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Bacterial community composition and colored dissolved organic matter in a coastal upwelling ecosystem

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ABSTRACT: The aim of the present study was to correlate changes in dissolved organic matter (DOM) composition, as characterized through humic- and protein-like fluorescence, with changes in the abundance of major bacterial groups (Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria and Bacteroidetes) and bacterial activity in an upwelling system during the season of low productivity. Sampling was conducted under 2 contrasting periods characterized by low (February) and high (October) precipitation. In October, the mean humic-like DOM fluorescence in surface waters (3.1 ppb equivalents of quinine sulphate [ppb QS]) was higher than the annual average for this coastal zone (2.2 ppb QS), which was attributed to enhanced continental runoff. Alphaproteobacteria and Bacteroidetes were the most abundant groups, accounting for about 13 and 16% of total bacterial abundance, respectively. Betaproteobacteria were detectable only during the rainy period, accounting for 2 to 9% of total bacterial abundance. The bulk dissolved organic carbon concentration similarly explained the relative abundance of Alphaproteobacteria, Betaproteobacteria and Gammaproteobacteria (ca. 50% of total variability). By contrast, a strong correlation was found between the humic-like DOM fluorescence and the relative abundance of the Betaproteobacteria group, explaining 68% of the total variability. Multivariate linear regression analyses revealed that the relative abundance of Betaproteobacteria has the greatest influence on bacterial carbon fluxes, explaining 61 and 65% of bulk bacterial activity and biomass variability, respectively. Despite their relatively low abundance, Betaproteobacteria might play a relevant biogeochemical role in this coastal transition ecosystem during the low productivity period as allochthonous DOM consumers.

KEY WORDS: Colored dissolved organic matter · Bacterial community composition · Bacterial production · Northwest Spain

INTRODUCTION

The importance of dissolved organic matter (DOM) for carbon and nutrient fluxes is enhanced in coastal waters where river inputs, together with an intense physical and biological activity, contribute to the accumulation of large amounts of DOM from both marine and terrestrial origin (Cauwet 2002). Paradoxically, despite terrestrial DOM having been traditionally classified as refractory, a relatively large fraction appears to be consumed by heterotrophic bacteria (Moran & Hodson 1994, McCallister et al. 2004). The bioavailability of colored DOM is enhanced upon sunlight exposure (Obernosterer & Benner 2004, Nieto-Cid et al. 2006); nevertheless, the potential role of photoproducts as bacterial carbon substrates remains poorly studied.

Addressing the links between diversity and functions of natural bacterial communities is essential to understand their roles in biogeochemical cycles. To date, little information exists on the role that particular bacterial taxa play in DOM decomposition in marine planktonic systems (Kirchman 2004). Culture-independent molecular tools, such as the combination of

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microautoradiography and fluorescence *in situ* hybridization (FISH) (e.g. Cottrell & Kirchman 2000, Elifantz et al. 2005, Malmstrom et al. 2005, Alonso-Sáez & Gasol 2007), have been used to unravel the links between bacterial function and diversity in a wide variety of marine pelagic environments. These types of studies have revealed systematic differences in the uptake patterns of specific organic compounds among major phylogenetic groups. Alphaproteobacteria seem to mostly contribute to low molecular weight (LMW) DOM utilization (glucose, amino acids, DMSP), whereas Bacteroidetes appear to be proficient in the use of proteins and other plankton-derived high molecular weight (HMW) compounds. Several studies in lakes and estuaries have also reported changes in bacterial community structure and function associated to shifts in terrestrial organic matter influx. By comparison, little is known about the patterns of utilization of allochthonous carbon sources, such as those derived from continental runoff, in marine environments. A recent study by Kisand et al. (2008) suggests that bacterial decomposition of riverine humic-rich DOM might be significantly higher in marine than in estuarine waters.

The northwest Iberian coastal transition zone is an area affected by a marked seasonal cycle of coastal winds, which divides the annual cycle in an upwelling season (March to September) with short relaxation intervals that enhance productivity, and a downwelling season (from October to March), characterized by low phytoplankton biomass and primary production (Álvarez-Salgado et al. 2003). Continental runoff is most intense during this low productivity period, potentially transporting large amounts of allochthonous carbon into this coastal zone.

Fluorescence has proven to be a simple, sensible and quick tool for characterizing DOM in marine systems (Nieto-Cid et al. 2005). There are 2 main classes of DOM that may be quantified through fluorescence analysis: (1) the labile protein-like material and (2) the refractory humic-like substances from both terrestrial and marine origin. Nieto-Cid et al. (2005) reported seasonal variations in DOM fluorescence in shelf waters off northwest Spain and attributed humic-like DOM fluorescence maxima in surface waters to enhanced continental runoff.

Within this context, the aim of the present study was to correlate changes in DOM composition, as characterized by means of fluorescence, with changes in the relative abundance of major bacterial groups (Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria and Bacteroidetes [also known as Cytophaga-Flavobacteria-Bacteroides, or CFB]) and bacterial production and biomass in the coastal transition zone of the northwest Iberian peninsula during the low productivity phase. Sampling was conducted during 2 contrasting periods characterized by low (February) and high (October) continental runoff.

MATERIALS AND METHODS

Sampling area. The coastal transition zone of the northwest Iberian Peninsula was sampled during 2 cruises conducted from 7 to 14 February and 23 to 30 October 2005 on board RV 'Cornide de Saavedra'. The sampling stations are presented in Fig. 1. In February, we sampled along a latitudinal transect centered at 41.92° N, close to the mouth of the River Miño. We did not complete this transect in front of the River Miño during October due to severe weather conditions. A total of 5 to 6 stations were occupied during each cruise. Temperature and salinity profiles were obtained with a SBE9/11 CTD device incorporated into a rosette sampler equipped with 24 Niskin bottles (12 l volume). CTD temperature and salinity sensors were calibrated at the factory.

At each station, we took samples at 5 depths within the euphotic zone (depth reached by 1% of surface irradiance) to measure chlorophyll *a* (chl *a*) concentration, bacterial abundance and bacterial production, and to study the taxonomic composition of the bacterial community using FISH techniques. Samples for chemical analysis (nutrients, dissolved organic carbon [DOC] concentration and DOM fluorescence) were also sampled at each station and depth, including 1 to 2 additional depths below the euphotic zone.

Dissolved inorganic nutrients. Collected water samples were transferred into 50 ml polyethylene flasks directly from the Niskin bottles and kept frozen (–20°C) until concentrations of ammonium, nitrite, nitrate, phosphate and silicate were determined by means of standard segmented flow analysis with standard colorimetric methods (Grashoff 1983).

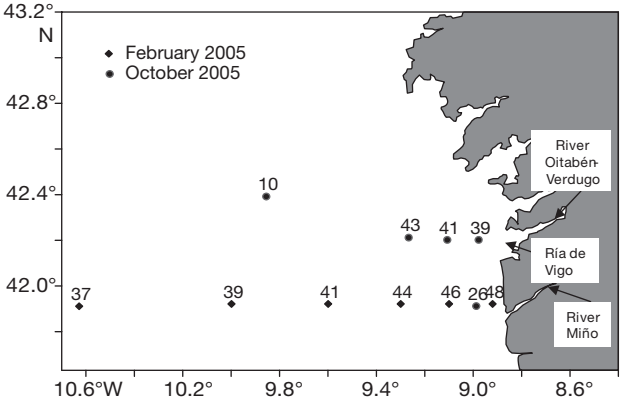

Fig. 1. Study area showing locations of the stations sampled during the cruises conducted in February and October 2005

Chl *a* concentration. Chl *a* was measured fluorimetrically after concentrating particulate matter by filtering 250 ml of seawater trough 0.2 µm polycarbonate filters. The filters were immediately frozen and kept at –20°C until further analysis onshore. In the laboratory, chl *a* was extracted by adding 5 ml of 90% acetone to the filters. Extraction was done at 4°C overnight. Chl *a* fluorescence was determined with a TD700 fluorometer that was calibrated with pure chl *a* (Sigma).

DOC concentration. Samples for DOM were collected in 500 ml acid-cleaned flasks and filtered through precombusted (450°C, 4 h) 47 mm Ø GF/F filters (Whatman) in an acid-cleaned glass filtration system under low N_2 flow pressure. Aliquots for the analysis of DOC were collected into 10 ml precombusted (450°C, 12 h) glass ampoules. After acidification with H_3PO_4 to pH <2, the ampoules were heat-sealed and stored in the dark at 4°C until analysis. DOC was measured with a TOC-5000 organic carbon analyzer (Shimadzu) as described in Nieto-Cid et al. (2005). The system was standardized daily with potassium hydrogen phthalate. The concentrations of DOC were determined by subtracting the average peak area from the instrument blank area and dividing by the slope of the standard curve. The precision of measurement was ±0.7 µmol C l^{-1}. The accuracy was tested daily with reference materials provided by D. Hansell (University of Miami).

DOM fluorescence. Samples for fluorescence of DOM (FDOM) determination were filtered through precombusted (450°C, 4 h) 47 mm Ø GF/F filters (Whatman) in an acid-cleaned glass filtration system under low N_2 flow pressure and preserved at 4°C until determination with a LS 55 luminiscence spectrometer (Perkin Elmer) within 1 h of sample collection. The instrument was equipped with a xenon discharge lamp, equivalent to 20 kW for an 8 µs duration, and a 1 cm quartz fluorescence cell. Milli-Q water was used as a reference for fluorescence analyses, and the intensity of the Raman peak was checked regularly. Discrete excitation/emission (Ex/Em) pair measurements were performed at peaks M (marine humic substances, Ex/Em 320/410 nm; $FDOM_M$) and T (aromatic amino acids, Ex/Em 280/350 nm; $FDOM_T$). Basically we determined the fluorescence at the wavelengths proposed by Coble et al. (1990) and confirmed by Nieto-Cid et al. (2005) in the Iberian upwelling system. Four replicate measurements were performed for each Ex/Em pair. A 4-point standard curve was prepared daily with a mixed standard of quinine sulphate (QS) and tryptophan (Trp) in 0.05 M sulphuric acid (Nieto-Cid et al. 2005). The equivalent concentration of every peak was determined by subtracting the average peak height from the blank height and dividing by the slope of the standard curve. Fluorescence units were expressed in ppb equivalents of quinine sulphate (ppb QS) for $FDOM_M$ and ppb equivalents of Trp (ppb Trp) for $FDOM_T$. The precision was ±0.1 ppb QS and ±0.6 ppb Trp. The ratio (±SE) between DOC and $FDOM_M$, after several replicate measurements at different concentrations of a commercial fulvic acid, was 2.67 ± 0.06 µM C (ppb QS)$^{-1}$ (Nieto-Cid et al. 2005).

Bacterial abundance and assemblage composition. Immediately after collecting the samples from the Niskin bottles, water samples of 5 to 10 ml were fixed by adding 0.2-µm-filtered paraformaldehyde (2% final conc.) and were stored at 4°C in the dark for 4 to 12 h. Thereafter, the sample was filtered through a 0.2 µm polycarbonate filter (Millipore, GTTP, 25 mm diameter) supported by a cellulose nitrate filter (Millipore, HAWP, 0.45 µm), washed twice with Milli-Q water, dried and stored in a microfuge vial at –20°C until further processing in the home laboratory at Universidade de Vigo.

Filters for catalyzed reporter deposition FISH (CARD-FISH) were embedded in low-gelling-point agarose and incubated with lysozyme. Filters were cut in sections and hybridized with horseradish peroxidase (HRP)-labeled oligonucleotide probes (Eub338 for bacteria, Alf968 for Alphaproteobacteria, Gam42a for Gammaproteobacteria, Bet42a for Betaproteobacteria, CF319a for Bacteroidetes and NON-Eub338 as a negative control) and tyramide-Alexa488 for signal amplification following the protocol described in Teira et al. (2008). It is important to mention that the FISH probe used for Bacteroidetes (CF319a) targets mainly the class Flavobacteria (formerly known as order Cytophagales), which comprises the most abundant class of planktonic marine Bacteroidetes (Alonso et al. 2007). Cells were counter-stained with a DAPI-mix (5.5 parts of Citifluor [Citifluor], 1 part of Vectashield [Vector Laboratories] and 0.5 parts of phosphate buffered saline [PBS] with DAPI [final concentration 1 µg ml^{-1}]).

The slides were examined under a microscope (Leika) equipped with a 100 W mercury lamp and appropriate filter sets for DAPI and Alexa488. More than 800 DAPI-stained cells were counted per sample. For each microscope field, 2 different categories were enumerated: (1) total DAPI-stained cells and (2) cells stained with the specific probe. Negative control counts (hybridization with HRP-NON-Eub338) were always below 0.5% of DAPI-stained cells. The mean counting error, expressed as the percentage of SE between replicates, was <2% for DAPI counts and <9% for FISH counts. Bacterial biomass was estimated using a conversion factor of 12 fg C $cell^{-1}$ (Fukuda et al. 1998).

Bacterial production. Bulk prokaryotic activity was measured by incubating 5 to 10 ml of water in duplicate and one formaldehyde-killed blank with 40 nmol

l^{-1} [^{3}H]-leucine (final conc., specific activities of 595.7 and 558.7 GBq $mmol^{-1}$, Amersham) in the dark at *in situ* (±1°C) temperature for 1 to 1.5 h. Thereafter, the incubation was terminated by adding formaldehyde (2% final conc.) to the samples and filtering them through 0.2 µm cellulose nitrate filters (Millipore, 25 mm diameter). Subsequently, the filters were rinsed 3 times with 5% ice-cold trichloroacetic acid, placed in scintillation vials and stored at –20°C until they were counted in a liquid scintillation counter. The disintegrations per minute (dpm) of the formaldehyde-fixed blank were subtracted from the samples and the resulting dpm converted into leucine incorporation rates. Two previous experiments were conducted, one with coastal water and one with oceanic water, to determine the saturation leucine concentration. A conversion factor to convert leucine to carbon was also determined experimentally once during each cruise with water from a shelf station (Stn 46 in February and Stn 26 in October). The derived conversion factors were 2.34 and 2.03 kg C mol leucine^{-1}, in February and October, respectively.

Meteorological variables. Daily Ekman transport values (–Qx, $m^3\ s^{-1}\ km^{-1}$) were calculated as described by Nieto-Cid et al. (2005). Precipitation data (mm) were obtained from the hydrological bulletin of the Spanish Ministerio de Medio Ambiente. The River Oitabén-Verdugo, the main tributary to the Ría de Vigo, is a combination of regulated and natural flows. The flow from the Eiras Reservoir was provided by the company in charge of the management of urban waters (Seraguas S.A.).

Statistical analysis. Variables that did not comply with normality were logarithmically transformed. An arcsine transformation was applied to percentages. The Pearson coefficient was used to analyze correlations between bacterial community composition and both environmental and biological variables. Multivariate linear regression analysis was conducted to explore the relationship between bacterial community structure and both bacterial production and biomass.

RESULTS

Meteorological conditions and hydrography

The February cruise took place under upwelling-favorable (northerly) winds (–Qx = 379 $m^3\ s^{-1}\ km^{-1}$, Table 1), whereas the October cruise took place during downwelling conditions (–Qx = –2036 $m^3\ s^{-1}\ km^{-1}$, Table 1). There was a period of relaxed northerly winds during the 10 d previous to the October cruise. Rainfall was abnormally low, almost nil, during the February cruise, but was extremely high in October (Table 1), about twice the historical mean for the region, which implies enhanced continental runoff. The measured flow from the River Oitabén-Verdugo was, on average, 5 $m^3\ s^{-1}$ during the February cruise and 28 $m^3\ s^{-1}$ during the October cruise (Table 1). In addition, the mean river flow during the 10 d previous to the October cruise was as high as 42.6 $m^3\ s^{-1}$, considerably higher than the annual mean of 15.9 $m^3\ s^{-1}$. The meteorological conditions were reflected in the hydrography of the region during each sampling period. In February, the water column was well mixed with temperatures averaging 13°C (Fig. 2A,C, Table 1) and salinities averaging 35.81. The prevailing northerly winds are responsible for the upwelling of subpolar Eastern North Atlantic Central Water (ENACW, salinity 35.69, temperature 12.3°C) over the shelf (Fig. 2A,C). By contrast, in October, an upper mixed layer of 20 to 60 m, with a mean temperature of 16.9°C and a mean salinity of 35.77, was observed. Below the thermocline the temperature decreased to 13.5°C and the salinity increased to 35.9 (Fig. 2B,D). This salinity gradient can be only explained by freshwater inputs. Strong rainfall before and during the October cruise together with the previous period of relaxed northerly winds, which favor positive Ekman transport, supports the freshwater origin of this low-salinity surface water layer.

Table 1. Summary of meteorological and hydrological conditions during the 2 sampling periods, February and October 2005

Variable	February 2005	October 2005
–Qx ($m^3\ s^{-1}\ km^{-1}$)	379	–2036
Precipitation (mm)	0.4	105.0
River Oitabén flow ($m^3\ s^{-1}$)	5.0	28
Mixed layer (ML) (m)	40–140	20–60
ML temperature (°C)	13.0	16.9
ML salinity	35.81	35.77

Vertical distribution of nutrients, chl *a*, DOC and FDOM

The vertical distribution of nutrient salts (Fig. 3) reflected the structure of the water column. The nitrate, phosphate and silicate concentrations in the mixed layer were significantly lower in October than in February (*t*-test, $p < 0.001$). There were no significant differences in the ammonium concentration between both sampling periods.

The vertical profiles of DOC were distinct between the 2 sampling periods (Fig. 4A,B). Significantly higher values (*t*-test, $p < 0.001$) and higher variability were recorded in October compared with February. The same pattern was observed in the vertical profiles of

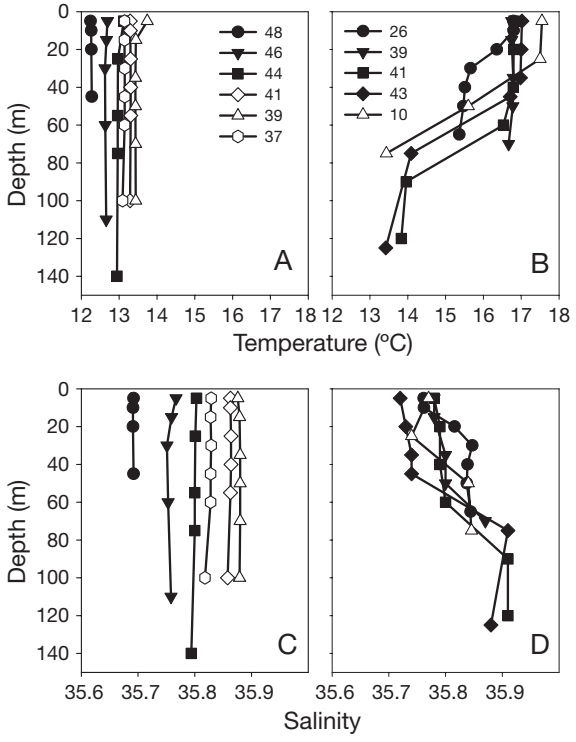

Fig. 2. Vertical distribution of (A,B) temperature and (C,D) salinity in (A,C) February 2005 and (B,D) October 2005. Black and open symbols represent shelf and offshore stations, respectively. Legend in graph A also applies for graph C. Legend in graph B also applies for graph D

$FDOM_M$ and $FDOM_T$ (Fig. 4E–H). In February, the inner shelf stations showed a relatively high $FDOM_M$ signal associated with a low $FDOM_T$ signal, which is characteristic of nutrient-rich, DOC-poor ENACW. In October, the mean $FDOM_M$ showed an increase with depth, while DOC and $FDOM_T$ decreased with depth. Maximum variability of these characteristics occurred in the upper 60 m (Fig. 4B,F,H). Highest $FDOM_M$ values were observed at Stn 26, whereas highest $FDOM_T$ values were found at Stn 41. Mean chl *a* concentration did not significantly differ between the 2 sampling periods (Fig. 4C,D).

Vertical distribution of bacterial biomass and production

Both bacterial biomass and production were significantly higher in October than in February (*t*-test, $p < 0.001$) (Fig. 5). In October, a great variability was observed in the upper 60 m for both variables, which showed higher values at the shelf than at the offshore station. Maximum biomass and production was measured at Stn 26.

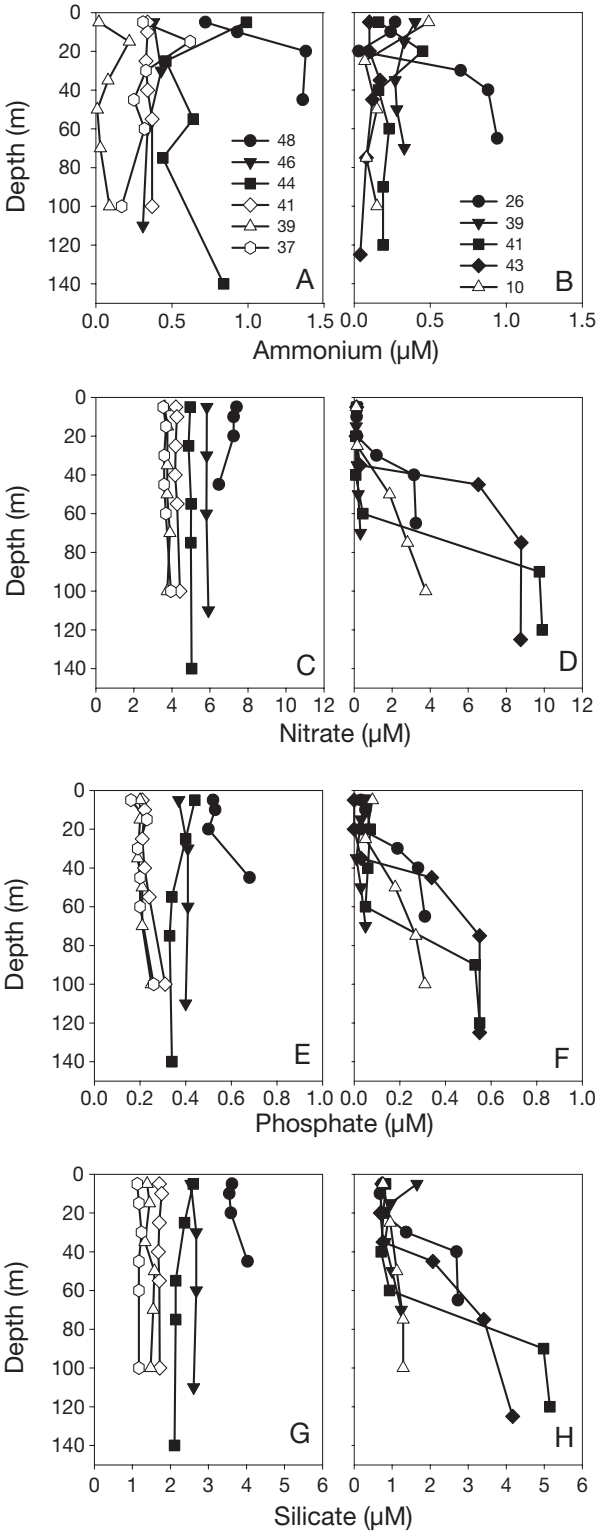

Fig. 3. Vertical distribution of (A,B) ammonium (C,D) nitrate, (E,F) phosphate and (G,H) silicate in (A,C,E,G) February 2005 and (B,D,F,H) October 2005. Black and open symbols represent shelf and offshore stations, respectively. Legend in graph A also applies for graphs C, E and G. Legend in graph B also applies for graphs D, F and H

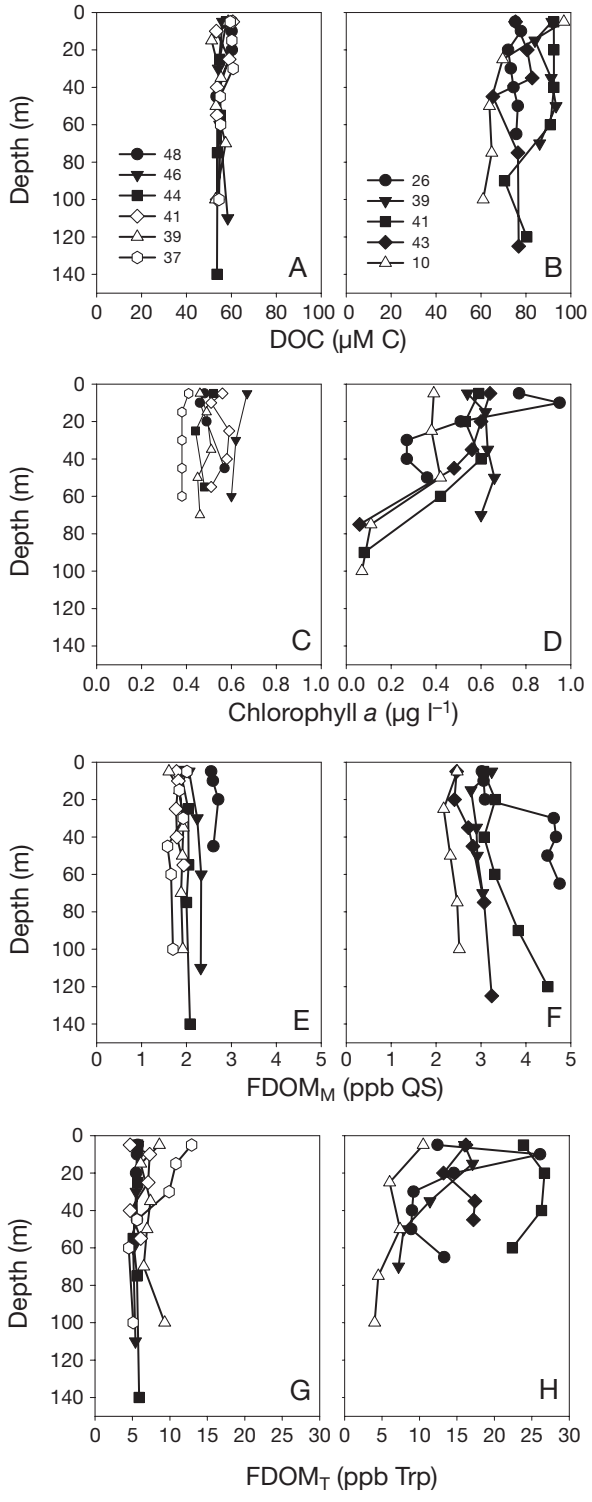

Fig. 4. Vertical distribution of (A,B) DOC, (C,D) chl *a*, (E,F) humic-like DOM fluorescence ($FDOM_M$) and (G,H) protein-like DOM fluorescence ($FDOM_T$) in (A,C,E,G) February 2005 (and (B,D,F,H) October 2005. Black and open symbols represent shelf and offshore stations, respectively. Legend in graph A also applies for graphs C, E and G. Legend in graph B also applies for graphs D, F and H. QS: quinine sulphate; Trp: tryptophan

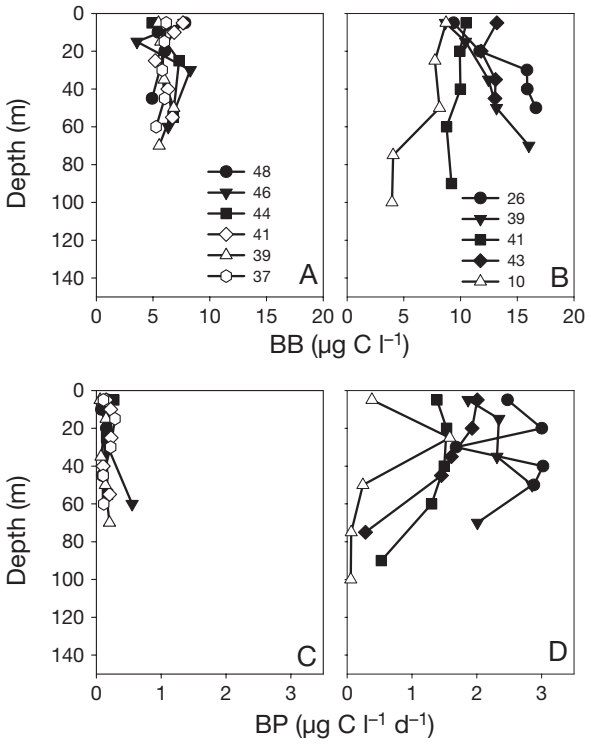

Fig. 5. Vertical distribution of (A,B) bacterial biomass (BB) and (C,D) bacterial production (BP) in (A,C) February 2005 and (B,D) October 2005. Black and open symbols represent shelf and offshore stations, respectively. Legend in graph A also applies for graph C. Legend in graph B also applies for graph D

Vertical distribution of major bacterial groups

On average, the percentage of cells that hybridized with the Eub338 probe (referred to as bacteria) was 63, and this proportion was slightly higher in October (68%) than in February (58%). The total percentage of bacteria recovered by the 4 selected probes was, on average, 41%, and was higher in October (49%) than in February (33%). The most abundant groups in the area were Alphaproteobacteria and Bacteroidetes (Fig. 6A,B,G,H), which accounted, on average, for 8.5 and 10.5% of total DAPI-stained cells (13.5 and 16.5% of total bacterial abundance), respectively. The least abundant groups, Gammaproteobacteria and Betaproteobacteria (Fig. 6C–F), accounted for ~4 and ~1% of total DAPI counts (~7 and ~2% of total bacterial abundance), respectively. The relative abundance of Bacteroidetes did not differ significantly between sampling periods (*t*-test, $p > 0.05$), whereas the relative abundance of Alphaproteobacteria was significantly higher in October (11% of DAPI counts) than in February (6% of DAPI counts) (*t*-test, $p > 0.01$). The relative abundance of Betaproteobacteria (0.2% of DAPI counts) in February (Fig. 6C) was below the detection

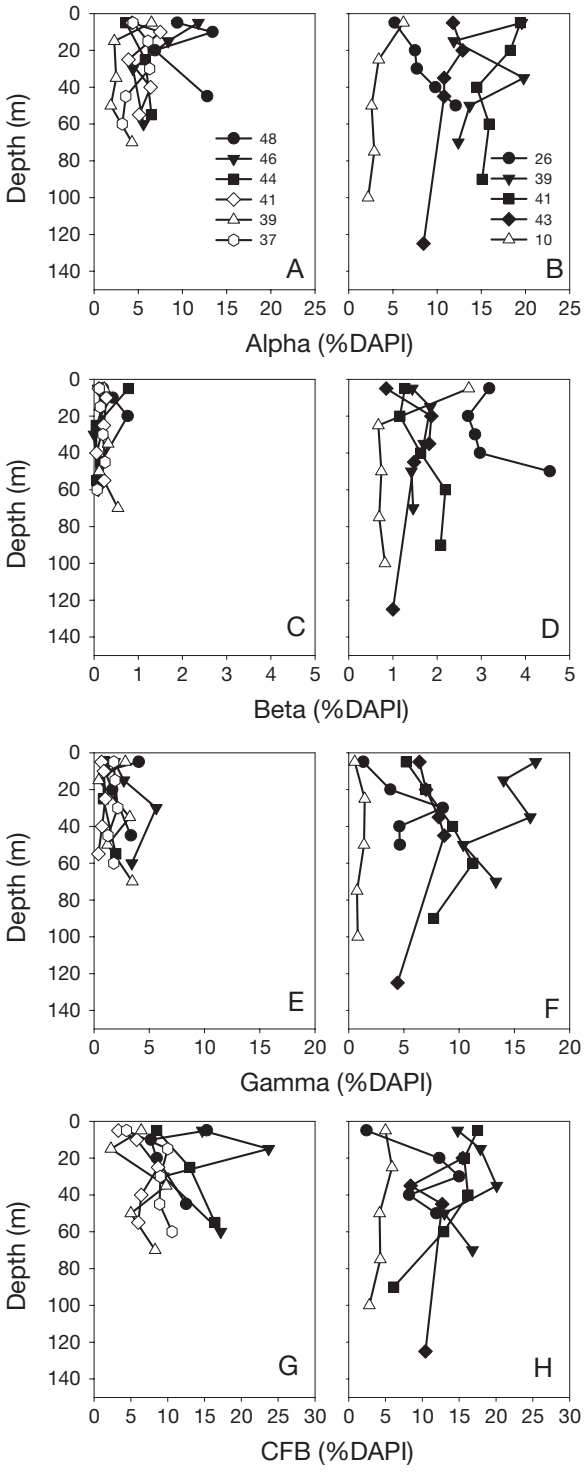

Fig. 6. Vertical distribution of the relative abundance (expressed as percentage of DAPI-stained cells) of (A,B) Alphaproteobacteria (Alpha), (C,D) Betaproteobacteria (Beta), (E,F) Gammaproteobacteria (Gamma) and (G,H) Bacteroidetes (CFB) in (A,C,E,G) February 2005 and (B,D,F,H) October 2005. Black and open symbols represent shelf and offshore stations, respectively. Legend in graph A also applies for graphs C, E and G. Legend in graph B also applies for graphs D, F and H

limit (0.5% of DAPI counts). By contrast, the relative abundance of Betaproteobacteria ranged from 0.7 to 4.6% of DAPI counts (2.1 to 8.6% of total bacterial abundance) in October. The highest relative abundance of Betaproteobacteria occurred at Stn 26. The relative abundance of Gammaproteobacteria was also significantly higher in October (10.5% of total bacterial abundance) than in February (3.4% of total bacterial abundance).

Relationship between major bacterial groups, chemical variables and bulk bacterial biomass and production

Using the entire dataset, we conducted a correlation analysis to identify any potentially relevant chemical variable explaining the observed variability in the abundance of major bacterial groups (Table 2). None of the groups showed a significant correlation with ammonium or silicate. Both Betaproteobacteria and Gammaproteobacteria showed negative correlations with nitrate and phosphate. Only the Bacteroidetes groups correlated positively with chl *a*. All the groups correlated positively with DOC, which similarly explained ca. 50% of the observed variability in the relative abundance of Alphaproteobacteria, Betaproteobacteria and Gammaproteobacteria, and only 18% in the case of Bacteroidetes. All the groups showed positive correlations with both $FDOM_M$ and $FDOM_T$, although the percentage of their respective relative abundances explained by FDOM was variable. $FDOM_M$ explained only 11 to 32% of the variability in Alphaproteobacteria, Gammaproteobacteria and Bacteroidetes, but as much as 68% in Betaproteobacteria. $FDOM_T$ explained 44% of the variability in the relative abundance of Alphaproteobacteria. All the groups showed positive correlations with both bacterial biomass and bacterial production (Table 2). We conducted a multivariate linear regression analysis using the bulk bacterial production or bacterial biomass as dependent variables and the abundance of the 4 major bacterial groups as independent variables to explore the relationship between bacterial community structure and bacterial carbon fluxes (Table 3). Betaproteobacteria has the greatest influence on bacterial carbon fluxes, explaining 61 and 65% of bulk bacterial activity and biomass variability, respectively.

DISCUSSION

Over the last 2 decades, growing efforts have been devoted to the analysis of DOM utilization by specific prokaryotic groups following a variety of methodolo-

Table 2. Pearson correlation coefficients between the abundance of major bacterial groups (expressed as % of DAPI-stained cells) and several chemical and biological variables. $FDOM_M$, fluorescence at peak marine humic substances (M); $FDOM_T$, fluorescence at peak aromatic amino acids (T); Chl *a*, chlorophyll *a* concentration; DOC, dissolved organic carbon concentration; BP, bacterial production; BB, bacterial biomass; Alpha, Alphaproteobacteria, Beta, Betaproteobacteria; Gamma, Gammaproteobacteria; and CFB, Bacteroidetes. Numbers in **bold** represent correlations (r values) higher than 0.6; ***p < 0.001, **p < 0.01, *p < 0.05; ns: not significant

N = 47–50	NH_4	NO_3	PO_4	SiO_4	Chl *a*	DOC	$FDOM_M$	$FDOM_T$	BP	BB
Alpha	ns	ns	ns	ns	ns	**0.713*** **	0.567***	**0.667*** **	0.600***	0.520***
Beta	ns	−0.506***	−0.423**	ns	ns	**0.703*** **	**0.825*** **	0.433***	**0.758*** **	**0.754*** **
Gamma	ns	−0.427**	−0.399**	ns	ns	**0.714*** **	0.525***	0.554**	**0.686*** **	**0.618*** **
CFB	ns	ns	ns	ns	0.386***	0.428**	0.333*	0.419**	0.428**	0.330*

Table 3. Linear regression analysis of the contribution of the major bacterial groups to the variability in bacterial production (BP) and bacterial biomass (BB). Standardized coefficients measure the contribution by the bacterial group to explaining variation in the dependent variables. ns: not significant

N = 48	Standardized coefficients				Adjusted	
Dependent variables	Alpha	Beta	Gamma	CFB	r^2	p
BP	ns	0.614	0.318	ns	0.74	<0.001
BB	ns	0.645	0.318	ns	0.67	<0.001

gies. Most of these studies used either single compounds (e.g. leucine, glucose, N-acetylglucosamine, chitin, ATP) (Cottrell & Kirchman 2000, Malmstrom et al. 2005, Alonso-Sáez et al. 2008) or different DOM components or fractions (e.g. low and high molecular weight fractions) (Covert & Moran 2001). The use of a single compound has the constraint that it may not model a given component of the DOM. On the other hand, the different size-fractions of DOM have a variable composition (Kirchman 2004). In addition, the isolation of a given component from natural DOM has several technical difficulties (large sample volumes, 10^2 to 10^3 l) and limitations (changes in physical and chemical properties during the procedure) (Benner et al. 1992). A complementary approach comes from the combination of data on DOM composition and bacterial community structure. There are several direct chemical approaches to studying DOM composition that do not involve isolation, such as the analysis of elemental composition, the molecular characterization of DOM or fluorescence spectroscopy, which provide valuable information about the chemical nature (fluorescence functional groups) of DOM (Nieto-Cid et al. 2005).

To achieve our objective of correlating changes in DOM optical properties and bacterial community structure we studied the coastal transition zone of the northwest Iberian Peninsula during the period of low productivity (from October to March). This coastal zone is particularly interesting during this season since the sources of DOM can greatly vary (e.g. semi-labile DOM accumulated during the previous productive period, refractory DOM of terrestrial origin introduced via enhanced runoff) (Doval et al. 1997), which ensures a wide range of variation in DOM composition.

Primary production (~380 mg C m^{-2} d^{-1}) and chl *a* concentration (0.45 mg m^{-3}) were low and did not differ between the sampling periods (Teira et al. 2009). By contrast, rainfall was intense before and during the October cruise, and almost nil before and during the February cruise, and the mean flow from the River Oitabén, the main tributary to the Ría de Vigo, was 5- to 8-fold higher in October than in February (Table 1). As a consequence, a higher influence of freshwater inputs was expected in October than in February. The hydrographic data support the influence of freshwater in October. During this period there was a clear thermal stratification (Fig. 2B); therefore, the low salinity in surface waters (Fig. 2D) can only be explained by the influence of freshwater inputs. On the other hand, humic substances in the marine environment can be either of terrestrial origin or are generated *in situ* as a byproduct of microbial respiration processes. A significant inverse correlation was found between salinity and $FDOM_M$ in the mixed layer ($r = -0.55$, $p < 0.01$, $n = 44$), which supports the freshwater origin of the humic material accumulated in the mixed layer. Both marine and terrestrial humic substances are sensitive to natural UV radiation, which provokes intense photobleaching of these substances in the surface layer. Therefore, a vertical profile of humic-like fluorescence in a coastal area depends on the relative importance of continental runoff (which increases the fluorescence of surface waters), UV radiation intensity (which decreases the fluorescence of surface waters) and apparent oxygen utilisation (which increases the fluorescence of subsurface waters) (Nieto-Cid et al.

2005, 2006). Therefore, the high mean $FDOM_M$ measured in the upper layer (down to 60 m depth) in October (~3.1 ppb QS), compared with the mean annual value reported for this area (~2.2 ppb QS, Nieto-Cid et al. 2005) reinforces the hypothesis of a continental origin of surface humic-like DOM. Furthermore, the Betaproteobacteria group, which is typically abundant in freshwater environments (Methé et al. 1998, Bouvier & del Giorgio 2002, Kirchman et al. 2005), accounted for 2 to 9% of the total bacterial abundance in October, and was undetectable in February. The presence of these bacteria in marine waters has been typically regarded as an indicator of freshwater inputs. Garneau et al. (2006) also found a significant contribution of Betaproteobacteria (6% of DAPI counts) in a river-influenced offshore coastal Arctic station. The relative abundance of Betaproteobacteria in our study was not correlated to salinity, which was higher overall than 35.5, indicating an important dilution of the freshwater inputs along the coastal transition zone. Kisand et al. (2008) demonstrated that the bacterial degradation of terrestrial humic substances might preferentially occur at high salinities (in polyhaline estuarine regions and coastal adjacent seas), with only a minor fraction of riverine DOC degradation occurring during the estuarine mixing.

Bacterial community structure and CARD-FISH: methodological considerations

Both the percentage of DAPI-stained cells detected by the general bacterial probe (Eub338) and the total percentage of bacteria identified with the set of probes were remarkably low in our study. The sum of the 4 groups accounted for only 15 to 70% of total detected bacteria. A similar mean recovery rate (15 to 91%) was also obtained by Alonso-Sáez et al. (2008) in the Arctic using CARD-FISH and the same set of probes. Low detection may be attributed to both a poor coverage of the oligonucleotide probes (see details in Amann & Fuchs 2008) or to a low level of bacterial activity. The coverage of CF319a probe is 38%. This probe mainly fails to detect the class Bacteroides, the members of which are strictly anaerobic intestinal bacteria. Thus, the coverage of the probes used, as assessed by Amann & Fuchs (2008), ranges from 76 to 90% for marine bacterioplankton.

Major bacterial groups and DOM quality assessed by fluorescence

The fact that the group Bacteroidetes did not show strong correlations with DOC, $FDOM_M$ and $FDOM_T$ (Table 2) during the low productivity season agrees with the widely reported tendency of this group to proliferate during the decay of phytoplankton blooms (Alderkamp et al. 2006, Teira et al. 2008). In fact, despite the low levels of phytoplankton biomass found in the region during the downwelling period, only this group showed a significant positive correlation with chl *a*.

The bulk DOC concentrations strongly correlated with Alphaproteobacteria, Betaproteobacteria and Gammaproteobacteria, which explains a similar percentage of their variability (ca. 50%). More interesting insights come from the analysis of the relationship between bacterial community structure and the FDOM.

The group Alphaproteobacteria showed a strong positive correlation with the protein-like fluorescence ($FDOM_T$), explaining 44% of its variability. The $FDOM_T$ only explains 31% of the variability in Gammaproteobacteria and <19% of that in Betaproteobacteria and Bacteroidetes. The FDOM at peak T has been found to be a tracer of labile dissolved amino acids (Yamashita & Tanoue 2003), which are liberated mainly during phytoplankton exudation, cell lysis and zooplankton grazing (Nagata 2000). Studies combining microautoradiography and FISH (Micro-FISH), and selected radiolabeled components of DOM revealed that Alphaproteobacteria (both SAR11 and *Roseobacter*) tend to dominate the utilization of labile low molecular weight DOM (glucose and free amino acids) (Cottrell & Kirchman 2000, Malmstrom et al. 2005, Alonso-Sáez & Gasol 2007), which agrees with our results.

The strong correlation between Betaproteobacteria and $FDOM_M$ indicates the potential allochthonous nature of this group, and that they are probably living on terrestrial DOM. The fact that this group is absent in February, when the continental runoff is extremely reduced, further supports the freshwater origin of this bacterial group. DOC of freshwater origin in the coastal transition of the northwest Iberian Peninsula is probably a minor fraction of the bulk DOC, and, thus, it is not surprising that the bulk DOC similarly explains the abundance of the 3 proteobacterial groups. Pernthaler et al. (1998) showed that the abundance of Betaproteobacteria was linked to the input of allochthonous organic carbon in lakes. It has been recently demonstrated that only a few species of bacteria, belonging to Bacteroidetes and Betaproteobacteria, are important players in the decomposition of riverine DOM in the northern Baltic Sea (Kisand et al. 2002, Kisand & Wikner 2003). Subsequent studies have suggested that riverine bacterioplankton may successfully migrate to seawater and play an important biogeochemical role by decomposing allochthonous DOM

(Langenheder et al. 2004, Kisand et al. 2005). Covert & Moran (2001) used ultrafiltration to identify groups of estuarine bacteria using high- and low-molecular weight fractions of surface DOM. They found that Betaproteobacteria were only present in the HMW enrichment. In surface waters, the HMW fraction is composed mainly of labile polysaccharides (Benner et al. 1992) and refractory humic substances, proportions of which may vary depending on environmental factors. For example, polysaccharides accumulate after upwelling episodes in the northwest Iberian upwelling system (Nieto-Cid et al. 2004), whereas humic substances may accumulate in surface seawater as a result of intense freshwater discharge (Chen et al. 2002, Nieto-Cid et al. 2005). Covert & Moran (2001) speculated that high molecular weight DOM in their study was mainly composed of humic substances. Therefore, our finding of a strong positive correlation between the relative abundance of Betaproteobacteria and $FDOM_M$ (Table 3) agrees with the importance of Betaproteobacteria presence in the HMW enrichment observed by Covert & Moran (2001).

Bacterial community structure and bulk bacterial biomass and production

Although several recent studies suggest the influence of bacterial community composition, even as depicted from the abundance of major bacterial groups, on bacterial carbon cycling (Kirchman 2004, Bertilsson et al. 2007, Teira et al. 2008), the links between the distribution and abundance of bacterial taxa and ecosystem function is still poorly understood for natural microbial plankton communities. Multivariate linear regression analysis (Table 3) indicates that bacterial community structure explains a significant amount of the variation in bacterial production and biomass. It is remarkable that the most abundant groups, Alphaproteobacteria and Bacteroidetes, did not significantly explain variations in bulk bacterial activity or biomass. This is in agreement with several studies in both temperate and polar pelagic systems, which have shown that the most abundant groups are not the most active ones (Elifantz et al. 2005, Alonso-Sáez et al. 2008). Betaproteobacterial abundance was the most significant variable included in the regression models, which suggests a potentially relevant role of these bacteria in carbon cycling during the sampling period. The strong positive correlation between Betaproteobacteria and the humic-like DOM fluorescence further supports an important contribution of allochthonous DOM remineralization to carbon cycling in this coastal zone. A similar relationship between Betaproteobacterial abundance and bacterial production was also reported by Garneau et al. (2006) and Zhang et al. (2006). Grossart et al. (2008) observed that both the abundance and the specific growth of Betaproteobacteria in lakes were largely controlled by the availability of humic substances. Bano et al. (1997) estimated that bacterial growth efficiency on humic substances was as high as 22%. Unfortunately, we do not have estimates of bacterial growth efficiencies; however, an efficient use of terrestrial humic substances by Betaproteobacteria would explain bulk bacterial production and biomass that would result from the high contribution of bacterial growth.

To summarize, a strong relationship has been found in the present study between the Alphaproteobacteria and Betaproteobacteria groups and DOM composition as assessed by fluorescence analysis. Whereas the bulk DOC concentration does not provide relevant information to explain the variability in the relative abundance of different major groups, fluorescence of humic and protein-like substances provided insightful information. The humic substances explained 68% of the variability on Betaproteobacterial abundance, whereas the protein-like substances explained 44% of the variability on Alphaproteobacterial abundance. Moreover, the abundance of Betaproteobacteria was the dominant variable in the regression models for bacterial production and biomass accounting for 61 and 65%, respectively, of the total observed variability. Our observations strongly suggest, despite their relatively low abundance, that consumers of allochthonous DOM have a potentially relevant biogeochemical role in the coastal transition zone of the northwest Iberian Peninsula during the low productivity period.

Although it is difficult to extrapolate from correlation analysis to causal relationships, our data add evidence to the increasingly popular notion that members of major bacterial groups (Alphaproteobacteria, Betaprotoebacteria, Bacteroidetes) could actually represent functionally relevant units in aquatic environments.

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