animal activity did not alter the sediment inertia for the radiotracer release: animal reworking of the surficial layer and/or excretion did not represented processes effective in releasing the radiotracer from the sedimentary compartment. An animal-related increase in microbial activity can represent a second, further explanation for the observed uptake and release patterns of southern sediments. The analysis of final ergosterol content in sediment surficial layers highlighted actually a significant, positive effect of suspensivores on the microfungal development at the water-sediment interface; the positive interaction between micro- and macrofauna may thus represent a further, even though not quantitatively analysed in our paper, factor in enhancing phosphorus sequestering in sediment surficial layers.

CONCLUSIONS

Phosphorus dynamics in the northern Adriatic Sea results from the combined influence of inputs from the Po River watershed, the degree and seasonality of water column stratification, the large-scale horizontal water circulation as they control and/or moderate assimilation and regeneration processes in water column and in the benthic system (Degobbis and Gilmartin, 1990; Gilmartin *et al.*, 1990). The benthic phosphorus fluxes across the water-sediment interface, in particular, have been recently estimated as comparable to the input from the Po River, even though a high spatial heterogeneity has been clearly indicated in relation to the sampling area or the degree of macrofaunal irrigation (Giordani *et al.*, 1992). Our laboratory study quantitatively assessed the potential role in benthic phosphorus dynamics of the microbial component in relation to different areas of the basin and to the occurrence of the pelagic frontal system; moreover, it provided original information on the role of different tropho-functional groups dominating the macrobenthic assemblages in northern and central

Adriatic soft bottoms, together with an advanced resolution of the interaction between micro- and macrofauna.

In turn, our results showed a significant effect on benthic phosphorus dynamics exerted by both micro- and macrofauna; in this perspective, further investigations are needed to incorporate explicitly in northern Adriatic Sea phosphorus budget determinations of benthic fluxes and exchange rates between sediments and overlying waters comprehensive of effects of the biotic component of benthic systems.

The actual resolution of the whole spectrum of factors affecting phosphorus dynamics will provide crucial information for any action of monitoring and management of large-scale processes (*e.g.*, eutrophication) in the northern Adriatic Sea.

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References

Asmus, R.M. and Asmus, H. (1991) "Mussel beds: limiting or promoting phytoplankton?", *J. Exp. Mar. Biol. Ecol.* **148**, 215–232.

Boers, P.C.M., Cappenberg, T.E. and van Raaphorst, W. (1993) "The third international workshop on phosphorus in the sediments", *Hydrobiologia* **253**, 11–18.

Bortoluzzi, G., Frascari, F., Frignani, M., Giordani, P., Guerzoni, S., Rovatti, G. and Turci, C. (1984b) "Influenza del Po e dei fiumi minori sul contenuto di sostanze nutrienti nei sedimenti marini dell'Emilia Romagna", *Mem. Soc. Geol. It.* **27**, 483–497.

Bortoluzzi, G., Frascari, F. and Ravaioli, M. (1984a) "Ricerche sedimentologiche in area marine adriatiche e tirreniche finalizzate alla comprensione dei fenomeni di inquinamento costiero", *Mem. Soc. Geol. It.* **27**, 499–525.

Brambati, A., Ciabatti, M., Fanzutti, G.P., Marabini, F. and Marocco, R. (1984) "Distribuzione dei sedimenti nel Mar Adriatico: confronto tra le classificazioni tessiturali di Shepard e di Nota", *Mem. Soc. Geol. It.* **27**, 391–392.

Buljan, M. and Zore-Armanda, M. (1976) "Oceanographical properties of the Adriatic Sea", *Oceanogr. Mar. Biol. Ann. Rev.* **14**, 11–98.

Carpenter, S.R., Cottingham, K.L. and Schindler, D.E. (1992) "Biotic feedbacks in lake phosphorus cycles", *Tree* **7**, 332–336.

Clavero, V., Fernandez, J.A. and Niell, F.X. (1992) "Bioturbation by *Nereis* sp. and its effects on the phosphate flux across the sediment-water interface in the Palmones River estuary", *Hydrobiologia* **235/236**, 387–392.

Degobbis, D. and Gilmartin, M. (1990) "Nitrogen, phosphorus, and biogenic silicon budgets for the northern Adriatic Sea", *Oceanol. Acta* **13**, 31–45

Einsele, W. (1936) "Über die Beziehunger des Eisenkreislaufes zum Phosphatkreislauf im eutrophen See", *Arch. Hydrobiol.* **29**, 664–686.

Einsele, W. (1937) "Physikalisch-chemische betrachtung einiger probleme des limnischen mangan-und eisenkreislaufs", *Vehr. Int. Verein. Limnol.* **5**, 69–84.

Einsele, W. (1938) "Über chemische und kolloidchemische Vorgänge in Eisen-Phosphat – Systemen unter limnochemischen und limnogeologischen Gesichtspunkten", *Arch. Hydrobiol.* **33**, 361–387.

Fleischer, S. (1986) "Aerobic uptake of Fe(III)-precipitated phosphorus by microorganisms", *Arch. Hydrobiol.* **107**, 269–277.

Franco, P. and Michelato, A. (1992) "Northern Adriatic Sea: oceanography of the basin proper and of the western coastal region", *Sci. Total Environ.* Suppl, pp. 35–62.

Gardner, W.S., Nalepa, T.F., Quigley, M.A. and Malczyk, J.M. (1981) "Release of phosphorus by certain benthic invertebrates", *Can. J Fish. Aquat. Sci.* **38** 978–981.

Gätcher, R., Meyer, J.S. and Mares, A. (1988) "Contribution of bacteria to release and fixation of phosphorus in lake sediments", *Limnol. Oceanogr.* **33**, 1542–1558.

Gessner, M.O., Bauchrowitz, M.A. and Escautier, M. (1991) "Extraction and quantification of ergosterol as a measure of fungal biomass in leaf litter", *Microb. Ecol.* **22**, 285–291.

Gessner, M.O. and Chauvet, E. (1993) "Ergosterol-to-biomass conversion factors for aquatic hyphomicetes", *Appl. Environ. Microbiol.* **59**, 502–507.

Gilmartin, M., Degobbis, D., Revelante, N. and Smodlaka, N. (1990) "The mechanism controlling plant nutrient concentrations in the Northern Adriatic Sea", *Int. Revue ges. Hydrobiol.* **75**, 425–445.

Giordani, P., Hammond, D.E., Berelson, W.M., Montanari, G., Poletti, R., Milandri, M., Frignani, M., Langone, L., Ravaioli, M., Rovatti, G. and Rabbi, E. (1992) "Benthic fluxes and nutrient budgets for sediments in the Northern Adriatic Sea: burial and recycling efficiencies", *Sci. Total Environ.* Suppl, pp. 251–275.

Graf, G. (1992) "Benthic-pelagic coupling: a benthic view", *Oceanogr. Mar. Biol. Annu. Rev.* **30**, 149–190.

Heilmann, J., Richardson, K. and Æertebjerg, G. (1994) "Annual distribution and activity of phytoplankton in the Skagerrak/Kattegat frontal region", *Mar. Ecol. Prog. Ser.* **112**, 212–223.

Holdren, G.C. and Armstrong, D.E. (1980) "Factors affecting phosphorus release from intact lake sediment cores", *Envir. Sci. Technol.* **14**, 79–87.

Hupfer, M. and Uhlmann, D. (1991) "Microbially mediated phosphorus exchange across the mud-water interface", *Verh. Internat. Verein. Limnol.* **24**, 2999–3003.

Josefson, A.B. and Conley, D.J. (1997) "Benthic response to a pelagic front", *Mar. Ecol. Prog. Ser.* **147**, 49–62.

Kamp-Nielsen, L.H., Mejer, H. and Sorensen, S.E. (1982) "Modelling the influence of bioturbation on the vertical distribution of sediment phosphorus in L. Esrom", *Hydrobiologia* **91**, 197–206.

Karner, M., Fuks, D. and Herndl, G.J. (1992) "Bacterial activity along a trophic gradient", *Microb. Ecol.* **24**, 243–257.

Kautsky, N. and Evans, S. (1987) "Role of biodeposition by *Mytilus edulis* in the circulation of matter and nutrients in a Baltic coastal ecosystem", *Mar. Ecol. Prog. Ser.* **38**, 201–212.

Kristensen, E. and Blackburn, T.H. (1987) "The fate of organic carbon and nitrogen in experimental marine sediment systems: influence of bioturbation and anoxia", *J. Mar. Res.* **45**, 231–257.

Mancinelli, G., Fazi, S. and Rossi, L. (1998) "Sediment structural properties mediating dominant feeding types patterns in soft-bottom macrobenthos of the Northern Adriatic Sea", *Hydrobiologia* **367**, 211–222.

Mortimer, C.H. (1941) "The exchange of dissolved substances between mud and water in lakes (Parts I and II)", *J. Ecol.* **29**, 280–329.

Mortimer, C.H. (1942) "The exchange of dissolved substances between mud and water in lakes (Parts III and IV)", *J. Ecol.* **30**, 147–201.

Mortimer, C.H. (1971) "Chemical exchanges between sediments and water in the Great Lakes – Speculations on probable regulatory mechanisms", *Limnol. Oceanogr.* **16**, 387–404.

Mulholland, P.J., Elwood, J.W., Newbold, J.D. and Ferren, L.A. (1985) "Effect of a leaf-shredding invertebrate on organic matter dynamics and phosphorus spiralling in heterotrophic laboratory streams", *Oecologia* **66**, 199–206.

Nalepa, T.F., Gardner, W.S. and Malczyk, A.G. (1991) "Phosphorus cycling by mussels (Unionidae: Bivalvia) in Lake St. Clair", *Hydrobiologia* **219**, 239–250.

Newell, S.Y., Arsuffi, T.L. and Fallon, R.D. (1988) "Fundamental procedures for determining ergosterol content of decaying plant material by liquid chromatography", *Appl. Environ. Microbiol.* **54**, 1876–1879.

Nichols, F.H. (1985) "Increased benthic grazing: an alternative explanation for low phytoplankton biomass in northern San Francisco Bay during the 1967–1977 drought", *Estuar. Coast. Shelf Sci.* **21**, 379–388.

Ott, J.A. (1992) "The Adriatic benthos: problems and perspectives", In: Colombo, G, Ferrari, I, Ceccherelli, VU, Rossi, R (eds). *Marine Eutrophication and Population Dynamics*, Olsen, Olsen, Fredensborg, Denmark, pp. 367–377.

Park, D. (1972) "Methods for detecting fungi in organic detritus in water", *Trans. Brit. Mycol. Soc.* **58**, 281–290.

Prins, T.C. and Smaal, A.C. (1994) "The role of the blue mussel *Mytilus edulis* in the cycling of nutrients in the Oosterchelde estuary (The Netherlands)", *Hydrobiologia* **282/283**, 413–429.

Qin, J. and Threlkeld, S.T. (1990) "Experimental comparison of the effects of benthivorous fish and planktivorous fish on plankton community structure", *Arch. Hydrobiol.* **119**, 121–141.

Sinke, A.J.C. and Cappenberg, T.E. (1988) "Influence of bacterial processes on the phosphorus release from sediments in the eutrophic Loosdrecht Lakes", The Netherlands. *Arch. Hydrobiol. Beih. Ergebn. Limnol.* **30**, 5–13.

Whittaker, R.H. (1961) "Experiments with radiophosphorus tracer in aquarium microcosms", *Ecol. Monogr.* **31**, 137–186.