RESEARCH ARTICLE

Assemblages' structure and activity of bacterioplankton in northern Adriatic Sea surface waters: a 3-year case study

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Abstract

The bacterial community, both in terms of community structure (denaturing gradient gel electrophoresis fingerprinting) and activity (exoenzymatic hydrolysis of proteins, polysaccharides and phosphorylated molecules and leucine uptake), was investigated seasonally for 3 years (2004–2006) in a large-scale grid in the northern Adriatic Sea. A high variability characterized the spatial structure of bacterial assemblages and a scarce seasonality was found in all the nine studied stations. Bacterial communities were substantially diverse in the same season of the 3 years, in contrast to what was reported previously for oceanic sites. Assemblages were in general strongly affected by river inputs, especially in spring, when freshwater loads were higher. Finally, a close relationship was found between given assemblages and their patterns of degradation/production activities by applying a multivariate analysis (linear discriminant analysis) to the dataset. The high variability of bacterial community structures and patterns of activity may indicate an ecological response to the high dynamism that characterizes the basin both on a physical and on a biological basis.

Introduction

Over the last 15 years, after the development and utilization of fingerprinting techniques targeting the prokaryotic 16S rRNA gene, it became clear that marine microbial communities undergo temporal and spatial variations as most of the planktonic assemblages do. On a global scale, marine bacteria were found to be distributed according to actual biogeographical patterns (Pommier *et al.*, 2007), experiencing, for example, differentiations following latitudinal gradients (e.g. Schattenhofer *et al.*, 2009). In the open ocean, Hewson *et al.* (2006) reported that surface community similarity was high for samples collected within about 2 km, but assemblages located tens to hundreds of kilometres apart had the same level of dissimilarity. Distances from coasts and terrestrial inputs are also important factors in shaping bacterial communities (e.g. Zhang *et al.*, 2009). From a temporal point of view, bacterial assemblages often experience seasonal successions (see Zhang *et al.*, 2009 and references therein), and at some locations, they were found to reoccur annually, thus being defined as temporally 'predictable' (Fuhrman *et al.*, 2006). Naturally, local features within a given environment contribute to the definition of specific patterns or the recognition of random schemes (an all the ways through these two opposite poles).

Recent studies have also taken into account the relationship between microbial composition and ecosystem functioning (e.g. Reed & Martiny, 2007). Because microbial communities are made up of functional groups (C and N fixers, ammonia oxidizers, sulphur reducers, etc.) the absence or the presence of given organisms would affect the biogeochemical activities occurring in a given environment, by impacting elemental cycling rates.

Taking into account the bacterioplankton community structure variations generally described in the literature (a comprehensive overview is provided by Fuhrman & Hagström, 2008), we have analysed the surface bacterial assemblages' variations [through denaturing gradient gel electrophoresis (DGGE) fingerprinting] occurring in a marine ecosystem that is characterized by high spatial and temporal variations occurring at different scales. We have seasonally investigated nine stations located in a large-scale grid comprising most of the northern Adriatic basin for 3 years (2004–2006) aiming (1) to understand the temporal

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successions of bacterial communities occurring in the basin, considering the possibilities of seasonal patterns and potential annual reoccurrence of similar assemblages; (2) to highlight the spatial scale of community differentiation in a highly dynamic environment; and (3) to illustrate the presence or absence of links between community structure and patterns of activities [exoenzymatic activities and prokaryotic carbon production (PCP)] expressed by surface bacterioplankton.

Hydrological features and atmosphere–sea interactions are the main drivers of the high dynamisms of the northern Adriatic Sea. It is characterized by the presence of a main core of dense water formation (North Adriatic Dense Waters – Gačić *et al.*, 2001), intrusions of high-salinity waters from SE (Modified Levantine Intermediate Water) and a relevant contribution of freshwater and atmospheric forcing factors. The main contribution to the circulation is made by the Po River (Socal *et al.*, 2008), which is the major freshwater source, with an annual mean discharge rate of 1500–1700 $m^3 s^{-1}$, accounting for 51% of the total riverine input to the basin (Degobbis & Gilmartin, 1990). The prevailing winds, 'bora' (ENE) and 'scirocco' (SE), trigger the modification of hydrological properties by altering the water column stability (Franco & Michelato, 1992) and by changing the physical features of the basin in general (e.g. Bergamasco & Gačić, 1996). All these factors can have either a local or a basin-scale influence and are known to be highly variable both on a seasonal and on an interannual time scale (Socal *et al.*, 2008). This broad variability is inherently projected on the planktonic system. High interannual variations were found for zooplankton, microphytoplankton (Fonda Umani *et al.*, 2005; Pugnetti *et al.*, 2008), nanoplankton (Pugnetti *et al.*, 2008) and bacterial biomass (Del Negro *et al.*, 2008), together with primary production (Fonda Umani *et al.*, 2007) and prokaryotic metabolism (Del Negro *et al.*, 2008). Seasonal fluctuations of many planktonic fractions (i.e. prokaryotes, phytoplankton and protozoans) have been observed at different sites within the basin (Del Negro *et al.*, 2008; Pugnetti *et al.*, 2008), and marked spatial physical and chemical gradients (which identify the northwestern sector as the most eutrophic, whereas oligotrophy characterizes the eastern coasts; Solidoro *et al.*, 2009) were also found to be involved in shaping the plankton community structure (e.g. Del Negro *et al.*, 2008; Pugnetti *et al.*, 2008). However, to date, no information concerning bacterial large-scale variability is available in the literature.

Materials and methods

Sampling and analytical determinations

Nine stations (A1, 13°06′00″E, 45°37′12″N; A5, 13°08′24″E, 45°30′36″N; A7, 13°10′48″E, 45°25′48″N; C1, 12°18′46″E, 45°15′00″N; C10, 12°46′00″E, 45°15′00″N; C13, 13°00′42″E, 45°14′59″N; E1, 12°33′36″E, 44°57′36″N; E6, 12°46′20″E, 44°57′50″N; E10, 13°00′39″E, 44°57′30″N) were selected in order to have a large-scale grid made up of transects influenced by the main rivers in the basin (Fig. 1). Transect A is located in front of the Tagliamento River mouth (annual mean discharge = 70 $m^3 s^{-1}$); transect C is influenced both by the Adige River (annual mean discharge = 235 $m^3 s^{-1}$) and by the Lagoon of Venice, whereas transect E is the most influenced by freshwater, being often comprised in the Po River (the major tributary of the basin) plume. Samplings were carried out during nine seasonal

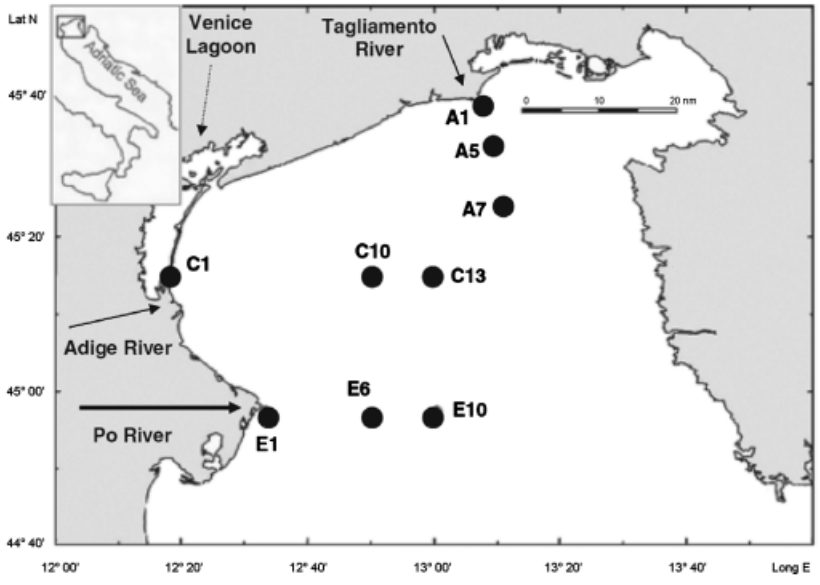

Fig. 1. Location of the sampling stations in the Northern Adriatic Sea.

surveys: May, July, November 2004; March, May, July, 2005; and March, May, July 2006.

At each sampling, temperature and salinity were measured by means of an Idronaut Ocean Seven 316 multiprobe. Water samples for microbial analyses were collected at the surface (-0.5 m) with a 5-L Niskin bottle, equipped with silicon elastic and red silicon O-rings.

Bacterial community structure

After prefiltration through 20-μm nylon screens, 1-L samples for bacterial community structure analysis were filtered onto ∅25-mm, 0.2-μm polyethersulphone membrane filters (Supor 200, PALL Corporation) and the filters were frozen at $-20\,^{\circ}$C until DNA extraction, which was then performed according to Boström *et al.* (2004). Bacterial 16S rRNA genes were PCR-amplified using a reverse universal primer complementary to position 517–534 (5′-ATT ACC GCG GCT GCT GG-3′) and a forward bacterial primer complementary to position 341–358 plus a GC clamp (5′-CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GCC TAC GGG AGG CAG CAG-3′) (Muyzer *et al.*, 1993; Celussi *et al.*, 2008). PCR products were visualized on a 1% (w/v) agarose gel stained with ethidium bromide using a UV transilluminator to confirm the presence of the proper length products. A total of 18 DGGE were performed. A first set of nine DGGEs was run to determine the temporal evolution at each single station (nine samples collected during the nine cruises for each of the nine stations). Another set of nine DGGEs to analyse the spatial structure of surface bacterioplankton assemblages was performed by comparing all nine stations during each sampling time (nine cruises). Five hundred nanograms of PCR products were loaded on 8% polyacrylamide gels (acrylamide : *N*,*N*′-methylenebisacrylamide 37 : 1) containing denaturant gradients of 35–65%, top to bottom (where 100% is defined as 7 M urea and 40% v/v formamide). Electrophoresis was performed with a hot bath DGGE unit (CBS Scientific, Del Mar, CA) using $0.5\times$ TAE running buffer (20 mM Tris, 10 mM acetate, 0.5 mM Na_2EDTA, pH 8.2) at $60\,^{\circ}$C for 16 h at 85 V. Gels were stained for 1 h in SYBR Gold nucleic acid stain (Molecular Probes) and photographed with UV transillumination. DGGE banding patterns were used to determine the bacterial community structure. Bands in different lanes corresponding to the same operational taxonomic unit (OTU) were identified using the EquiBands applet (Huber & Peduzzi, 2004) and band patterns were converted into a presence/absence matrix. Each sample was first run in triplicate (PCR products from three separate PCR reactions) and then used for comparative community structure analysis if a Sørensen Index ≥ 0.98 was detected among the three banding patterns.

Bacterioplankton assemblages' fingerprints were compared by taking into account the presence or absence of different OTUs in different lanes by means of the Sørensen Index, calculated according to the equation:

$$\text{Sørensen} = 2W/(a_1 + a_2),$$

where W is the number of shared OTUs between populations 1 and 2, and a_1 and a_2 are the total OTU numbers in populations 1 and 2, respectively.

The 18 DGGE banding patterns' matrixes were analysed using the PRIMER 5 v5.2.9 (Plymouth Marine Laboratory, UK) program. The Bray–Curtis similarity coefficient was calculated for all pairs of samples within one matrix; cluster analysis using the group average linkage method was then used to visualize the similarities between samples. Nonmetrical multidimensional scaling (MDS) was performed using the same software in order to detect general similarities among stations by merging the nine matrixes used for the spatial approach. The similarity matrix was calculated and constructed by the Bray–Curtis similarity coefficient on the basis of the binary data. The band patterns with the higher similarity are plotted closer and the band patterns with the lower similarity are located further apart. To determine the degree to which this ordination matches the similarity matrix, the stress value of MDS was examined. Stress value = 0.06 indicated a good ordination, with a weak risk of misinterpretation of banding patterns (Clarke & Warwick, 1994).

Prokaryotic activities

Prokaryotic activities were measured along transect A only in May and July 2004, whereas analyses were always performed along transects C and E. Hydrolytic exoenzyme activities were measured with fluorogenic analogues of natural substrates (Hoppe, 1993) derived from 7-amino-methyl-coumarin (AMC) and 4-methyl umbelliferone (MUF). Aminopeptidase activity (AMA) was assayed as the hydrolysis rate of L-leucine-AMC. β-Glucosidase (BGLU) and alkaline phosphatase (APA) were assayed using MUF derivatives. Enzyme activities measured by means of fluorogenic substrates were expressed in terms of the rate of MUF or AMC production. After evaluating the saturating concentrations, hydrolysis rates were measured by incubating 2.5-mL subsamples with 200 μM (final concentration) MUF-β-glucoside and leucine-AMC substrates and 50 μM (final concentration) MUF-phosphate for 1 h at *in situ* temperature in the dark. The fluorescence released by enzymatic cleavage of the artificial substrates was measured fluorometrically, in triplicate, at 380/365 nm excitation and 440/455 nm emission for AMC/MUF substrates using a Shimadzu RF 1501 fluorometer. Standard

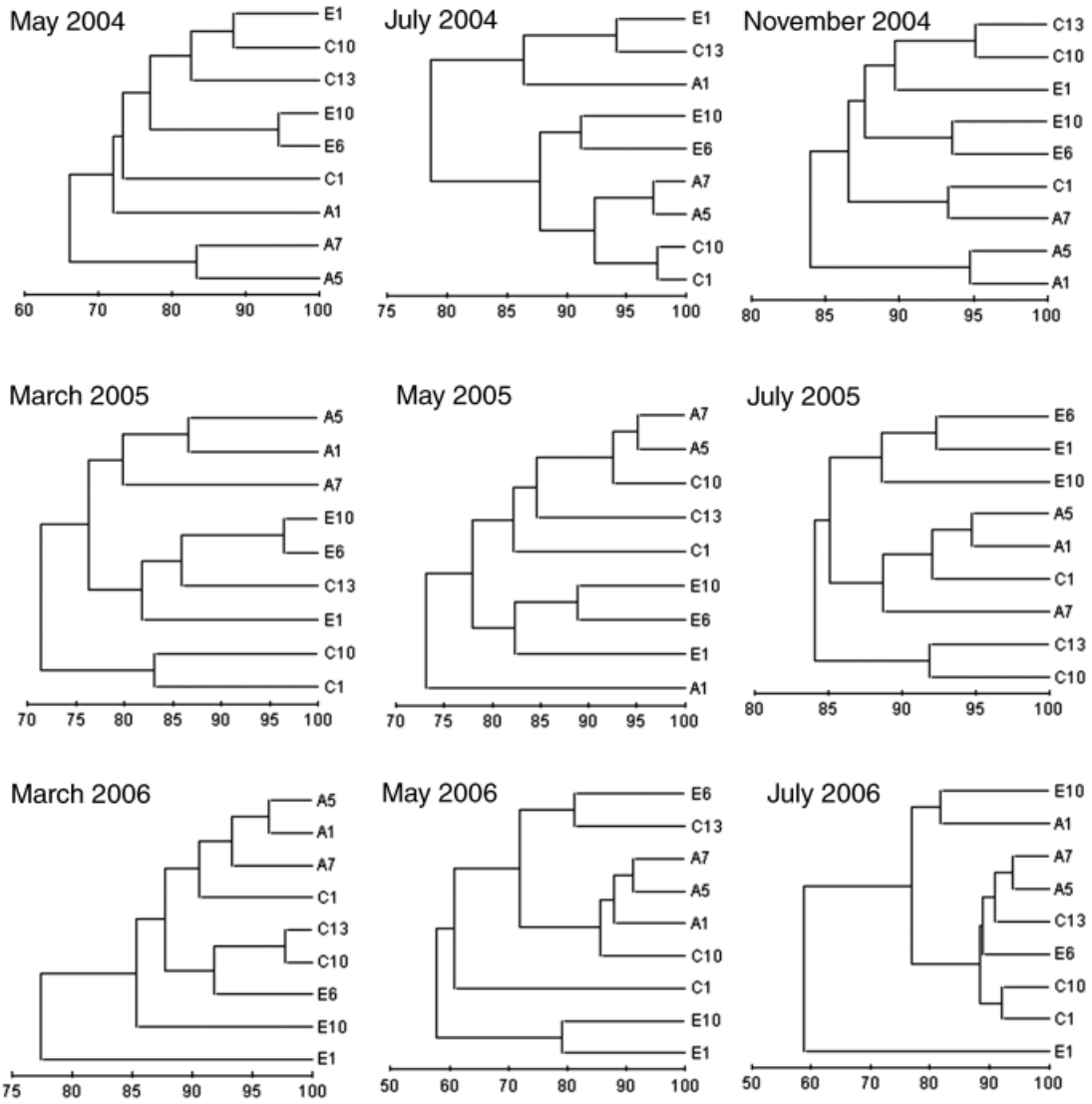

Fig. 2. Cluster analysis dendrograms of nine DGGE fingerprints, performed to analyse the spatial variations of bacterioplankton assemblages during nine cruises (May, July, November 2004; March, May, July 2005; March, May, July 2006) in the northern Adriatic Sea. Clusters were formed by calculating the Bray–Curtis similarity coefficient (horizontal scale bars) for all pairs of sampling within each DGGE matrix before the group average linkage method between similarities was used to build the dendrograms.

solutions of MUF and AMC were used to calibrate the fluorometer.

PCP was estimated by the incorporation of ^{3}H-leucine (Leu) (Kirchman *et al.*, 1985). Triplicate 1.7-mL aliquots and two killed controls [90 μL 100% trichloracetic acid (TCA)] were amended with a 20 nM radiotracer and incubated at *in situ* ($\pm 2\,^{\circ}$C) temperature in the dark. Incubations were stopped with 100% TCA after 1 h. The extraction with 5% TCA and 80% ethanol was carried out using the microcentrifugation method (Smith & Azam, 1992). Activity in the samples was determined using a β-counter (Packard Tri-Carb 300) after the addition of 1 mL scintillation cocktail (Ultima Gold MV; Packard). Incorporation of ^{3}H-leucine was converted into carbon produced via bacterial protein production according to Simon & Azam (1989), assuming a twofold isotope dilution for leucine.

Linear discriminant analysis (LDA)

LDA is a method used in statistics to find a linear combination of features that characterize or separate classes of objects or events and attempts to model the difference between the classes of data. LDA, performed using the R software (R foundation, Vienna, Austria), was used to identify the relative contribution of microbial activities (BGLU, APA, leucine AMA and PCP) in discriminating

among DGGE band patterns of bacterial communities. The *a priori* defined groups (classes) were obtained by subclusters in Fig. 2. In this approach, if clouds of points represented by the same symbol (which correspond to discrete subclusters) are grouped together and separated by the other symbols in the LD1 vs. LD2 plots, it means that similar assemblages (corresponding to subclusters) exert similar patterns of activity (a combination of degradation and production processes) that differ from the ones expressed by more dissimilar communities.

Because of significant pairwise correlation between AMA and PCP, only the latter was used in the analysis. A total of nine analyses were performed by considering from three to five *a priori* subclusters derived from the cluster analysis, according to the spatial approach described in the previous section.

Results

Bacterial community structure

The results of the cluster analysis applied to the nine gels used for the spatial approach and to the nine used for the temporal approach are reported in Figs 2 and 3, respectively. In the latter case, no clear temporal pattern was observed in any of the nine dendrograms. No robust season- or month-related grouping was formed and no consensus among the nine stations for their temporal variations was evidenced (i.e. no subcluster grouping the same month of different years was clearly formed). Generally, in the spatial analysis, stations closely located or belonging to the same transect were grouped together, even though some outgroups

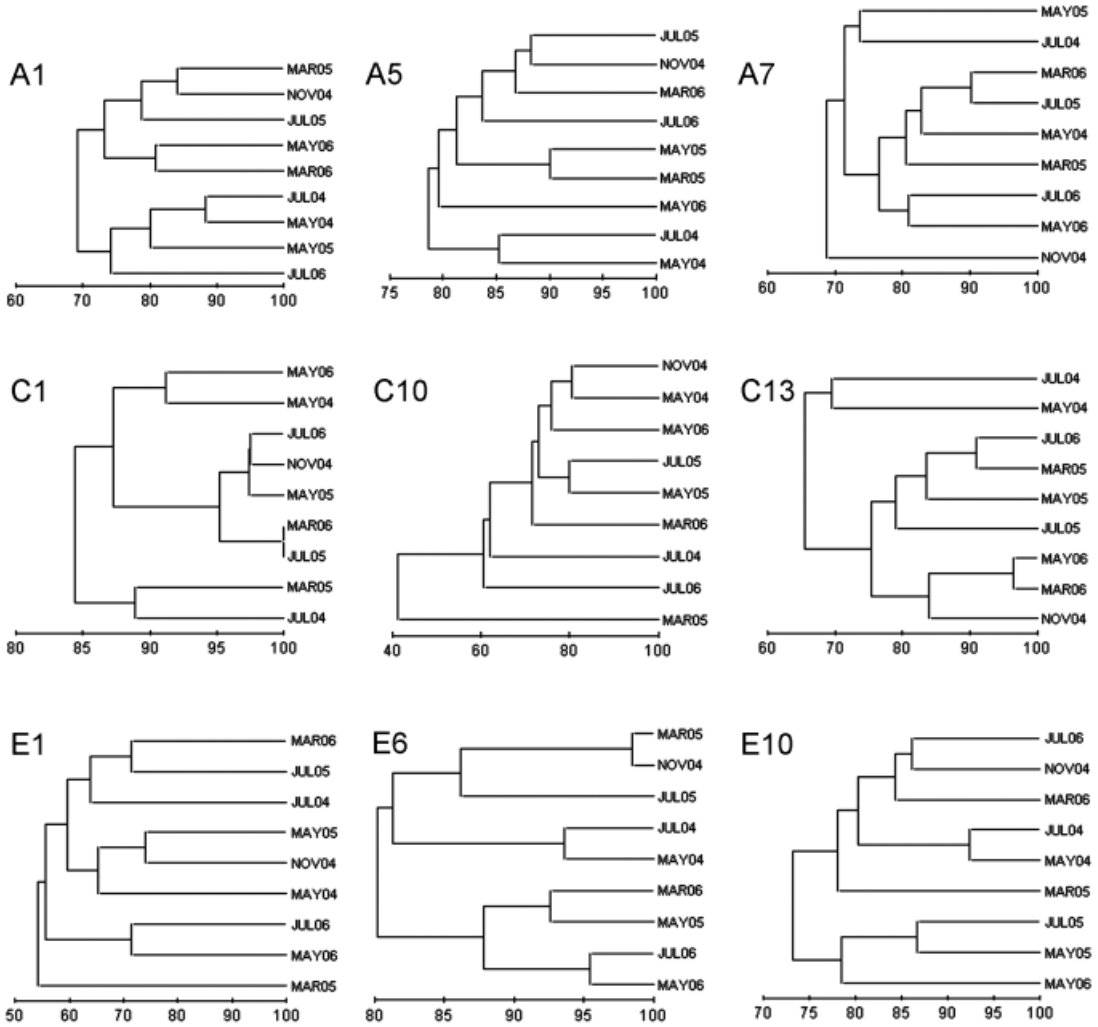

Fig. 3. Cluster analysis dendrograms of nine DGGE fingerprints, performed to analyse the temporal variations of bacterioplankton assemblages at nine stations (A1, A5, A7, C1, C10, C13, E1, E6 and E10) in the northern Adriatic Sea. Clusters were formed by calculating the Bray–Curtis similarity coefficient (horizontal scale bars) for all pairs of sampling within each DGGE matrix before the group average linkage method between similarities was used to build the dendrograms.

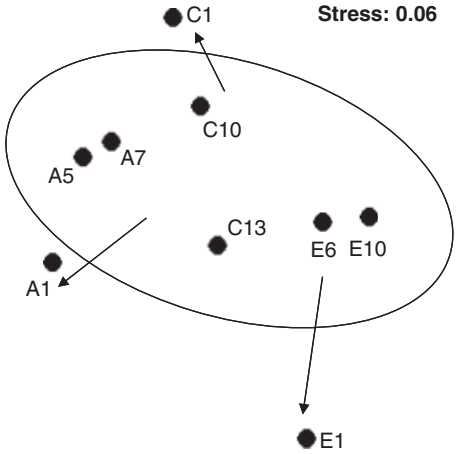

Fig. 4. MDS plot obtained by merging nine DGGE matrixes used to analyse the spatial variations of bacterioplankton assemblages in the northern Adriatic Sea from 2004 to 2006. The MDS was formed by calculating the Bray–Curtis similarity coefficient for all pairs of sampling.

corresponding to the estuarine/most coastal stations were formed in May 2005, March 2006 and July 2006. All the presence/absence matrixes used in the spatial analysis were merged vertically in order to obtain a general spatial pattern of assemblages' similarity. This procedure allowed us to compare stations against each other by also following their temporal evolution. The resulting MDS plot is reported in Fig. 4. A marked separation of the southern transect (E) was evident.

Prokaryotic activities

All the measured activities were particularly high during May and July 2004 and 2005 (Fig. 5). Pronounced BGLU activities were recorded at station C1 during the spring and the summer cruises. However, the highest hydrolysis rate ($114.4 \pm 14.5\,nM\,h^{-1}$) was measured during the first cruise at station E1. In March and November, BGLU was always rather low, presenting values $< 10\,nM\,h^{-1}$ at every station. Similarly, phosphatase activity never exceeded $50\,nM\,h^{-1}$ in autumn and winter. Extremely high APA characterized the entire basin during May 2004, reaching the maximum at station E6 equal to $1.7 \pm 0.1\,\mu M\,h^{-1}$. Also, in this case, station C1 presented the highest rates in the basin, especially in spring and summer 2005 and 2006. The fastest protein degradation occurred at station E1 during every sampling, except May 2005, reaching rates of 1.4 ± 0.1, 1.5 ± 0.02 and $1.7 \pm 0.0\,\mu M\,h^{-1}$ in May 2004, July 2004 and July 2005, respectively. Similar to protease, the highest PCP values were recorded at station E1 during most of the cruises and reached their maxima in summer (7.2 ± 0.3, 6.1 ± 0.4 and 3.4 ± 0.05 in 2004, 2005 and 2006, respectively).

LDA

The LDA applied to prokaryotic activities showed a distribution of variables according to new linear combinations (Table 1; Fig. 6). In every case, the proportion of trace (percent) of the first linear discriminant (LD1) or the sum of the first and the second linear discriminants (LD1+LD2) was > 99.5. The diagrams of the projections of the sampling cases on the LD1 vs. LD2 plane showed that in general, different distributions of activities occurred in different bacterial assemblages, which were highlighted by the cluster analysis. The separation of clouds of points in Fig. 6 indicates that each community expressed (to some extent) peculiar schemes of C degradation/utilization, even if some overlaps indicate that structurally different assemblages expressed the same metabolic requirements (as in the case of May 2004, May 2006 and July 2006).

Discussion

Temporal changes in bacterioplankton assemblages

The cluster analysis applied to the nine DGGE matrixes utilized to visualize the relationship among samplings at each station did not highlight any peculiar seasonal or interannual scheme. Each station presented a temporal pattern hardly comparable to the other one's dendrogram. In general, rare groupings of samplings performed during the same month of following years were identified (some examples at station C1 and C10) and subclusters collecting contiguous samplings (such as autumn/winter or spring/summer) were formed occasionally (e.g. E6 and E10 dendrograms). These 'random' groupings are not commonly found in studies on the temporal variations of bacterial assemblages. Clear seasonal patterns are in fact found in very different environments, ranging from the Baltic Sea (Pinhassi & Hagström, 2000), through the temperate Mediterranean (e.g. Schauer *et al.*, 2003; Celussi & Cataletto, 2007) and Atlantic Ocean (Kan *et al.*, 2006) to the subtropical Atlantic (Morris *et al.*, 2005) and Pacific Ocean (Fuhrman *et al.*, 2006). Additionally, some ecosystems harbour specific features that lead to the reoccurrence of peculiar assemblages during the same period of the following years (Fuhrman *et al.*, 2006; Kan *et al.*, 2006). However, Kan *et al.* (2006), by comparing the literature findings, underlined that some contrasting results may mirror the intrinsic features of a given aquatic ecosystem. Our calculation of similarity indexes between samples collected during the same months of different years (Fig. 7) confirmed these differences by extending a nonrepeated annual pattern to the northern Adriatic Sea surface bacterial assemblages. The Sørensen Indexes are indeed rather low, especially at one of the offshore stations (C10) and at the most estuarine site

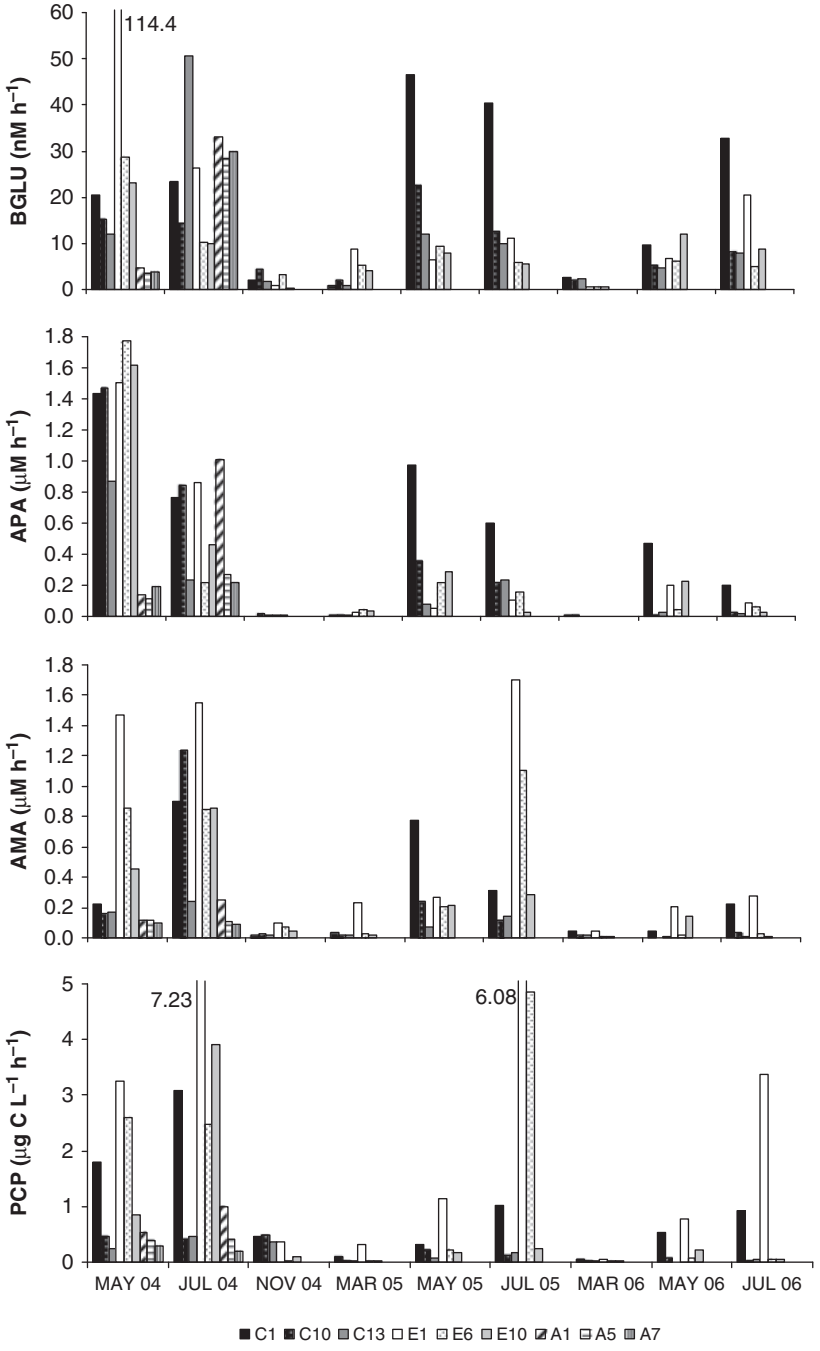

Fig. 5. β-Glucosidase (BGLU), alkaline phosphatase (APA), leucine aminopeptidase activities (AMA) and prokaryotic carbon production (PCP) at nine stations during nine cruises in the northern Adriatic Sea. Each value represents the mean of three determinations ± SD < 10%.

(E1), whereas a repetition of monthly assemblages would be pinpointed by values approaching 1. Surprisingly, the most stable station in this context was station C1, which is highly affected by terrestrial inputs of different origins (one of the Venice Lagoon outlets and the Adige river mouth). However, we cannot rule out the possibility that the sampling design was not properly suited to analyse seasonal or interannual trends, because a previous study carried out in the north-eastern sector of the Adriatic based on a monthly sampling scheme indicated that bacterial assemblages experience a defined seasonal cycle both at a freshwater influenced site and at a more typically marine station (Celussi & Cataletto, 2007). The reduced sampling within 1 year and the 3-year approach used in the present study may be the causes for the differences between these results and previous analyses. The extreme variability of the physical features of the entire northern basin can in fact lead to variations that are not repeatable every year. Changes in the monthly river outflows

Table 1. Coefficients of linear discriminants (for LD1 and LD2) and percent proportion of traces (PT %) for each diagram in Fig. 4

	May 2004		July 2004		November 2004	
	LD1	LD2	LD1	LD2	LD1	LD2
PCP	−1.58	−1.36	0.12	1.45	−1.57	
APA	1.44	1.07	1.97	−0.94	5.04	
BGLU	2.33	1.77	3.00	0.04	3.60	
PT (%)	96.9	3.1	88.48	11.00	100	
	March 2005		May 2005		July 2005	
	LD1	LD2	LD1	LD2	LD1	LD2
PCP	−1.02		−0.47		−7.07	0.79
APA	0.44		−3.48		18.58	−2.10
BGLU	0.14		4.82		19.30	2.46
PT (%)	100		100		99.95	0.05
	March 2006		May 2006		July 2006	
	LD1	LD2	LD1	LD2	LD1	LD2
PCP	−1.81	0.57	−2.70		−88.36	−0.27
APA	1.72	−0.53	−3.81		9.12	−2.28
BGLU	−0.48	1.26	−0.97		15.42	1.93
PT (%)	89.90	10.10	100		99.99	0.01

(especially from the Po River), for example, can cause ecosystem changes such as the anticipation or the delay in the spring phytoplankton bloom, consequently modifying the bacterial communities' structure. Bernardi Aubry *et al.* (2006) reported that the spring bloom occurs in this area between February and April (although high interannual variations were identified), suggesting that a phytoplankton bloom delay may be the cause for the low similarities between the March and the May bacterial assemblages observed in Fig. 7. It must also be noted that during June 2004, a large-scale phenomenon of mucilage formation characterized the entire basin, probably modifying 'typical' summer assemblages and the organic matter pool available for the July 2004 assemblage, thus making these assemblages different from the other summer ones (July 2005 and 2006).

Spatial patterns of bacterial diversity

Similar to what emerged from the clustering of samples according to the temporal changes approach, Fig. 2 revealed that no strict grouping of close stations or of stations belonging to the same transect occurred during the nine sampling cruises. The three transects are located along the direction of freshwater flow of the three main tributaries of the basin, but according to the cyclonic circulation characterizing the basin, the river plumes may follow the coastline westward (transect A) and southward (transects C and E). Studies on the bacterial composition along estuarine gradient have highlighted a spatial bacterial community shift from the river-influenced areas to the open waters (e.g. Hewson & Fuhrman, 2004). This diversity gradient is basically composed by organisms living at low salinities or stenohaline bacteria in the proximity of the freshwater inputs, shifting towards typically marine offshore communities (Rappé *et al.*, 2000). Such a defined pattern was rarely found in our samples (Fig. 2), as transect-related subclusters were formed in November 2004 and during the three 2005 cruises only. Local features other than terrestrial inputs might thus lead to a community selection in different times of the year and in different years, which is hard to be recognized. Given the high heterogeneity of the system, it is possible to hypothesize that different sources of organic material can act as an important (local) feature in shaping bacterial communities. Autochthonous organic matter is produced mainly by phytoplankton that, in the area, is known to be very variable both in time and in space, because bloom supported by different species have been reported for different sites in the basin and during different periods (Bernardi Aubry *et al.*, 2006). Also, it is noteworthy that the northwestern coast of the basin is highly inhabited and industrialized; thus, marine bacterial communities might be affected by different organic matter spectra originating from anthropogenic activities (Zhang *et al.*, 2009).

In order to depict a general spatial pattern, we merged the nine DGGE matrixes that were used to describe the spatial variations during each cruise. This approach minimizes the occurrence of sporadic ribotypes (unique bands represented from 0% to 13% within the entire dataset), allowing the comparison among stations together with their temporal evolution, as every matrix represents a temporal frame of the

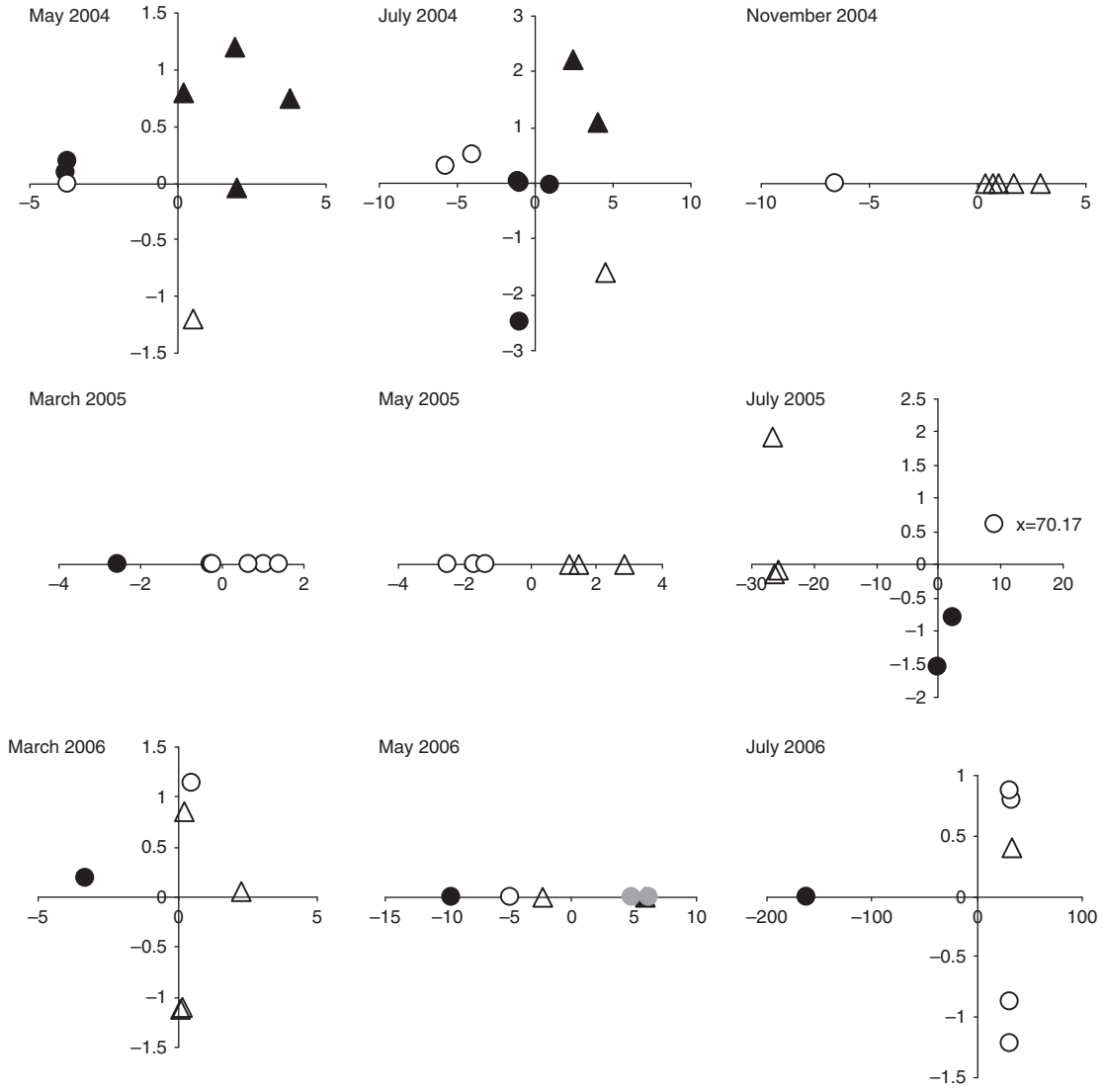

Fig. 6. Diagrams of the first and the second linear discriminants (LD1 vs. LD2) obtained from the microbial activities data for each DGGE presented in Fig. 3. Coefficients of linear discriminants and proportion of traces for each diagram are reported in Table 1. Different symbols in the LDA plots indicate samples from different subclusters in Fig. 2.

surface bacterial assemblages' distribution (Fig. 4). The MDS derived from the new matrix separated the offshore stations (grouped together in the centre of the diagram) from the coastal ones. The stations' plotting in the MDS resembled their geographical location with the coastal sites being separated along three different gradients, corresponding to the three transects. Socal *et al.* (2008) have previously used a similar approach using thermohaline data on a broader period (2003–2006) including our time interval (fig. 11 in Socal *et al.*, 2008). In their analysis, the coastal stations C1 and E1 are primarily separated from the others because of reduced salinity values, a second cluster comprises stations that sometimes are influenced by riverine plumes (C10, C13 and E6) and a third group is made up of typically offshore sites (E10 in our dataset; transect A is not considered). Our general scheme did not strictly mirror the hydrological clustering, although river discharges seem to have a major influence on bacterial assemblages' structure. Other factors such as top-down control or competition for resources may have played an important role, as depicted by the differences between patterns in Fig. 2 and the MDS in Fig. 4.

A different approach, following the one proposed by Hewson *et al.* (2006), took into account the comparison between the pairwise similarity index among samples and the spatial distance among stations (applied to each sampling period). No relationship was found between similarity and distance during summer, as similarities did not decrease

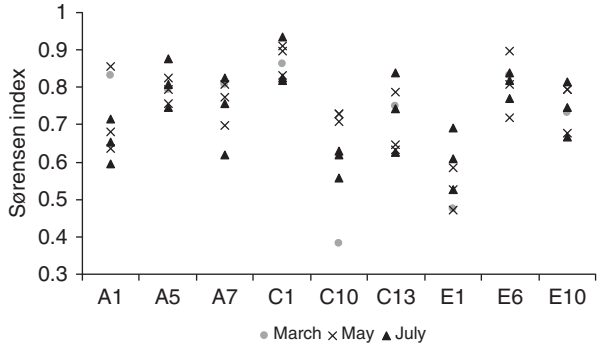

Fig. 7. DGGE-based assemblage similarity (Sørensen Index) comparisons of samples collected at each station, during the same month of different years. Grey circles indicate the pairwise index values between March 2005 and March 2006; crosses indicate the pairwise index values between May 2004 and May 2005, May 2005 and May 2006 and May 2004 and May 2006; full triangles indicate the pairwise index values between July 2004 and July 2005, July 2005 and July 2006 and July 2004 and July 2006.

for increasingly distant stations. An opposite situation occurred in May, when a negative correlation (Spearman coefficient) was always found between pairwise similarities between bacterial assemblages and the distance between stations (2004, $\rho = -0.42$ $P < 0.01$; 2005, $\rho = -0.42$, $P < 0.01$; 2006, $\rho = -0.55$, $P < 0.001$; $n = 36$). During the other periods, divergent results were obtained, with weakly significant correlations ($P < 0.05$) occurring only in November 2004 and March 2005. If we consider that the higher concentration of close stations is present in the centre of the basin, we can hypothesize that when the correlation is significant (May), an increasing diversification is in place while approaching toward the eastern and the northern coast of the basin in a radial-like scheme. In other words, because May is generally the month in which higher riverine discharges are recorded (Socal *et al.*, 2008), very diverse coastal/estuarine bacterial assemblages tend to mix while approaching the centre of the basin, increasing the level of similarity between samples. The completely different situation that emerged from the summer samples indicates that these months are characterized by a more pronounced patchiness, likely due to other factors such as local selective predation/infection and/or resources' availability.

Links between assemblage structure and patterns of microbial activities

We analysed BGLU, AMA and APA because these activities are primarily responsible for the degradation of polysaccharides, polymeric amino acids and phosphorylated molecules in the oceans, together with leucine uptake rates as a means of prokaryotic biomass production. In order to establish whether the given assemblages did perform the same patterns of activities, we analysed the activity data set through an LDA. The different similarity values obtained by the clusters in Fig. 2 did not allow us to select a unique threshold similarity for the discrimination of subclusters, and for this reason, we applied the multivariate analysis in order to have from two to five subgroups. Also, transect A was not considered in the analysis as no activity measurement was performed in this area. What emerged from the LDA plot (Fig. 6) is that the different assemblages determined by the cluster analysis formed rather separate clouds of points in the LD1 vs. LD2 diagrams, meaning that each community expressed (to some extent) peculiar schemes of C degradation/utilization, even when similar assemblages were spatially distant. These results support the idea that bacteria affiliated to different phylogenetic groups can express different patterns of activity (Martinez *et al.*, 1996) and thus similar assemblages have analogous metabolic requirements that are reflected in their degradation and production processes (Pinhassi *et al.*, 1999). Such a link has been found previously in very diverse environments (e.g. Pinhassi *et al.*, 1999; Boucher & Deboras, 2009; Celussi *et al.*, 2009a), although it does not represent an absolute dogma. In the close Lagoon of Venice, in fact, Celussi *et al.* (2009b) by considering an extensive data set of exoenzymatic activities together with leucine and thymidine incorporation, did not find any relationship between bacterial community structure and patterns of degradation/production, and attributed the uncoupling to the high heterogeneity of the environment, which affects the nutrient composition on short spatial and temporal time scales and thus the microbial response. In the Northern Adriatic, the structure–activity coupling detected in the present study would be the result of concomitant factors, from physical through chemical to biological. The general (although not strict) importance of terrestrial influence on bacterial composition can in fact rely on the type of allochtonous nutrients transferred to the marine systems by rivers and bacterial response, namely their metabolism, is consequently controlled by these substrates (Caruso, 2010). The local features (possibly organisms' interaction, viral infections, substrate patchiness, etc.) that generated the 'anomalies' (Figs 2 and 3) from the general scheme (Fig. 4) would thus similarly act as a control on microbial activities, mediated by assemblages' composition.

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