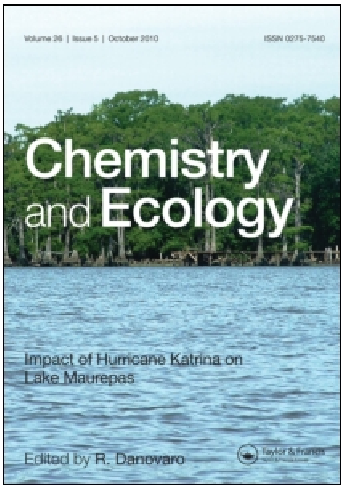

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SEDIMENTARY ORGANIC MATTER AND BACTERIAL COMMUNITY IN MICROTIDAL MIXED BEACHES OF THE LIGURIAN SEA (NW MEDITERRANEAN)

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The quantity and quality of organic matter, and bacterial density and frequency of dividing cells were investigated in six microtidal mixed beaches of the Ligurian Sea (NW Mediterranean) to evaluate their main trophodynamic features. Concentrations of biopolymeric carbon (average $88.5 \pm 89.0\ \mu gC/g$) and the protein:carbohydrate ratio (on average lower than 1) were very low and classified these beaches as highly oligotrophic. The study of biochemical composition highlighted the nature of organic matter as being mainly refractory; furthermore, the quantitative differences observed along the across-beach gradient together with the unchanged pattern in qualitative features suggest that the organic matter in Ligurian beaches is prevalently of marine origin. This implies a negligible contribution of allochtonous and anthropogenic terrestrial input or *in situ* autochthonous production. Bacterial density displayed values ranging from 0.1–9.0 cell $\times 10^8/g$ DW in the top 2 cm layer and showed a significant correlation with the quantity of organic matter. In addition, the frequency of dividing cells showed a positive correlation with the protein:carbohydrate ratio, suggesting that the biochemical composition of organic matter also has an influence on the active bacterial fraction. Because of the exposed nature, a strong coupling was found between the beach and the marine systems, and this seems to be of fundamental importance in terms of material and energy supply for the beach ecosystem. A shortage within this linkage was observed in summer owing to the strong environmental constrains leading to a sort of "beach desertification" and to a marked oligotrophy. Summer also has an effect of smoothing for spatial variability occurring within the biochemical and microbiological variables among the different beaches. The linkage observed between the sea and the land is the main factor controlling the origin and nature of sediment organic matter in these beaches also regulating bacterial abundances and the frequency of dividing cells.

Keywords: Sand; Detritus; Bacteria; Beach coastal ecosystem

1 INTRODUCTION

Beaches are transitional ecosystems situated between the sea and the land (Bird, 1988; Zann, 1997) and are subject to strong environmental forces. Physical, chemical and biological pressures make beaches highly dynamic systems, characterized by intense and complex biogeochemical processes (Blanchard *et al.*, 2001; Guarini *et al.*, 2000; Sundbäk *et al.*,

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1996). Beach ecosystems are also under continuous stress because of waste disposal, over-engineering, urbanization and tourism pressure (Defeo and De Alava, 1995; Lercari and Defeo, 1999; Lubchenco *et al.*, 1995; Schoeman *et al.*, 2000; Zann, 1997). In particular, Ligurian beaches are heavily affected by high urbanization and tourism. Liguria is a highly populated region mainly concentrated along the coastline, and the high levels of human activities, such as agriculture, aquaculture and industrial activities, are often a major threat to the marine environment. Tourism is by far the major economic resource, and management for recreational purposes has urged the need for certain nourishment practices to deal with the problem of physical erosion. All these factors may finally contribute to the alteration of the main ecological pathways and, ultimately, a potential decrease in ecosystem resilience (Fabiano *et al.*, 2002). Multidisciplinary integrated knowledge of beaches is thus essential for habitat preservation to help maintain natural features and to allow for a sustainable human exploitation (Lercari *et al.*, 2002).

The scientific literature has repeatedly addressed beach topics, such as geomorphology and management (Hanson *et al.*, 2002; Kroon and Masselink, 2002; Rodriguez-Ramirez *et al.*, 2003; Stépanian and Levoy, 2003; Thomalla and Vincent, 2003), but ecological studies are mainly related to the study of macrofauna (Contreras *et al.*, 1999; Frouin *et al.*, 1998; Jaramillo *et al.*, 1996; Lercari *et al.*, 2002; Nakashima and Taggart, 2002; Schoeman and Richardson, 2002; Wilber *et al.*, 2003), meiofauna and phytobenthos (Ansari and Ingole, 2002; Delgado *et al.*, 1991; Guarini *et al.*, 1999; Raffaelli, 1982). In addition, all these studies mainly refer to macrotidal environment, while microtidal Mediterranean beaches have been scarcely investigated (Marques *et al.*, 2003; Scapini *et al.*, 1992). Studies on the entity and ecological role of lower trophic levels are generally lacking for the beach ecosystem (Fabiano *et al.*, 2002; Jedrzejczak, 2002; Koop and Griffiths, 1982; Novitsky and Macsween, 1989; Sundbäk *et al.*, 1996). The organic matter–bacterial community system represents a major component within the detritus food chain and plays a pivotal role in the overall biogeochemical cycles. In fact, the quality and quantity of organic matter in beach sediments represent a primary nutritional source for the living community (Brown and McLachan, 1990; Colombini *et al.*, 2000; Fabiano *et al.*, 1995; Griffiths *et al.*, 1983; Inglis, 1989; Jedrzejczak, 2002). Recently, the origin and biochemical composition of organic matter have been proposed as one of the key factors together with the physical environment (McLachlan, 1990) for the control of the beach fauna (Incera *et al.*, 2003). In particular, bacteria are the primary utilizers of organic matter and are net mineralizers (Jedrzejczak, 2002). In addition, due to the small size and the rapid turnover time, bacteria respond promptly to changes in environmental conditions and are successfully employed in environmental monitoring (Danovaro *et al.*, 1993; Fabiano and Danovaro, 1994; Vezzulli *et al.*, 2002; Kefalas *et al.*, 2003). For these reasons, the study of the organic matter and the bacterial community is of primary interest for a better understanding on the ecology and health of the beach ecosystem. In this study, we evaluated the quantity and biochemical composition of organic matter in six mixed beaches of the Ligurian Sea in order to investigate their main trophodynamic features. Biochemical variables were evaluated throughout space and time covering the main patterns of variability. Bacteria density and frequency of dividing cells were finally assessed and related to the biochemical variables.

2 MATERIALS AND METHODS

2.1 Study Area

For the study, six sand and gravel mixed beaches (Lavagna—LAV, Pietra Ligure—PIE, Loano—LOA, Albisola—ALB, Varazze—VAE, Varigotti—VAI) (Figure 1) were selected

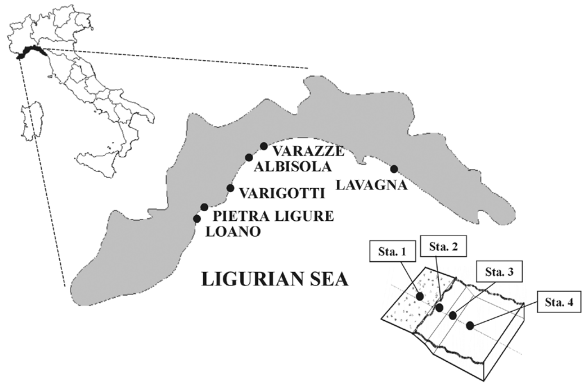

FIGURE 1 Study area and the six beaches sampled. Locations of the sampling stations along the across-beach gradient are also indicated.

along the Ligurian coast (Italy, NW Mediterranean) on the basis of their ecological relevance and because they have been affected by recreational and nourishment activities. These beaches have a small length and width and a shore slope lower than 5% (AA. VV, 1999). The beaches are microtidal (maximum tidal amplitude about 20 cm), characterized by moderate wave action (wave height generally lower than 3 m) and usually no macrofaunal burrows. According to McLachlan (1980), they are classified as “exposed beaches”. Following Hegge *et al.* (1996), the morphology is stepped at the surf break that is very close to the swash zone (Fabiano *et al.*, 2002). The sediment grain size generally varies from coarse sand to gravel, with the finer fraction mostly found in the submerged beach. Because of the predominance of the coarse grain fraction, oxic conditions were always found within the top 2 cm sediment layer.

2.2 Sampling

Sampling was carried out at two replicate transects selected in each beach in March, June and October 2000. In June, only the beaches of LOA, LAV, VAE and PIE were sampled. Nourishment was carried out in LAV, VAE, and PIE in May 2000. Transects were oriented perpendicular to the coast and were kept 500 m apart. In each transect, four stations were placed along the across-beach gradient: station 1 (Sta. 1) located in the 1–1.5 m above the extreme wave run-up; station 2 (Sta. 2) located in the swash zone, station 3 (Sta. 3) located in the surf zone (depth ~ -1 m) and station 4 (Sta. 4) located in the submerged beach (depth ~ -5 m) (Figure 1).

For each sampling operation, three replicates sediment samples were collected manually using Plexiglas cores (inner diameter 5 cm) by scuba divers. Only the top sediment layer (0–2 cm depth) was taken. The biochemical composition of the sedimentary organic matter was determined by frozen samples at -20 °C. For microbial analyses, samples were collected using sterile syringes, fixed with 2% prefiltered and sterilized formalin solution and stored at 4 °C.

2.3 Laboratory Analysis

2.3.1 Biochemical Analysis

Carbohydrates (CHO) were analysed according to Dubois *et al.* (1956): D(+) glucose solution was used as standard. Protein (PRT) analyses were carried out following Hartree (1972), modified by Fabiano *et al.* (1995): albumin solution was used as standard. Lipids (LIP) were extracted according to Bligh and Dyer (1959) and measured following Marsh and Weinstein (1966): tripalmitine solution was used as standard. For each analysis, controls were performed following the same method, with sediment pre-treated in a muffle furnace (550 °C, 4 h). Concentrations were expressed as μg/g sediment dry weight. Carbon biopolymeric fraction (BPC) was calculated according to Fabiano and Danovaro (1994) as the sum of lipids, proteins and carbohydrates converted to carbon equivalents according to Fichez (1991): values were expressed as μgC/g.

2.3.2 *Bacterial Analysis*

Samples were sonicated three times (Sonifier Labor 2000, 195 W for 1 min). Counts were performed using 0.2 μm black Nuclepore filters after Acridine Orange staining (Hobbie *et al.*, 1977), as described by Danovaro and Fabiano (1995), by means of epifluorescence microscopy (Zeiss Universal Microscope).

Bacterial density (TBN) (cell $\times\ 10^8$/g sed DW) was normalized to dry weight after desiccation at 60 °C for 24 h. The number of dividing bacterial cells, defined as cells with a clearly visible invagination, was determined and expressed as a percentage of the total density (FDC).

2.4 Data Analysis

Pearson's correlation analysis was carried out to test for correlation among microbial and biochemical variables in the six beaches during the three sampling periods pooled together. Spatial and temporal variability of biochemical and bacterial variables in the beach ecosystems was assessed by a multifactorial ANOVA with two factors crossed ("Beach, 6 level, fixed" and "Period, 3 levels, fixed") and two factors nested ("Transect, 2 levels, random" plot in "Beach" and "Station, 4 levels, random" plot in "Transect"). In the multifactorial design three replicates were collected for each variable. Factors that scored significant were analysed further using Tukey's post hoc test for a multiple comparison of means.

To assess the degree of linkage between the beach and marine systems (as defined in Section 3), a Mantel test with 1000 permutations was carried out on Euclidean distances matrices calculated for the six beaches using biochemical (PRT, CHO, LIP) and bacterial measures (TBN, FDC) as the input variables. The rationale to this approach is that if the two systems are tightly connected, we should expect a significant covariation of the investigated variables along the across-beach gradient.

Non-metric MDS (ordination of samples on a two-dimensional space) was finally employed to assess differences between stations and periods using BPC (OM quantity), PRT (OM quality), TBN (bacterial density) and FDC (bacterial frequency of dividing cells) as input variables. In this frame, the impact of nourishment was investigated considering the beaches of Pietra Ligure (nourishment), Varazze (nourishment), Lavagna (nourishment) and Loano (no nourishment) that were sampled for all periods in March (Spring), June (Summer) and October (Fall). Input matrices were averaged for transects and stations before analysis. All statistical tests and correlation analysis were made using the statistics toolbox, R12, of MATLAB. MDS was carried out using PRIMER 6 (Plymouth Marine Laboratory).

3 RESULTS AND DISCUSSION

Biopolymeric carbon (BPC) displayed values ranging from 9.1 to 438.3 μgC/g sed DW in the top 2 cm layer. These data represent the first available data on the quantity and composition of sediment organic matter in Ligurian beach ecosystems (Tab. I). Comparison with data collected in shallow marine sediments in the same geographical area (Ligurian sea) showed that BPC values in beaches were always very low (average 88.5 $\pm$ 89.0 μgC/g sed DW) (Danovaro *et al.*, 1994; Fabiano *et al.*, 1995). The PRT : CHO ratio gives a measure of the quality of organic matter (Fabiano *et al.*, 1993) and showed an average value lower than 1, similar to that observed in shallow sediments of the Ligurian Sea (Danovaro *et al.*, 1994; Fabiano *et al.*, 1995). The low PRT:CHO ratio, together with the low concentrations of BPC, classified these beaches as highly oligotrophic (*sensu* Dell'Anno *et al.*, 2002). In addition, since carbohydrates constituted the dominant fraction of BPC (on average 42%), the organic matter in Ligurian beaches was mostly of detrital and allochthonous origin (Fabiano *et al.*, 1995). Comparison with other flats and beach areas highlighted as organic matter concentrations in Ligurian beaches were among the lowest reported in the literature (Tab. II).

On average, very similar BPC concentrations (Tukey post hoc $P =$ n.s.) were observed among the different beaches, with the exception of Varigotti, where significantly lower concentrations were found (Tab. IV, Tukey post hoc $P < 0.05$) (Tab. I). Varigotti beach is far less urbanized than the other beaches, and this seems a plausible reason for this finding. In contrast, the similar level of urbanization and similar OM concentrations supplied by the nearshore environment (discussed later) may explain the lack of any detectable differences among the beaches. This reflects a peculiar feature of Ligurian beaches that are characterized by a reduced spatial heterogeneity also due to the lack of important river outflows and the ubiquity of anthropic impact mainly derived from tourism and urbanization.

Bacterial density (TBN) values ranged from 0.1 to 9.0 cell $\times$ 10^8/g sed DW in the top 2 cm layer (Tab. III) and showed a significant positive correlation with BPC indicating a bottom-up control played by food availability on the bacterial community ($n = 62$, $P < 0.01$). In particular, TBN showed a high correlation with carbohydrates ($n = 62$, $P < 0.01$) and proteins ($n = 62$, $P < 0.01$), while no significant correlation was found with the lipid fraction ($n = 62$, $P =$ n.s.). Thus, proteins and carbohydrates were the main organic compounds affecting the bacterial density in these beaches (Fabiano and Danovaro, 1994). In addition, the significant positive correlation observed between the frequency of dividing cells and the PRT:CHO ratio ($n = 62$, $P < 0.01$) showed that the biochemical composition of organic matter also has an influence on the active bacterial fraction.

The distribution of organic matter and bacterial density along the across-beach gradient highlighted a significant difference between Sta. 4 and Sta. 1, 2 and 3 for all six beaches (Tab. V, Tukey post hoc, $P < 0.05$). Sta. 4, situated in the subtidal zone (-5 m), showed the highest concentrations of CHO (172.2 $\pm$ 67.5 μg/g sed DW), PRT (112.4 $\pm$ 59.7 μg/g sed DW), LIP (86.0 $\pm$ 105.4 μg/g sed DW) and BPC (201.1 $\pm$ 90.6 μg/g sed DW), whose values were within the range reported for shallow coastal sediments in the Ligurian Sea (Danovaro *et al.*, 1994; Fabiano *et al.*, 1995) (Figure 2). In contrast, Sta. 1, Sta. 2 and Sta. 3 showed lower concentrations, CHO (41.7 $\pm$ 42.3 μg/g sed DW), PRT (29.4 $\pm$ 24.9 μg/g sed DW), LIP (23.0 $\pm$ 21.7 μg/g sed DW) and BPC (51.3 $\pm$ 38.4 μgC/g sed DW), and no significant differences were observed among the three stations (Tukey post hoc, $P < 0.05$). The differences observed between Sta. 1, 2, 3 and 4 in terms of BPC concentration did not reflect any evident change in the qualitative composition of organic matter. In fact, all stations showed a similar OM composition and no difference in the PRT:CHO ratio. The quantitative differences observed among stations together with the unchanged pattern in qualitative features suggest that the OM in Ligurian beaches is prevalently of marine origin. This, together with the detrital nature of OM, implies a negligible contribution of allochtonous and anthropogenic terrestrial input or *in situ* autochthonous production.

TABLE I Mean concentrations of biochemical parameters (average among transects, stations and months $\pm$ standard deviations) in the top 2 cm sediment of the six Ligurian beaches during the study period.

Beach	*BPC* $\mu gC/g$ *sed DW*	*SD*	*PRT* $\mu g/g$ *sed DW*	*SD*	*CHO* $\mu g/g$ *sed DW*	*SD*	*LIP* $\mu g/g$ *sed DW*	*SD*	*PRT:CHO*	*SD*
LAV	90.4	117.8	36.6	40.4	69.1	75.6	52.1	99.1	0.7	0.4
LOA	102.1	81.5	55.7	71.0	100.1	104.2	36.3	33.2	1.0	1.0
PIE	106.8	81.4	65.9	55.3	102.8	94.6	34.8	50.1	0.9	0.9
VAI	50.7	52.9	28.8	34.5	34.5	30.6	27.1	32.1	0.8	0.4
ALB	106.1	120.1	55.4	50.5	65.7	90.7	63.6	102.5	2.0	2.0
VAE	72.7	54.4	47.2	46.4	74.3	65.8	19.5	16.1	0.8	0.4

Note: BPC: biopolymeric carbon; PRT: protein; CHO: carbohydrate; LIP: lipid; PRT:CHO: protein:carbohydrate ratio.

TABLE II Comparison of biopolymeric organic matter concentrations from different flats and beach areas.

Location		*CHO* *µg/g sed DW*	*PRT* *µg/g sed DW*	*LIP* *µg/g sed DW*	*Source*
Cape Henlope (Delaware, USA)	Sandflat		420–950		Bock and Miller (1995)
Bay of Mont-Saint-Michel (France)	Intertidal flat			30–200	Meziane *et al.* (1997)
Barraña (Galicia, Spain)	Intertidal falt	30–670	40–4100	50–1480	Cividanes *et al.* (2002)
Iberian Peninsula (Spain)	Macrotidal sandy beach	4.5–537.19	93.8–1115.0	18.2–486.3	Incera *et al.* (2003)
Ligurian Coast (Italy)	Microtidal mixed beach	7.2–283.1	5.0–287.2	3.2–366.1	Present study

Note: CHO: carbohydrate; PRT: protein; LIP: lipid.

TABLE III ANOVA table for the multifactorial design used to investigate the spatial and temporal variability of biopolymeric carbon (BPC) in Ligurian beaches.

Source of variation	*Sum sq.*	*d.f.*	*Mean sq.*	*F*	*P*
Beach [B]	494,666.8	5	98,933.36	134.0	*
Transect [T(B)]	4430.6	6	738.4	0.02	n.s.
Station [S(T(B))]	1,544,575.1	36	42,904.9	28.2	*
Period [P]	16,683.3	2	8341.65	11.5	*
Period × Beach [P*B]	151,905.6	10	15,190.6	20.8	*
Period × Transect [P*T(B)]	8746.8	12	728.9	0.4	n.s.
Period × Station [P*S(T(B))]	125,619	72	1744.7	1.1	n.s.
Error	438,629.8	288	1523.0		
Total	2,827,436.7	431			
Cochran's test					n.s.

*$P < 0.05$.

The exposure and the microtidal nature of these beaches may both contribute to these findings (Tab. I).

Similar considerations can be drawn from the study of bacterial density that displayed higher values at Sta. 4 (1.4 ± 0.8 cell $\times 10^8$/g sed DW) than at Sta. 1, 2 and 3 (3.2 ± 2.1 cell $\times 10^8$/g sed DW) (Tukey post hoc $P < 0.05$) (Figure 2), also comparable with the density found in coastal sediments of the Ligurian Sea (Albertelli *et al.*, 1999; Danovaro and Fabiano, 1995; Manini *et al.*, 1997). Therefore, from the very similar patterns observed for the biochemical and bacterial variables at Sta. 1, 2 and 3, these three stations could be considered as representative of the beach system. In contrast, Sta. 4, which displayed similar organic matter concentrations and bacterial density to those reported for the nearshore environment, is mainly representative of the marine system. A significant linkage was indeed found between the beach and marine systems in March and October (Mantel statistic, $P < 0.05$), while no correlation was found in June (Mantel statistic, $P =$ n.s.) probably due to the reduced hydrodynamic regime. This and the very low OM concentrations detected in October (Tukey post hoc, $P < 0.05$) suggest that the supply of organic matter from the sea may fall sharply in summer, thus leading to a marked oligotrophy. October represents the final stage of the summer season, characterized by a lack of sea input, dryness, high temperature and solar radiation leading to a sort of "desertification" of the beach ecosystem. At this time, a change in the quality of organic matter in terms of a decrease in the PRT:CHO ratio was also observed, indicating that the origin of the OM was largely detrital. One possible explanation may be the increase in decomposition processes during summer (Danovaro *et al.*, 2002; Fabiano *et al.*, 1992) that caused a rapid consumption of the labile fraction (proteins and lipids) and an accumulation of the refractory component

TABLE IV Mean bacterial density (TBN) and frequency of dividing cells (FDC) (average among transects, stations and months ± standard deviations) in the top 2 cm sediment of the six Ligurian beaches during the study period.

Beach	*TBN (cell × 10^8/g sed DW)*	*SD*	*FDC %*	*SD*
LAV	1.0	1.2	5.8	2.1
LOA	2.2	2.0	3.4	1.7
PIE	2.4	1.5	4.6	1.6
VAI	1.8	1.5	3.7	2.0
ALB	1.9	1.6	6.1	3.6
VAE	1.6	1.5	5.4	1.5

TABLE V ANOVA table for the multifactorial design used to investigated spatial and temporal variability of bacterial density (TBN) in Ligurian beaches.

Source of variation	*Sum sq.*	*d.f.*	*Mean sq.*	*F*	*P*
Beach [B]	82.6	5	16.5	66	*
Transect [T(B)]	1.5	6	0.25	0.06	n.s.
Station [S(T(B))]	150.0	36	4.2	3.2	*
Period [P]	11.1	2	5.6	18.7	*
Period × Beach [P*B]	88.9	10	9.0	30	*
Period × Transect [P*T(B)]	3.2	12	0.3	0.6	n.s.
Period × Station [P*S(T(B))]	38.6	72	0.5	0.4	n.s.
Error	367.9	288	1.3		
Total	872.9	431			
Cochran's test					n.s.

$^{*}P < 0.05$.

(carbohydrates) (Papadakis *et al.*, 1996). Again, the spatial variability detected among beaches displayed a time-dependent interaction (Tabs III and V), with a greater similarity observed in October (Figure 3). No differences were observed between Loano and the other beaches where spring nourishment took place (Figure 3). This suggests that the differences observed in OM concentrations and bacterial density among the six beaches are

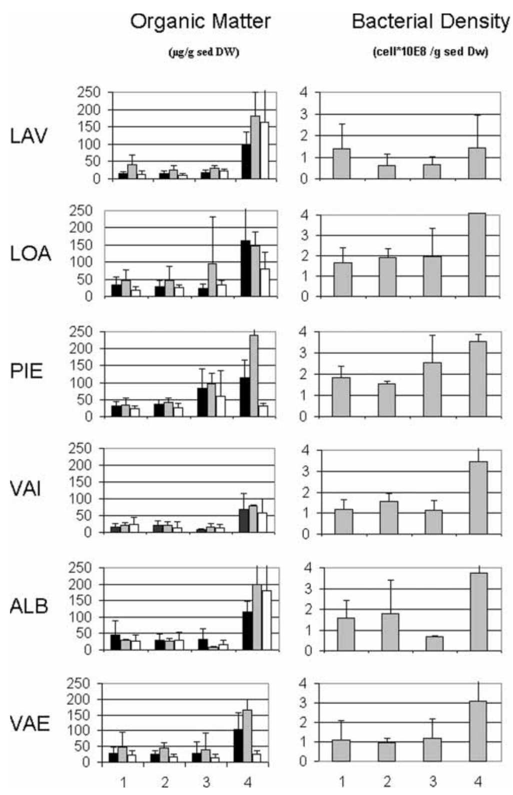

FIGURE 2 Three-month average of organic matter concentrations and bacterial density along the across-beach gradient for the top 2 cm of the sediment in the six Ligurian beaches (LAV: Lavagna; PIE: Pietra Ligure; LOA: Loano; ALB: Albisola; VAE: Varazze; VAI: Varigotti). PRT: protein; CHO: carbohydrate; LIP: lipid.

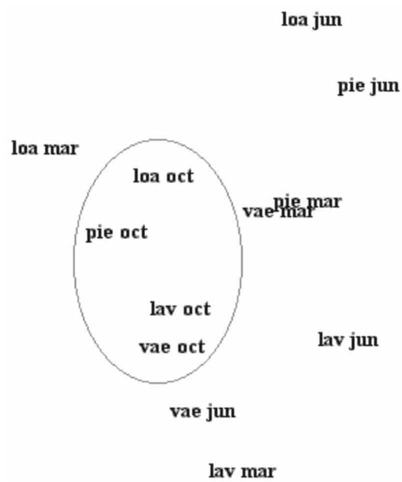

FIGURE 3 Multidimensional scaling ordination based on biopolymeric organic carbon, protein, bacterial density and frequency of dividing cells using beaches (LAV: Lavagna; PIE: Pietra Ligure; LOA: Loano; VAE: Varazze) and sampling periods (March, June and October) as the only factors of variability. October stations (circled) are clustered together, suggesting a high degree of similarity (see main text).

reduced in the summer, and anthropic activities carried out before summer seem to have a negligible role in affecting the quantity and distribution of these variables.

In conclusion, the linkage observed between the sea and the land is the main factor controlling the origin and nature of sediment organic matter in these beaches as well as regulating bacterial community structure. The nearshore marine environment represents a key component that needs to be addressed by ecological studies for a correct assessment of the health and recovery of Ligurian beach ecosystems.

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