

# Enzymatically Hydrolyzable Protein and Carbohydrate Sedimentary Pools as Indicators of the Trophic State of Detritus Sink Systems: A Case Study in a Mediterranean Coastal Lagoon

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**ABSTRACT:** In order to classify the trophic state of detritus sink systems, instead of the conventional indicators based on inorganic nutrient availability and algal biomass and productivity in the water column, we used new biochemical descriptors based on the amount of sedimentary organic carbon (C) and nitrogen (N) potentially available to heterotrophs. We investigated spatial and temporal changes in microphytobenthic biomass, organic matter biochemical composition, and enzymatically hydrolyzable protein and carbohydrate pools along a north-south transect in the Marsala lagoon (Mediterranean Sea, Italy) at three stations characterized by different hydrodynamic conditions and organic matter content in the sediment. In the Marsala lagoon water currents decreased from north to south and this pattern was reflected by organic matter distribution and composition. Sediment organic matter concentrations were among the highest reported in the literature and, in the central area where large meadows of the seagrass *Posidonia oceanica* were present, display a strong dominance of highly refractory carbohydrates. The protein to carbohydrate ratio was always  $< 1$ , indicating the dominance of aged organic detritus. Microphytobenthic biomass displayed an increasing pattern southward, and its contribution to the biopolymeric C pools ranged from negligible in the central sector of the lagoon to 50% in its northern part, indicating that sources of sediment organic C also changed along the hydrodynamic gradient. The percentage contribution of the enzymatically hydrolyzable fraction of proteins and carbohydrates was inversely related to total protein and total carbohydrate concentrations, respectively, suggesting that bioavailability of organic C and N increased with decreasing organic matter content in the sediment and with increasing hydrodynamic regime. Microphytobenthic contribution to biopolymeric C (as a proxy of autotrophic organic C) and the ratio of the enzymatically digestible fraction to biopolymeric C (as an indicator of organic matter lability) were significantly correlated, suggesting that chlorophyll *a* sediment content might be used as an indicator of food promptly available to consumers. The present study also highlighted that the ratio of labile (i.e., enzymatically digestible) versus biopolymeric organic C in the sediments tends to decrease with increasing organic matter content, due to the increase of the refractory fraction of organic C.

## Introduction

The classification of the trophic state of aquatic systems is a fundamental task in applied ecological research, but the scientific community currently has only a limited set of tools to help guide ecosystem management in the field of eutrophication (Cloern 2001; Cognetti 2001). Classification of different aquatic ecosystems on the basis of their trophic state has been generally based on water column variables, such as increased inorganic nutrient concentrations, algal biomass, and primary production (Kardis et al. 1983; Giovanardi and Tromellini 1992;

Ignatiades et al. 1992; Zurlini 1996; Vollenweider et al. 1998). Trophic state classification is now evolving from a static perspective to an input-response point of view (Cloern 2001). In this frame, Nixon (1995) has recently proposed new criteria for the classification of the trophic state of marine systems based on the supply of organic carbon (C) to the system. Such an approach would appear particularly suitable in an ecological perspective because it is not based on the potential or actual primary productivity, but on the supply of organic matter to the system. The increasing input of organic matter to marine coastal systems is, indeed, the triggering mechanism leading to the ecological consequences of eutrophication (i.e., from oxygen reduction and

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hypoxia up to mortality of marine organisms and biodiversity loss; Cloern 2001).

Shallow, productive, and sheltered marine coastal systems, such as estuaries and coastal lagoons, are generally characterized by a strong spatial and temporal variability of physicochemical characteristics and productivity patterns (Newell 1982). In these environments, the magnitude and direction of the ecological responses to inorganic nutrient increase (i.e., eutrophication) are even more difficult to predict. Pelagic and benthic compartments of shallow marine ecosystems are tightly coupled, so that responses to nutrient enrichment could be seen as changes in the trophic state of the benthic environment.

Extending Nixon's concept of eutrophication from increasing organic supply to the benthos to the accumulation of organic C in the sediment (Jorgensen 1996), we could assume the latter as an effective indicator of the trophic state of marine coastal environments (Fabiano et al. 1995). Due to the conservative nature of sediment organic C (Danovaro et al. 2001), changes in trophic state could be more evident in terms of organic matter biochemical composition rather than in terms of its concentrations (Tselepidis et al. 2000).

Our hypothesis is that the relative importance of labile and refractory organic compounds would reflect the accumulation and removal of organic matter in the sediment and could be used as an indicator of trophic state of shallow marine systems. A universally accepted method to distinguish between the labile and refractory fractions of sedimentary organic matter is not yet available. Total protein, carbohydrate and lipid content (referred together as biopolymeric organic carbon [BPC]; Fabiano et al. 1995) have often been used as an estimate of the labile fraction of organic C, but it has been demonstrated that only a minor fraction of BPC in marine sediments is actually bioavailable (i.e., enzymatically digestible; Dauwe and Middelburg 1998; Dauwe et al. 1999a,b; Dell'Anno et al. 2000; Danovaro et al. 2001). These results also suggest that BPC is a weak descriptor of the trophic state of sediments. Such weakness could be more evident in estuaries and coastal lagoons that accumulate huge amounts of organic C, functioning as a sink of organic detritus (Troussellier et al. 1993; Pusceddu et al. 1999; Shi et al. 2001).

We used the Marsala lagoon as a model for investigating factors controlling organic matter accumulation and quality in detritus sink systems, using new biochemical descriptors of trophic state (i.e., in terms of food potentially available for heterotrophs). To do this we investigated spatial and seasonal changes in microphytobenthic biomass, organic matter biochemical composition, and en-

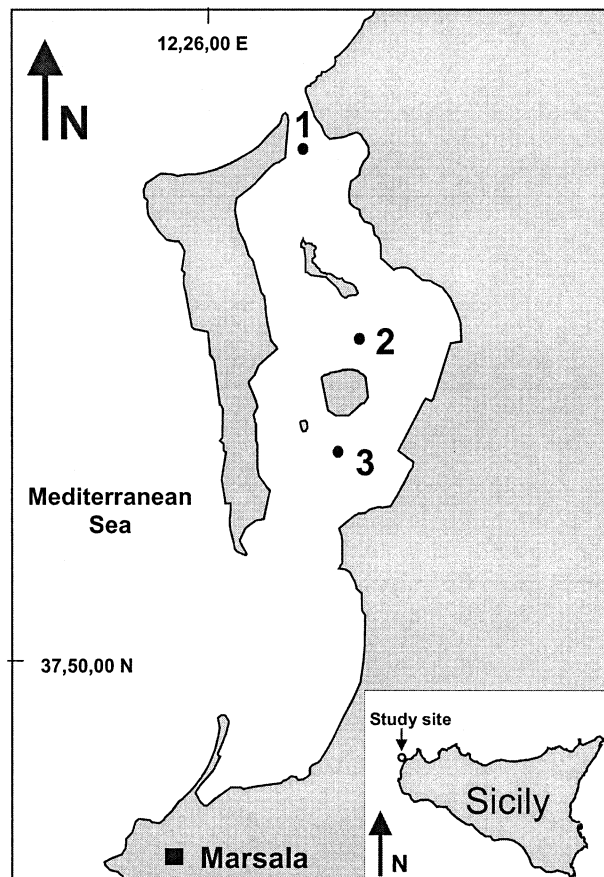


Fig. 1. Study area and location of the sampling stations.

zymatically hydrolyzable protein and carbohydrate pools at three stations characterized by different hydrodynamic conditions and organic matter content in the sediment.

## Materials and Methods

### STUDY SITE

The study was carried out in a Mediterranean coastal lagoon (Marsala lagoon, Fig. 1), characterized by water depth ranging between 0.5 (northern area) and 2.5 m (southern area).

Water exchanges with the open sea are ensured by currents with annual mean speeds between  $4.92 \pm 1.54 \text{ cm s}^{-1}$  in the south (northward direction) and  $2.34 \pm 0.87 \text{ cm s}^{-1}$  in the north (southward; Mazzola and Sarà 1995). In the northern mouth, seawater inputs are occasional and mainly due to the dominant southward winds (Pusceddu et al. 1997). In the southern area the ingression of seawater is partially hindered by a large reef of the seagrass *Posidonia oceanica*, characterized by almost surfacing leaves in spring and an average depth to surface not exceeding 0.3 m (Sarà et al. 1999). The Marsala lagoon is a marine-protected area, sur-

rounded by slightly used agricultural land, and characterized year-round by the virtual absence of riverine inputs and anthropogenic wastewater discharge. Sediments are dominated by a sandy fraction (up to 99% at the northern mouth), which in the central area becomes co-dominant with the pelitic fraction (53%, Vizzini 2000).

Physical features, such as wind-induced water dynamics, are the major forcing factors controlling suspended particle dynamics in this lagoon, which is not influenced by tide (Pusceddu 1999; Sarà et al. 1999; Fabiano et al. 2000). There is a southward decreasing pattern of hydrodynamic conditions concurrent with the increase in water depth and the presence of the *P. oceanica* reef (Pusceddu et al. 1997). The northern area of the Marsala lagoon is normally subjected to frequent sediment resuspension resulting in very high total suspended matter concentrations and the dominance of resuspended microphytobenthos (Maimone et al. 2000; Mazzola et al. 2001). The inner region of the basin is characterized by a strong accumulation of organic detritus mainly due to the deposition of particles deriving from decaying leaves of *P. oceanica* (Sarà et al. 1999).

#### SAMPLING

Sediment samples were collected monthly between March 1996 and February 1997 at three stations (Fig. 1). Three replicate cores per station at each sampling time were collected by scuba diving and manually inserting PVC tubes (4.7 cm i.d.). Samples were brought back to the laboratory and processed within 4 h. The top 1 cm of each core was immediately frozen at  $-20^{\circ}\text{C}$  and stored until analysis. Temperature and salinity were measured in situ using a Hydrolab multiprobe.

#### MICROPHYTOBENTHIC BIOMASS

Determinations of chlorophyll *a* (chl *a*) and phaeopigment (phaeo) were carried out, after extraction with 90% acetone (12 h in the dark at  $4^{\circ}\text{C}$ ), according to Plante-Cuny (1974). Chloroplastic pigment equivalents (CPE) were calculated as sum of chl *a* and phaeo concentrations. Microphytobenthic C was calculated by converting chl *a* concentrations to carbon content (Cchl *a*), using a conversion factor of 40 (De Jonge 1980).

#### BIOCHEMICAL COMPOSITION OF SEDIMENT ORGANIC MATTER

Total protein (PRT) analyses of three replicates were conducted following extraction with NaOH (0.5 M, 4 h) and were determined according to Hartree (1972), modified by Rice (1982) to compensate for phenol interference. Data are expressed as bovine serum albumin equivalents. To-

tal carbohydrates (CHO) of three replicate samples were analyzed according to Gerchacov and Hatcher (1972) and expressed as glucose equivalents. Blanks for each analysis were sediments that had been precombusted at  $450^{\circ}\text{C}$  for 2 h.

Protein and carbohydrate enzymatic hydrolysis of sediment samples were carried out according to Dell'Anno et al. (2000). Frozen sediment samples were homogenized in 0.1 M Na-phosphate buffer (pH 7.5, buffer:sediment ratio of 30 volume:weight), and sonicated three times for 1 min (with 30-s intervals) before enzyme addition. Triplicate samples of the slurry from each sediment sample (i.e., treated samples) were added to 100  $\mu\text{l}$  of proteinase-K (1 mg  $\text{ml}^{-1}$ ) and 100  $\mu\text{l}$  of protease (600  $\mu\text{g}$   $\text{ml}^{-1}$ ); an equal volume of Na-P buffer solution without enzymes (i.e., control samples) was added to another set of triplicates. All samples were incubated for 1 h at  $37^{\circ}\text{C}$  under gentle agitation and subsequently filtered onto GF/F filters and rinsed twice with 5 ml of cold 0.1 M Na-P buffer (pH 7.5) in order to remove the digested protein fraction and the enzymes from the sediments. Sediment sub-samples (0.1 g), muffled at  $550^{\circ}\text{C}$  for 4 h and processed as described above, were used as blanks. Protein analyses from these samples were carried out as described above. Differences between protein concentration of control and treated samples were assumed to represent the concentration of proteins actually hydrolyzed by proteases (hydrolyzed proteins, HPRT). Total protein concentrations from intact sediments (TPRT) and HPRT concentrations were normalized to sediment dry weight.

For enzymatic digestion of sedimentary carbohydrates, frozen sediment samples were homogenized with 0.1 M Na-P, 0.1 M EDTA (pH 5.0; sediment:buffer ratio of 2.5 volume:weight) and sonicated three times for 1 min (with 30-s intervals). Replicate samples of the slurry ( $n = 3$ , treated samples) were added to 100  $\mu\text{l}$  of  $\alpha$ -amylase, 50  $\mu\text{l}$  of  $\beta$ -glucosidase, 100  $\mu\text{l}$  of proteinase-K, and 100  $\mu\text{l}$  of lipase (stock solution of all enzymes was 1 mg  $\text{ml}^{-1}$ ). Another set of replicates treated adding 0.1 M Na-P instead of enzyme solutions was used as control. Samples were incubated for 1 h at room temperature under gentle agitation as for protein hydrolysis. Sediment sub-samples, muffled at  $550^{\circ}\text{C}$  for 4 h and processed as described above, were used as blanks. After incubation, all samples were centrifuged at  $2,000 \times g$  for 10 min and an aliquot of the supernatant was used to determine carbohydrates released from the sediments. Soluble carbohydrates were determined from the supernatant of the control sample. Carbohydrates from all supernatants and from intact sediments were analyzed spectrophotometrically as described above.

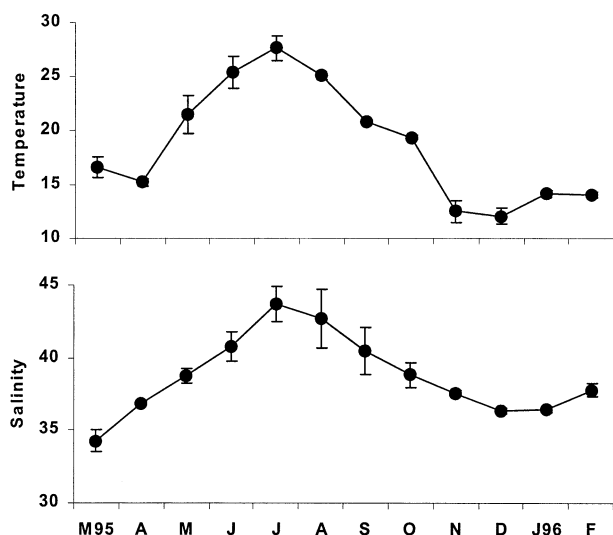


Fig. 2. Temporal changes in water temperature and salinity in the Marsala lagoon between March 1995 and February 1996. Values are means among the three stations ( $\pm$  SE).

The actual fraction of enzymatically hydrolyzed carbohydrates (HCHO) was obtained by difference between the carbohydrate concentrations determined in the supernatant of samples containing enzymes and the soluble fraction of the control. Total carbohydrate concentrations from intact sediments (TCHO) and HCHO concentrations were normalized to sediment dry weight.

After sonication in deionized water, total lipids (LIP) were extracted from about 1 g of sediment by direct elution with chloroform-methanol according to Bligh and Dyer (1959) and Marsh and Weinstein (1966). Analyses were performed spectrophotometrically and concentrations were calculated using standard tripalmitine solutions.

TCHO and HCHO and proteins and LIP were converted into C equivalents using 0.40, 0.49, and 0.75 g C g<sup>-1</sup> conversion factors, respectively, and normalized to sediment dry weight (Pusceddu et al. 2000). The sum of total protein, carbohydrate, and lipid C equivalents was reported as BPC. According to Danovaro et al. (2001) bioavailable organic carbon (BAOC), as a proxy of the organic C potentially available for benthic consumers, was defined as the sum of C equivalents of hydrolyzable carbohydrates and proteins (Danovaro et al. 2001).

#### STATISTICAL ANALYSIS

A Spearman-Rank correlation analysis on all biochemical variables was performed. Temporal and spatial changes in all the investigated variables were assessed by serial two-way analysis of variance (ANOVA) with time (mo) and space (stations) as sources of variation. When a significant difference

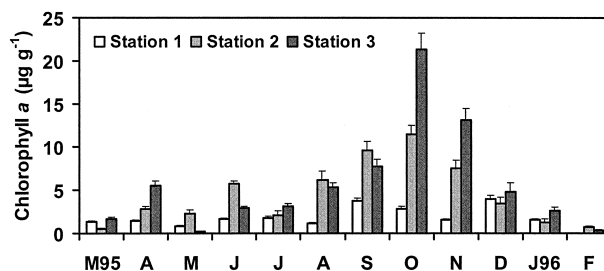


Fig. 3. Temporal and spatial changes in microphytobenthic biomass (expressed as chlorophyll *a* concentrations) in the Marsala lagoon during the study period (March 1995–February 1996). Bars indicate standard deviations among replicate samples.

for the main effect was observed ( $p < 0.05$ ) a Tukey's comparison test was also performed.

## Results

### ENVIRONMENTAL VARIABLES

Water temperature and salinity display clear seasonal patterns (Fig. 2) with highest values in July 1995 and lowest values in December 1995, while the lowest salinity was measured in March 1995. Both water temperature and salinity did not display significant changes among stations.

### CHLOROPLASTIC PIGMENTS AND MICROPHYTOBENTHIC BIOMASS

Sedimentary chl *a* concentrations display significant temporal and spatial changes (ANOVA,  $p < 0.01$  for both factors; Fig. 3). Significantly higher values were measured in October 1995 (Tukey's test,  $p < 0.01$ ), whereas lowest values were measured in February 1996. Chl *a* concentrations at station 1 were significantly lower than those at station 2 (Tukey's test,  $p < 0.01$ ) and station 3 (Tukey's test,  $p < 0.001$ ). Phaeo concentrations did not display significant temporal or spatial changes. Chl *a* accounted for  $65 \pm 5\%$  of CPE (as a sum of chl *a* and phaeo). Chl *a* contribution to CPE display significant spatial and temporal changes (ANOVA,  $p < 0.01$  for both factors) with values at station 1 significantly higher than at stations 2 and 3 (Tukey's test,  $p < 0.001$ ). Microphytobenthic biomass ranged from  $< 0.01$  mg C g<sup>-1</sup> (February 1996, station 1) to 0.9 mg C g<sup>-1</sup> (October 1995, station 3).

### BIOCHEMICAL COMPOSITION OF SEDIMENT ORGANIC MATTER

TPRT, TCHO, and TLIP concentrations did not display significant temporal changes, but were characterized by significant spatial changes (ANOVA,  $p < 0.01$  for the three compounds; Fig. 4). At station 1, TPRT and TCHO concentrations were

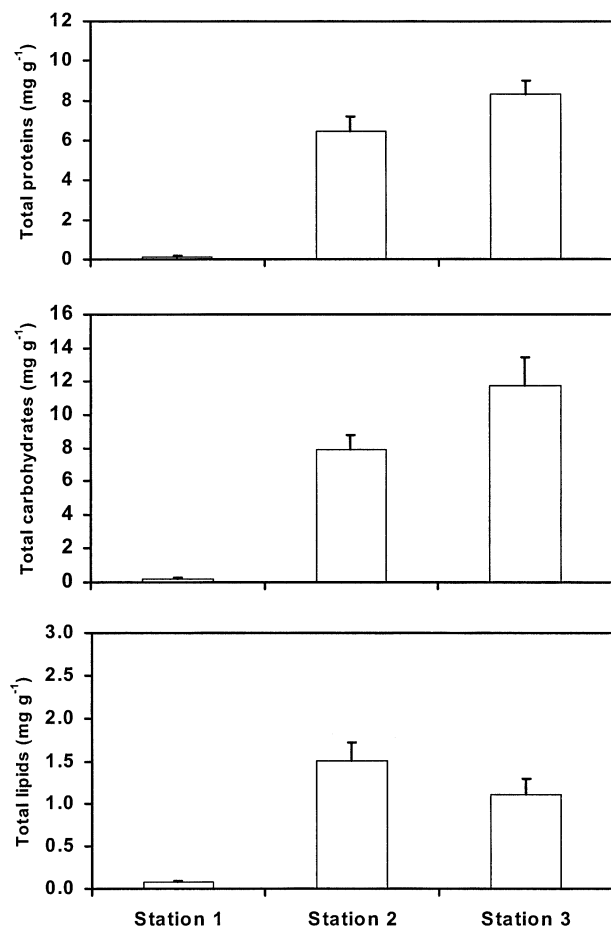


Fig. 4. Spatial changes (as annual average) in the biochemical composition of sediment organic matter in the Marsala lagoon at the three sampling stations during the study period (March 1995–February 1996). Bars indicate standard errors among sampling dates.

about 50 times lower than those at stations 2 and 3 (Tukey's test,  $p < 0.01$ , for both variables). TLIP concentrations at stations 2 and 3 displayed values 20 and 15 times higher, respectively, than those at station 1 (Tukey's test,  $p < 0.01$ ).

HPRT and HCHO concentrations (Table 1) did not display significant temporal changes. HPRT and HCHO concentrations exhibited significant spatial changes (ANOVA,  $p < 0.002$  and  $< 0.001$  for HPRT and HCHO, respectively; Fig. 5), and significantly increased from station 1 to 2 and 3 (Tukey's test,  $p < 0.001$ , for both variables).

HPRT and HCHO concentrations contributed,

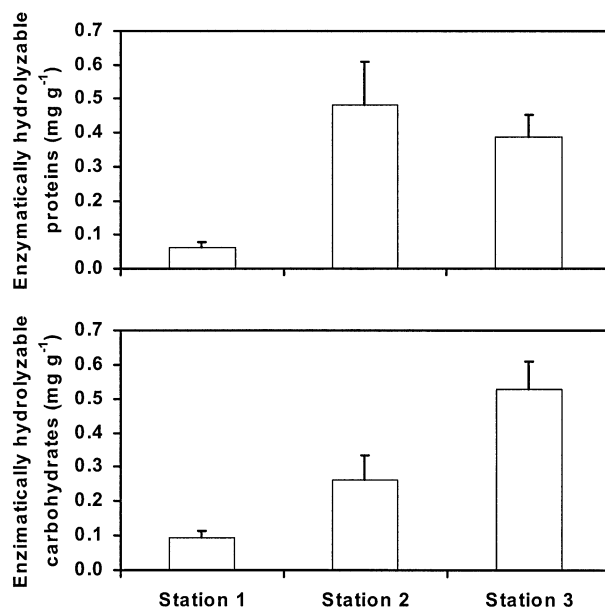


Fig. 5. Spatial changes in enzymatically hydrolyzable protein and hydrolyzable carbohydrate contents in the sediment of the Marsala lagoon at the three sampling stations during the study period (March 1995–February 1996). Bars indicate standard errors among sampling dates.

on annual average, 20% and 22% of total protein and carbohydrate pools. Such contributions did not display significant temporal changes, but exhibited values at station 1 (47% and 55% for HPRT and HCHO, respectively) significantly higher than those at stations 2 and 3 (Tukey's test,  $p < 0.001$ ).

The contribution of the enzymatically hydrolyzable protein and carbohydrate pools (i.e., the labile fraction of organic matter) to their total pools were significantly and negatively correlated with the total amount of sediment organic matter (Fig. 6).

Total carbohydrates represented the dominant fraction (annual average 42%) of BPC (as sum of protein, carbohydrate, and lipid carbon), followed by proteins (39%) and lipids (19%). The contribution of TCHO, TPRT, and TLIP to BPC displayed values at station 1 significantly lower than those at stations 2 and 3 (Tukey's test,  $p < 0.001$ , for the three compounds). Contribution of protein, carbohydrate, and lipid carbon to BPC did not display significant temporal changes.

TABLE 1. Hydrolyzable protein and carbohydrate content in the Marsala lagoon sediments. Reported are annual averages ( $\pm$  SE).

	Station 1	Station 2	Station 3
Hydrolyzable proteins ( $\text{mg g}^{-1}$ )	0.06 (0.02)	0.48 (0.12)	0.39 (0.06)
Hydrolyzable carbohydrates ( $\text{mg g}^{-1}$ )	0.09 (0.02)	0.26 (0.07)	0.58 (0.08)

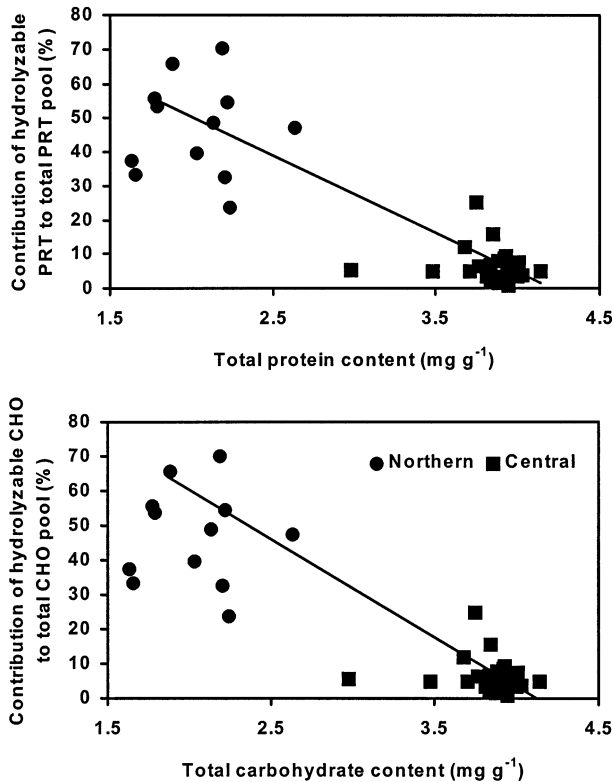


Fig. 6. Relationships between the enzymatically hydrolyzable fraction (%) of the total protein (PRT) pool and the total protein concentration; and enzymatically hydrolyzable fraction (%) of the total carbohydrate (CHO) pool and the total carbohydrate concentration in the northern (station 1) and central areas (stations 2 and 3 pooled together) of the Marsala lagoon.

#### BIOPOLYMERIC AND BIOAVAILABLE ORGANIC CARBON

Neither BPC nor BAOC concentrations exhibited significant temporal changes, but both displayed significant spatial changes (Fig. 7), with values at station 1 significantly lower than those at stations 2 and 3 (Tukey's test,  $p < 0.001$ , for both variables). BAOC contribution to BPC was significantly and positively correlated with the microphytobenthic contribution (Cchl *a*) to BPC (Fig. 8).

#### Discussion

##### ORIGIN AND BIOCHEMICAL COMPOSITION OF SEDIMENT ORGANIC MATTER

Marsala lagoon displays sediment organic matter concentrations among the highest reported in the literature (Pusceddu et al. 1999) and comparable with values reported from eutrophic areas (such as the northern Adriatic Sea; Manini et al. 2001) or organically enriched harbor sediments (Danovaro et al. 1999). This indicates that sediments of the Marsala lagoon exhibited characteristics typical of eutrophic systems.

The huge amounts of total carbohydrates in the

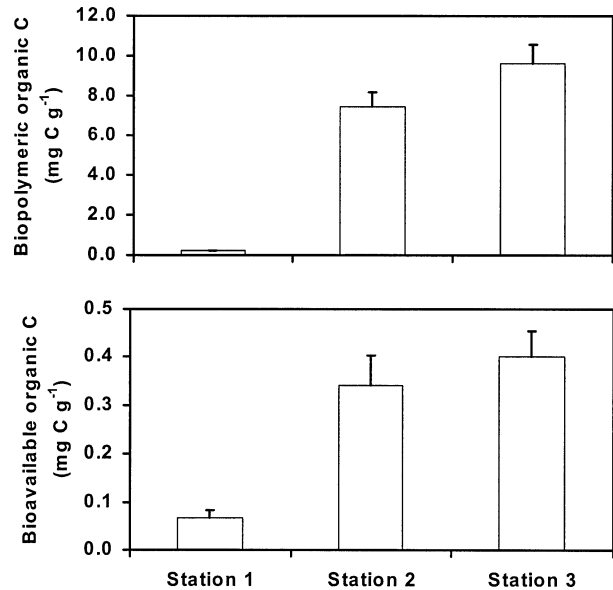


Fig. 7. Spatial changes in biopolymeric (as the sum of protein, carbohydrate, and lipid carbon equivalents) and bioavailable (as the sum of hydrolyzable protein and carbohydrate carbon equivalents) organic carbon contents in the sediment of the Marsala lagoon at the three sampling stations during the study period (March 1995–February 1996). Bars indicate standard errors among sampling dates.

central part of the lagoon were related to the accumulation of *P. oceanica* debris, which is known to be highly refractory (Kenworthy and Thayer 1984; Lawrence et al. 1989; Danovaro 1996).

Protein concentrations in the Marsala lagoon were extremely high, being comparable or higher than those reported in highly productive areas (such as the coastal Baltic Sea; Meyer-Reil 1983) or upwelling systems (Peru-Chile coast; Neira et al.

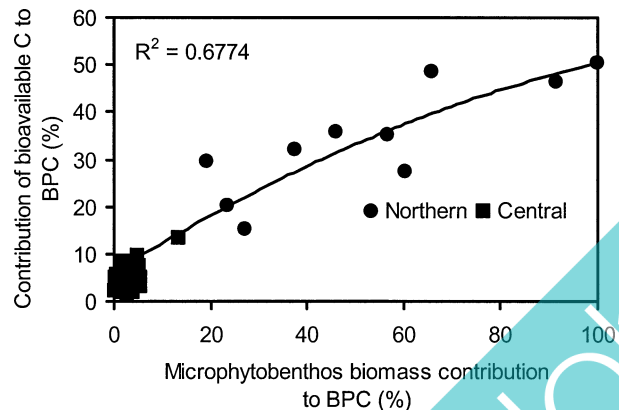


Fig. 8. Relationship between the microphytobenthic biomass contribution (%) to the biopolymeric organic carbon (BPC) and the bioavailable to biopolymeric organic carbon ratio (%) in the northern (station 1) and central (stations 2 and 3 pooled together) areas of the Marsala lagoon.

TABLE 2. Comparison of enzymatically hydrolyzable fraction of the sedimentary protein pool in different areas. \* indicates contribution of the enzymatically hydrolyzed amino acids to the total amino acid pool.

Sampling Area	Location	%	Studies
Gulf of Maine	estuarine-coastal transect	15*	Mayer et al. 1986
Mudflat	intertidal	26*	Mayer et al. 1995
North Sea	continental shelf	14–50*	Dauwe et al. 1999b
Porcupine abyssal Plain	abyssal	5–25	Dell'Anno et al. 2000
Marsala lagoon (northern area)	coastal lagoon	47	This study
Marsala lagoon (central area)	coastal lagoon	8	This study
Marsala lagoon (southern area)	coastal lagoon	5	This study

2001) and accounted for about 40% of sedimentary BPC. In all lagoon sediments, carbohydrates dominated the organic matter pool. Such dominance, resulting in a protein to carbohydrate ratio  $< 1$ , is a typical feature of detrital-heterotrophic environments (Danovaro 1996). Protein to carbohydrate ratios  $< 1$  have also been reported in extremely oligotrophic environments where proteins reach very low concentrations (Danovaro et al. 1993), so that differences between oligotrophic and detrital-heterotrophic systems cannot be easily identified. Since proteins are mobilized more rapidly than carbohydrates, low values of the protein to carbohydrate ratio would also indicate the presence of aged (i.e., not freshly produced) organic detritus (Pusceddu et al. 2000).

In the central area of the Marsala lagoon, primary organic C (i.e., microphytobenthos biomass) accounted for a negligible fraction of the BPC pools ( $< 0.1\%$ ), suggesting that most of the sediment organic C in this area was detrital and of heterotrophic origin. In the northern area, microphytobenthic C accounted, on annual average, for 57% of BPC, indicating that in this area microphytobenthos dominated the organic C pool. Although the use of a Cchl *a* ratio of 40 could introduce a bias, as this ratio should vary from 10 to 100 (on average 35 for phytoplankton; Cloern 1995), the spatial pattern of the contribution of primary organic C to sedimentary biopolymeric fraction of organic matter would remain the same, with low values in the central area (still negligible also using a factor 100) and higher values (up to 100%) in the northern area. Microphytobenthos biomass in the Marsala lagoon (on average  $0.16 \text{ mg C g}^{-1}$ ) was comparable with values reported for eutrophic Mediterranean coastal areas (Garrigue 1998; Conde et al. 1999; Danovaro et al. 2000). Values in the central area ( $0.20 \text{ mg C g}^{-1}$  as an average of stations 2 and 3) were significantly higher than in the northern area ( $0.07 \text{ mg C g}^{-1}$ ). The low contribution of primary organic C to the biopolymeric organic pool in the central area was due to the accumulation of organic detritus rather than to the lack of primary producers.

#### THE TROPHIC STATE OF DETRITUS SINK SYSTEMS: THE MARSALA LAGOON AS A MODEL

Assessment of organic matter bioavailability is a focal point of trophodynamic and geochemical studies, but it is still a recalcitrant and controversial problem. A universally accepted method to distinguish between the labile (bioavailable) and refractory fraction of sedimentary organic matter does not yet exist.

The amount of labile (bioavailable) organic matter in marine sediments could be mathematically extrapolated according to exponential decrease of organic C content with increasing depth in the sediments (Rice and Rhoads 1989; Berner 1995). Such a model does not take into account the fact that different classes of organic compounds might undergo degradation and utilization at different rates (Danovaro et al. 2001). Recent advances in estuarine science provided new approaches to discriminate labile and refractory fractions of organic matter. These were based on paired  $^{14}\text{C}$  and  $^{13}\text{C}$  measurements (Raymond and Bauer 2001), lipid biomarkers (Shi et al. 2001), fatty acids (Canuel 2001), molecular weight fractionation (Harvey and Mannino 2001), and enzymatic activities (Hopkinson et al. 1998). These investigations have also strengthened the coupling between systems productivity and organic matter biochemical composition and reactivity, opening new perspectives in the assessment of the trophic state by means of organic biogeochemical indicators (Bianchi and Canuel 2001).

An increasing number of studies has been devoted to the measure of organic matter quality through the analysis of its biochemical composition (Danovaro et al. 1993; Fabiano and Danovaro 1994; Fabiano et al. 1995; Danovaro 1996; Pusceddu et al. 1999; Tselepidis et al. 2000). It has been demonstrated that only a minor fraction of protein and carbohydrate pools in marine sediments can be mobilized enzymatically (Dauwe and Middelburg 1998; Dauwe et al. 1999a,b; Dell'Anno et al. 2000). For example, the fraction of proteins potentially mobilized by enzymatic digestion in marine



sediment ranges 5–50% of the total protein pool (Table 2). The results of the present study confirm other literature data and indicated that the contribution of the enzymatically hydrolyzable protein and carbohydrate pools (i.e., the labile fraction of organic matter) to their total pools was inversely related to the total amount of sediment organic matter (Fig. 6). In the central area, where large amounts of organic matter accumulated in surface sediments, hydrolyzable protein and carbohydrate pools accounted on average for about 5% of their respective total pools. In the northern area, where organic content was 40 times lower than in the central area, the contribution of the labile fraction to the total protein and carbohydrate pools was 10 times higher.

According to the conventional classification of the trophic state of water bodies based on the availability of inorganic nutrients, oxygen, and phytoplankton biomass (e.g., Vollenweider et al. 1998) the Marsala lagoon can be considered relatively oligotrophic (Pusceddu et al. 1997). The Marsala lagoon displays huge organic matter concentrations in the sediment even comparable to the most eutrophic areas ever reported in the literature. In this sense, the Marsala lagoon displays contrasting trophic conditions in the pelagic and benthic domains; the water column was relatively oligotrophic, but the sediments appeared eutrophic. Contrasting trophic conditions were also found in the northern and central sediments. Eutrophic conditions (high organic C load, low labile organic fraction, and negligible microphytobenthic contribution to BPC) were associated with the seagrass meadow, whereas oligotrophic conditions (low organic C load, higher labile organic fraction, and higher microphytobenthic contribution to BPC) were found in the northern sediments. The northern and central-southern sectors of the lagoon represent two different subsystems. Such a pattern was likely related to the differences in hydrodynamic regimes. Frequent sediment resuspension events in the northern area do not allow organic matter accumulation, whereas the central-southern area, characterized by lower hydrodynamic forcing, displays a stronger organic matter deposition (Pusceddu et al. 1997).

Sediment resuspension is a frequent phenomenon in shallow water systems characterized by a strong wind forcing and is an important factor controlling quantity of both suspended and sediment organic particles (Wainright and Hopkinson 1997). Resuspended organic particles are generally subjected to faster degradation rates and microbial respiration (Wainright 1987, 1990), so that patterns observed in the study site were likely related to upward and lateral export of bioavailable organ-

ic particles from the sediment to the water column (Mazzola et al. 2001).

Chl *a* concentrations in the sediments of the northern area of the Marsala lagoon were significantly lower (up to 3 times) than in the central area, but, conversely, the ratio of chl *a* to phytopigment content was about double (0.92 versus 0.52 and 0.51; at stations 1, 2, and 3, respectively). These results indicate that sediment resuspension exerted a strong control on microphytobenthic biomass, but also suggest that an active fraction of microphytobenthos persisted under turbulent regimes.

In the Marsala lagoon, the microphytobenthic contribution (Cchl *a*) to BPC and the BAOC contribution to BPC were significantly correlated (Fig. 8), suggesting that the chl *a* content of the sediment could also be a good descriptor of the trophic state of marine systems. This might be even more evident in shallow coastal ecosystems, in which most of the primary production is accounted by microphytobenthos and macroalgae rather than by phytoplankton (Lucas et al. 2000; Koester and Meyer-Reil 2001).

This study provides evidence that, especially in detritus sink systems (such as the central area of the Marsala lagoon), hydrodynamic conditions do not only control the accumulation and export of organic C in and from the sediments, but can also influence the bioavailability of sediment organic matter. Future conceptual models of trophic state assessment should not only take into account inputs-response mechanisms involving inorganic nutrient inputs and primary production response, but also should include hydrodynamic processes.

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