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Bacterial uptake of low molecular weight organics in the subtropical Atlantic: Are major phylogenetic groups functionally different?

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Abstract

We present measurements of glucose, amino acids, and adenosine triphosphate (ATP) bacterial uptake at tracer concentrations across an oceanic gradient from the Cape Blanc upwelling to the Northeast Atlantic subtropical gyre. The bulk uptake of the compounds increased in the upwelling, with amino acids being the most actively taken up substrate (up to 20 pmol L^{-1} h^{-1}). The single-cell activity of the bacterial groups also increased in the upwelling, particularly for *Rhodobacteraceae* (up to 94% of active cells), but this group had low activity in oligotrophic waters (< 10% of active cells), which suggests it is exclusively adapted to high-nutrient conditions. The percentage of SAR11 active cells was relatively high in the upwelling area, particularly for glucose and amino acid uptake (up to 53% of active cells), which suggests that some members of this group are also adapted to nutrient-rich environments. Of the broad phylogenetic groups tested, Bacteroidetes were the least active and Alpha- and Gammaproteobacteria showed similar percentages of active cells in amino acid uptake (~ 30%). Alphaproteobacteria had the highest percent of cells involved in glucose uptake, while Gammaproteobacteria dominated ATP uptake. This general pattern was confirmed in a broader analysis that included data from contrasting marine environments, which suggests that major phylogenetic groups of bacteria participate differently in the turnover of these low-molecular-weight organics. Our results support the view that broad phylogenetic groups can be identified within the bacterial 'black box' with different patterns in the cycling of organic matter. Analyzing them may help us understand, and ultimately predict, oceanic carbon processing.

The role diversity plays on ecosystem functioning is a major topic of debate that has recently been extended into the microbial world. The development of new approaches such as 'omics' technologies has become crucial for our understanding of the diversity, spatio-temporal distribution, and functional capabilities of marine microorganisms (Venter et al. 2004). Such advances are also importantly contributing to the identification of microbial functional groups and will possibly allow moving toward a trait-based microbial ecology in the near future (Green et al. 2008; i.e., the study of how microbial functional traits vary over spatio-temporal gradients and define the ecological niches of different taxa). Within marine planktonic communities, phytoplankton have been widely used as model systems in ecological studies, and various functional groups have shown different effects on biogeochemical fluxes (Legendre and Le Fèvre 1995). In the case of bacterioplankton, our knowledge about how functional traits vary among different bacterial taxa is still scarce despite their pivotal role on marine biogeochemical cycles.

Global dispersion of species and high functional redundancy among bacteria have been prevalent ideas supporting the view that bacterial community structure does not significantly affect the functioning of the ecosystem (Fenchel and Finlay 2004; Allison and Martiny 2008). However, changes in marine bacterial community structure have been detected even at a broad phylogenetic level across the depth vertical profile (DeLong et al. 2006), latitudinal (Wietz et al. 2010) and productivity (Alonso-Sáez et al. 2007) gradients, or through seasonal cycles (Mary et al. 2006*a*). Remarkably, significant changes in functional-genes abundance (DeLong et al. 2006), enzymatic activities (Pinhassi et al. 1999; Arnosti 2011), and general bacterial carbon-cycling parameters (Fuhrman et al. 2006; Alonso-Sáez et al. 2007) have also been detected associated with changes in community composition, which suggests that bacterial community structure may influence the cycling of organic matter.

Most relevant members of marine bacteria and archaea are extremely difficult to obtain in culture; therefore, the combination of single-cell activity techniques (such as microautoradiography or cell sorting) with phylogenetic analyses has proved a successful approach for studying how different groups participate in the turnover of organic and inorganic compounds (Cottrell and Kirchman 2000; Mary et al. 2006*b*). Differential in situ uptake of organic substrates by distinct bacterial groups has been observed by microautoradiography combined with fluorescence in situ hybridization (MAR–FISH [Cottrell and Kirchman 2000; Elifantz et al. 2005; Alonso-Sáez and Gasol 2007]), which reinforces the view that the composition of bacterial communities is relevant in order to understand the turnover of organic matter. However, most of the data obtained with this approach were from coastal environments and, thus, our knowledge of the single-cell activity of bacteria inhabiting the largest part of the ocean (i.e., oligotrophic oceanic waters) is still very limited.

Here, we provide measurements of the single-cell activity of major bacterial groups across a productivity gradient in the Subtropical Northeast Atlantic, including two extreme

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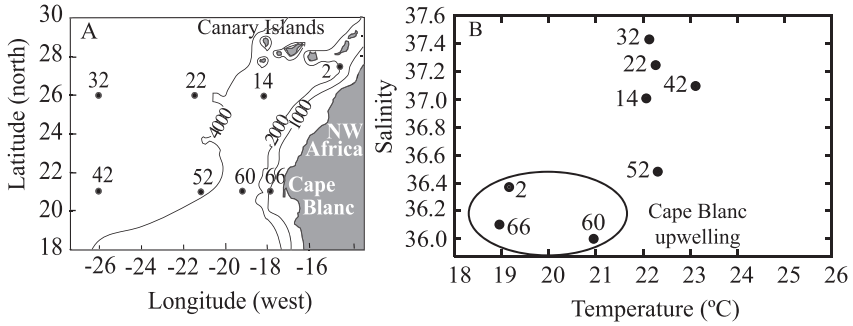

Fig. 1. (A) Map of the sampling area indicating the station numbers and location, and (B) temperature-salinity plot of surface waters. The stations that were located in the Cape Blanc upwelling area appear surrounded by a circle.

conditions: the nutrient-poor North Atlantic gyre, and the nutrient-rich Cape Blanc upwelling area. We targeted the analyses of the single-cell activities of different bacterial groups in these oceanic waters and how they were affected by this environmental gradient. Our results are also compared with existing data from other marine systems to explore whether major phylogenetic taxa could be regarded as functionally coherent 'boxes' in the turnover of labile low-molecular-weight (LMW) compounds.

Methods

Location and sampling—The study was conducted along two transects from Northwest African coastal waters to open waters of the North Atlantic subtropical gyre during a cruise aboard the R/V BIO-*Hespérides* (COCA-II: 20 May–10 Jun 2003). Water for the experiments was collected from the surface (5-m depth) at eight stations, which varied in hydrographic conditions (Fig. 1; Table 1). Bulk uptake rates of substrates were measured in all stations and MAR–FISH analyses were carried out in seven out of the eight stations. Further details of the sampling sites can be found in Alonso-Sáez et al. (2007).

Bulk uptake of amino acids, glucose, and adenosine triphosphate (ATP)—Bulk uptake of the three LMW substrates (i.e., [^{3}H] glucose [Amersham TRK85], [^{3}H]-amino acids mixture [Amersham TRK440], and [^{3}H]-ATP [Amersham TRK747]) was determined by measuring the radioactivity incorporated into the cells in incubations maintained at in situ temperature. The substrates were added to 0.5 nmol L^{-1} final concentration and the incubations lasted for 4–10 h. For each sample (20 mL), four aliquots (1.2 mL) were taken in Eppendorf tubes, and 120 μL of cold 50% trichloroacetic acid (TCA) was added at the end of the incubation to stop the incorporation and precipitate macromolecules. For every sample and compound, two extra aliquots killed with 50% TCA before the addition of the isotope were used as controls. Samples were kept frozen at -20°C until processing, which was carried out by the centrifugation method (Smith and Azam 1992). Finally, 1 mL of scintillation cocktail (Optimal HiSafe) was added to each Eppendorf tube, and samples were counted on a Beckman scintillation counter after 24 h.

Catalyzed reporter deposition fluorescence in situ hybridization (CARD–FISH)—CARD–FISH was carried out following the protocol described by Pernthaler et al. (2004). Samples were fixed overnight with formaldehyde (1.8%) at 4°C and gently filtered on 0.2-μm polycarbonate filters (Millipore, GTTP, 25-mm diameter). Filters were permeabilized with lysozyme (37°C, 1 h) and achromopeptidase (37°C, 30 min) and hybridizations were carried out overnight at 35°C. The following horseradish peroxidase-probes were used in order to characterize the composition of the microbial community in the samples: Eub 338-II-III (targets most Bacteria), Alf968 (targets most Alphaproteobacteria), Gam42a (targets most Gammaproteobacteria), Cf319 (targets many members of the Bacteroidetes group), Ros537 (targets members of the *Roseobacter–Sulfitobacter–Silicibacter* clade, hereafter referred to as *Rhodobacteraceae*) and SAR11-441R (targets the SAR11 cluster). The Eub

Table 1. In situ measurements of temperature, salinity, chlorophyll *a*, and phosphate and nitrate concentrations at the stations sampled for MAR-FISH analysis (depth of 5 m).

Sta.	Temperature (°C)	Salinity	Chlorophyll *a* (μg L^{-1})	Phosphate (μmol L^{-1})	Nitrate (μmol L^{-1})
2	19.26	36.38	0.45	0.22	0.20
60	20.88	35.99	3.33	0.25	1.09
66	18.95	36.13	4.27	0.30	6.66
14	22.15	37.02	0.10	0.42	0.18
22	22.34	37.26	0.66	0.03	0.22
42	23.39	37.11	0.15	0.20	0.19
52	22.33	36.50	0.27	0.09	0.29

antisense probe Non338 was used as a negative control. Specific hybridization conditions were established by addition of formamide to the hybridization buffers (20% formamide for Non338 probe, 45% formamide for Alf968 and SAR11-441R probes, and 55% for the other probes). Counterstaining of CARD–FISH preparations was done with 4,6-diamidino-2-phenylindole (DAPI, 1 μg mL^{-1}). Between 500 and 1000 DAPI-positive-cells were counted manually in a minimum of 10 fields.

Microautoradiography (MAR) combined with CARD–FISH (MAR-FISH)—This technique was performed following the protocol originally described by Alonso and Pernthaler (2005). Samples (20 mL) were incubated for 4–10 h at in situ temperature with the following tritiated substrates (0.5 nmol L^{-1} final concentration): [^{3}H] glucose (Amersham TRK85), [^{3}H]-amino acids mixture (Amersham TRK440), and [^{3}H]-ATP (Amersham TRK747). One replicate (for each compound and treatment) was killed with formaldehyde (1.8%) before the addition of the tritiated compounds and was used as control. After the incubation, the samples were fixed overnight with formaldehyde (1.8%) at 4°C, and gently filtered on 0.2-μm polycarbonate filters (Millipore, GTTP, 25-mm diameter). The filters were then hybridized following the CARD–FISH protocol explained above, and subsequently were glued onto glass slides with an epoxy adhesive (Uhu plus; Uhu GmbH). For microautoradiography, the slides were embedded in 46°C tempered photographic emulsion (Kodak NTB-2) containing 0.1% agarose, placed on an ice-cold metal bar for about 5 min, and finally placed inside black boxes at 4°C until development. The optimal exposure time was determined for each experiment and compound, which resulted in a range between 2.5 d and 9 d. For development, we submerged the exposed slides for 3 min in the developer (Kodak D19), for 30 s in distilled water, and 3 min in fixer (Kodak Tmax), followed by 5 min of washing with tap water. The slides were then dried in a dessicator overnight, stained with DAPI (1 μg mL^{-1}), and counted in an Olympus BX61 epifluorescence microscope.

Results

Surface samples were collected from stations selected along a gradient from the oligotrophic Atlantic Subtropical Gyre to waters affected by the Cape Blanc upwelling, which were characterized by lower surface temperature and salinities, and higher chlorophyll *a* concentrations, particularly in the South transect (Fig. 1; Table 1). The analysis of bacterial community structure after the MAR–FISH procedure showed the same distribution pattern that we had observed in the in situ samples of the same stations (Fig. 2, as compared with fig. 4 in Alonso-Sáez et al. 2007). Higher contributions of Bacteroidetes, Gammaproteobacteria, and *Rhodobacteraceae* were found at the stations affected by the upwelling (expressed as percentages of total DAPI counts), while the contribution of SAR11 slightly decreased in the upwelling area (Wilcoxon test, $p < 0.05$; Fig. 2).

The bulk uptake rates of the three different LMW compounds added at tracer concentration (i.e., 0.5 nmol L^{-1}) are shown in Fig. 3A. For each substrate, the highest uptake rates were found in the stations most directly affected by the Cape Blanc upwelling (Wilcoxon test, $p < 0.05$). In the upwelling area, amino acids were the substrate type most actively taken up by bacteria (up to 20 pmol L^{-1} h^{-1}), followed by glucose and ATP (up to 8 and 6 pmol L^{-1} h^{-1}, respectively). At the stations not affected by the upwelling, the uptake of ATP was almost below detection, while the uptake of glucose and amino acids was below 4 pmol L^{-1} h^{-1} (Fig. 3A).

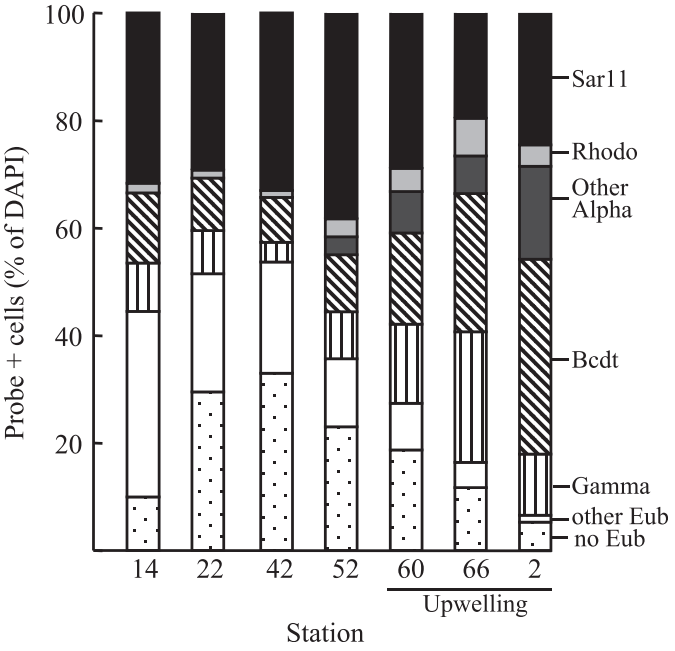

Fig. 2. Bacterial community composition in the samples, as analyzed by CARD–FISH. The abundances of the different bacterial groups are scaled to DAPI counts. Eub: Bacteria, Alpha: Alphaproteobacteria, Gamma: Gammaproteobacteria, Bcdt: Bacteroidetes, Rhodo: *Rhodobacteraceae*. 'no Eub' stands for cells that were not detected by the probe mix Eub338-II-III, while 'other Eub' stands for the fraction of cells that hybridized with the probe mix Eub338-II-III but not with any of the probes targeting the broad phylogenetic groups (i.e., Alpha-, Gammaproteobacteria, and Bacteroidetes) that we used.

The single-cell activity of the different bacterial groups was analyzed by MAR–FISH and expressed as percentages of probe+ cells active in the uptake of the different compounds. In the upwelling-affected area, the percentage of bacteria (i.e., Eub+ cells) taking up the organic compounds was generally higher (above 20% of active cells; Fig. 3B), in agreement with bulk uptake results. The percentage of bacteria taking up glucose and amino acids at the Cape Blanc upwelling was significantly higher than offshore (Wilcoxon test, $p < 0.05$), and the maximum percentage of active cells was found for amino acid uptake (average ± SD: 42% ± 3% Eub+ cells). At the stations not directly affected by the upwelling, the percentage of bacteria taking up glucose and ATP was similar (average ± SD: 8% ± 3% and 10% ± 5% active cells, respectively), while the percentage of active cells taking up amino acids was higher on average (19% ± 5% of active cells; Fig. 3B).

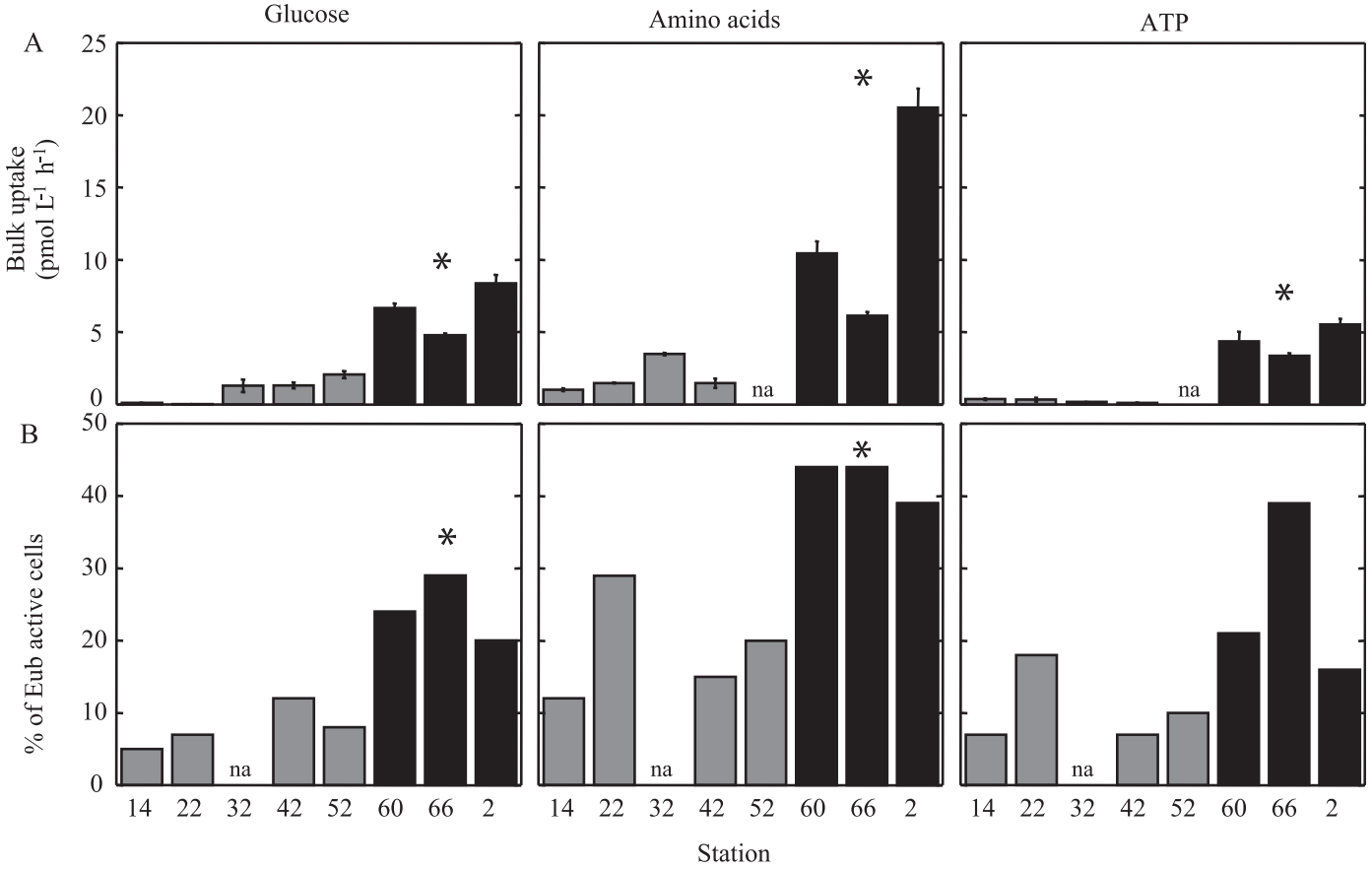

Fig. 3. (A) Bulk uptake of glucose, amino acids, and ATP, and (B) percentage of total Bacteria (Eub338-II-III positive cells) active in the uptake of the same compounds. The black bars correspond to samples collected in the Cape Blanc upwelling area. Error bars in the upper panels correspond to the standard deviation of four replicates. na: not available. Asterisks denote significant differences between upwelling and offshore samples (Wilcoxon test, $p < 0.05$).

In general, Alphaproteobacteria had the highest percent of cells involved in glucose uptake while Gammaproteobacteria dominated ATP uptake (Fig. 4). Both proteobacterial groups were also more active than Bacteroidetes in the uptake of amino acids, as analyzed by the Kruskal–Wallis test followed by Tukey's Honestly Significant Difference (HSD) test ($p < 0.05$). While the uptake activity of Alphaproteobacteria was significantly higher in upwelling waters for all substrates (Wilcoxon test, $p < 0.05$), no significant differences were found for glucose uptake in the case of Bacteroidetes, or ATP uptake in the case of Gammaproteobacteria (Wilcoxon test, $p > 0.05$; Fig. 4). Within Alphaproteobacteria, *Rhodobacteraceae* were the most active group in the uptake of the three compounds in the upwelling area (up to 94% of active cells), but their activity decreased importantly offshore and was not detectable in most stations in the case of ATP uptake (Fig. 4). The activity of SAR11 in glucose uptake was significantly higher in the upwelling region (Wilcoxon test, $p < 0.05$). Up to 32% and 53% of SAR11 cells were active in glucose and amino acid uptake in the upwelling, but the uptake of ATP was always low for this group (below 13% of active cells, Fig. 4).

In order to broaden our comparison of the single-cell activity of different bacterial groups, we performed an analysis by including published results for the uptake of the same compounds (glucose, amino acids and ATP) at tracer concentrations in a wide range of oceanic regions (i.e., Mediterranean, Atlantic, and polar environments [*see* references in Fig. 5]). Samples were classified according to coastal or open-ocean origin, and the oceanic upwelling samples from this study were considered a separate category due to their marked differences from oligotrophic oceanic waters. For each compound, the uptake activity of Gammaproteobacteria was not significantly different across the distinct oceanic regions (Kruskal–Wallis test, $p > 0.05$; Fig. 5A). In the case of Bacteroidetes, the activity was significantly different for ATP uptake, with higher values in coastal waters than offshore waters (Tukey's HSD test, $p < 0.05$, Fig. 5A).

Alphaproteobacteria were significantly more active in the uptake of ATP and glucose in upwelling and coastal waters as compared with offshore (Tukey's HSD test, $p < 0.05$; Fig. 5A), but no differences were found for amino acid uptake. Within Alphaproteobacteria, SAR11 showed a higher variability in their activity, particularly in offshore samples, and no significant differences were found among regions (Kruskal–Wallis test, $p > 0.05$). By contrast, *Rhodobacteraceae* showed significantly higher activity in

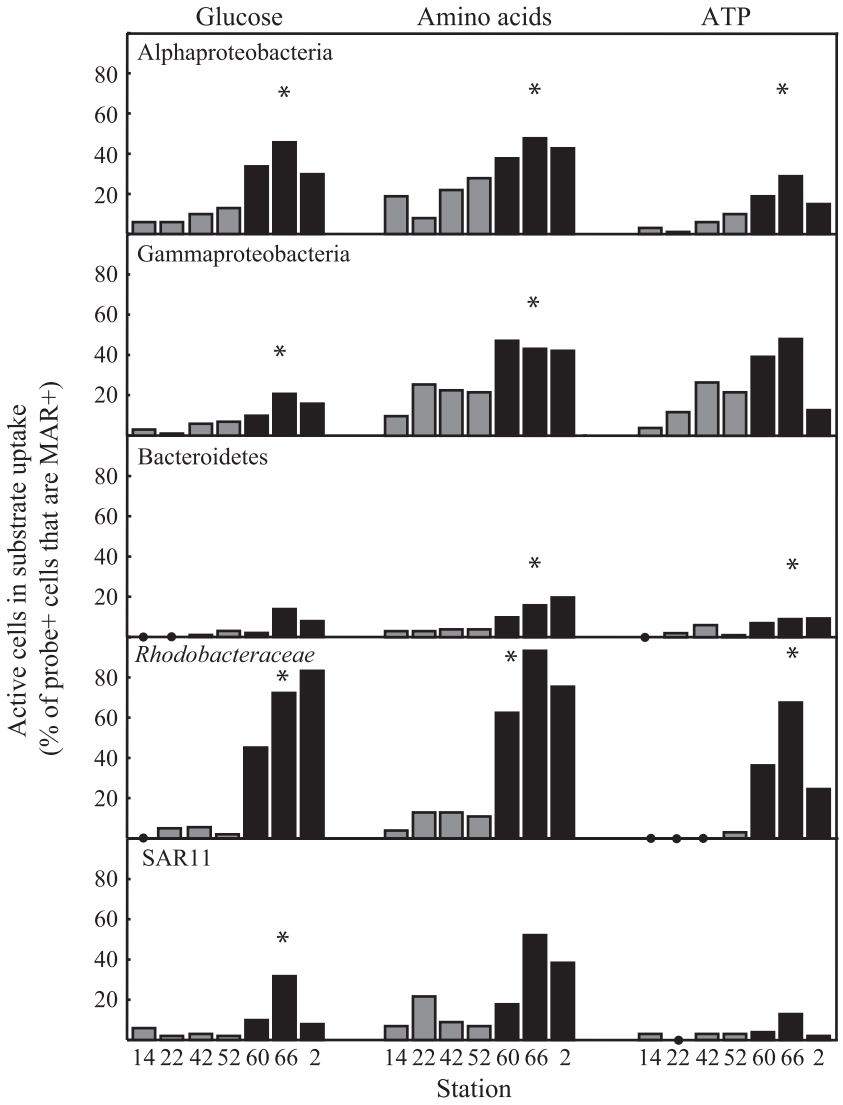

Fig. 4. Percentage of probe-positive cells affiliated with Alpha-, Gammaproteobacteria, Bacteroidetes, *Rhodobacteraceae*, and SAR11 active in the uptake of glucose, amino acids, and ATP as detected by MAR–FISH. The black bars correspond to samples collected in the Cape Blanc upwelling area. Asterisks denote significant differences between upwelling and offshore samples (Wilcoxon test, $p < 0.05$).

the uptake of the three compounds in coastal and upwelling samples, as compared with offshore samples (Tukey's HSD test, $p < 0.05$; Fig. 5B).

Finally, pooling together the already published data and this study, we analyzed the preferential uptake of different compounds by the bacterial phylogenetic groups by comparing the percentage of active cells within each group taking up the different substrates (Fig. 6). While Alphaproteobacteria showed more active cells than Gammaproteobacteria or Bacteroidetes in the uptake of glucose, Gammaproteobacteria were more active than Bacteroidetes in the uptake of ATP (Tukey's HSD test, $p < 0.05$; Fig. 6A). In the case of amino acid uptake, Bacteroidetes showed significantly lower percentage of active cells as compared with Alpha- and Gammaproteobacteria (Tukey's HSD test, $p < 0.05$; Fig. 6A). Within Alphaproteobacteria, *Rhodobacteraceae* were significantly more active than SAR11 in the uptake of the three LMW organic compounds (Kruskal–Wallis test, $p < 0.05$; Fig. 6B).

Discussion

Due to the central role of bacteria and archaea in the oceanic biogeochemical cycles, elucidating the specific functional roles of community members has become a challenge in microbial ecology studies. To what extent different bacterial taxa participate in dissolved organic matter (DOM) uptake and how environmental conditions influence their activity are vital pieces of information toward obtaining an integrated view of microbial DOM metabolism (Kujawinski 2011). A basic premise for establishing links between bacterial diversity and function

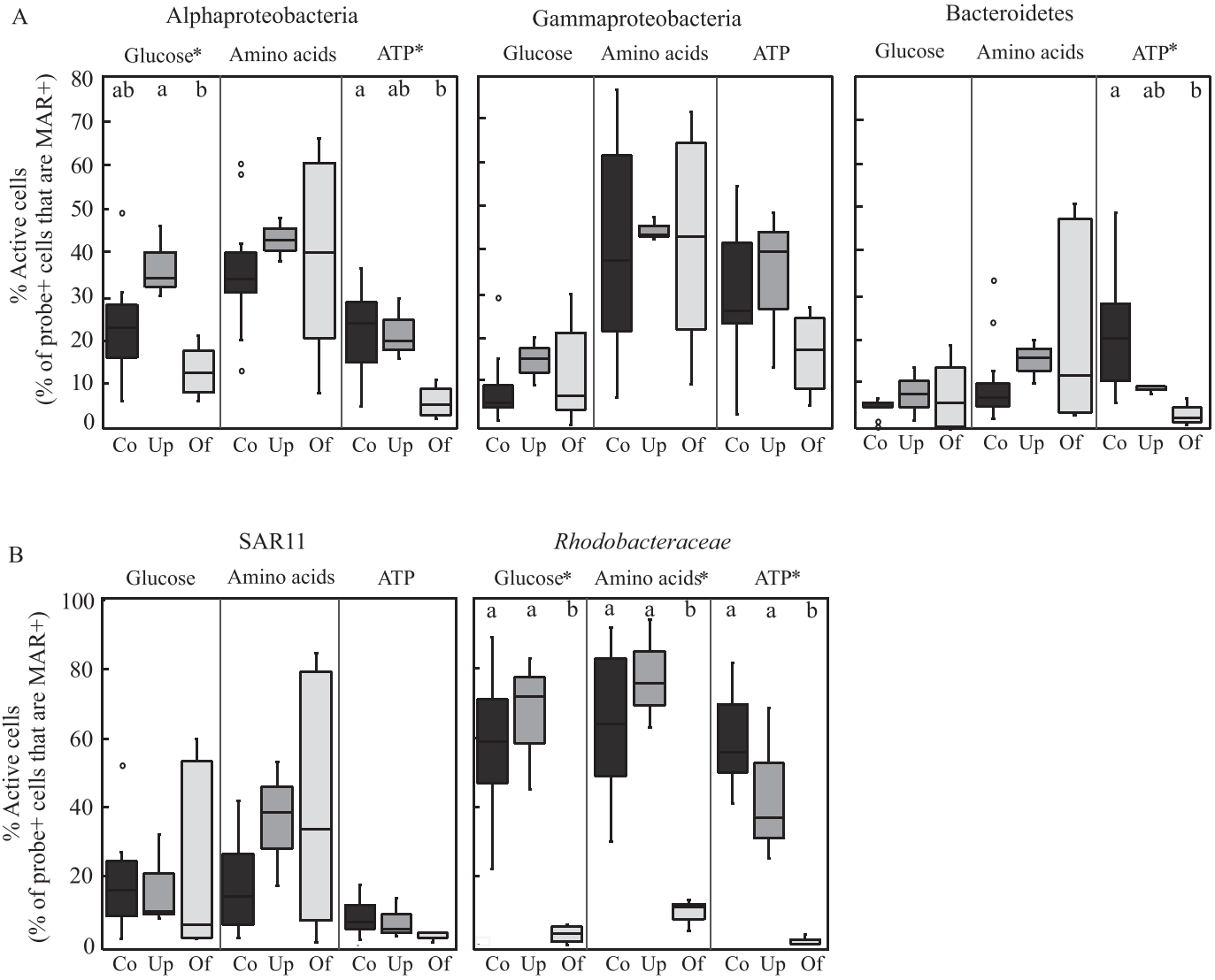

Fig. 5. Box plots representing the percentage of probe+ cells affiliated with (A) Alpha-, Gammaproteobacteria, Bacteroidetes, and (B) *Rhodobacteraceae* and SAR11, active in the uptake of glucose, amino acids and ATP in coastal (Co), upwelling (Up), or offshore samples (Of) as detected by MAR–FISH. The data collected in the present study were pooled together with published measurements from different systems such as the Mediterranean (Alonso-Sáez and Gasol 2007), the Arctic and Antarctic oceans (Elifantz et al. 2007; Alonso-Sáez et al. 2008; Straza et al. 2010), the North Sea (Alonso and Pernthaler 2006), and the Atlantic ocean (Malmstrom et al. 2004, 2005). In order to make a straight comparison, only studies that targeted the uptake of these particular compounds at tracer concentrations (up to 1 nmol L^{-1}) in marine samples were included in the analysis. When significant differences were found according to a Kruskal–Wallis test ($p < 0.05$, marked with asterisks, n ranges from 3 to 19), the Tukey's HSD test was used to determine which groups differed from each other. Within each panel, groups sharing the same letters are not significantly different.

would be to elucidate at what level of taxonomic resolution trait-based functional groups can be identified. In this sense, it is remarkable that differences in the utilization of enantiomeric forms of amino acids (D- vs. L-amino acids) were reported at the very broad domain level (i.e., *Bacteria* and *Archaea* in specific oceanic water masses; Teira et al. 2006).

Different patterns in the activity of class-level groups such as Alpha- and Gammaproteobacteria have also been described by MAR–FISH across temporal and spatial scales (Cottrell and Kirchman 2000; Alonso-Sáez and Gasol 2007), but most of these previous data were obtained from coastal environments. Also, while the preferential utilization of polymers vs. LMW monomers by different bacterial groups (such as Bacteroidetes and Alphaproteobacteria, respectively) is now recognized (Cottrell and Kirchman 2000), whether there are also trends in the utilization of various LMW, easy-to-degrade substrates has seldom been explored. Here, we analyzed several oceanic stations (bottom depth > 1000 m) along a natural trophic gradient from the Atlantic oligotrophic gyre to the Cape Blanc upwelling system. Bacterial single-cell activity measurements carried out in oligotrophic gyres, which account for the largest fraction of the surface ocean, or in

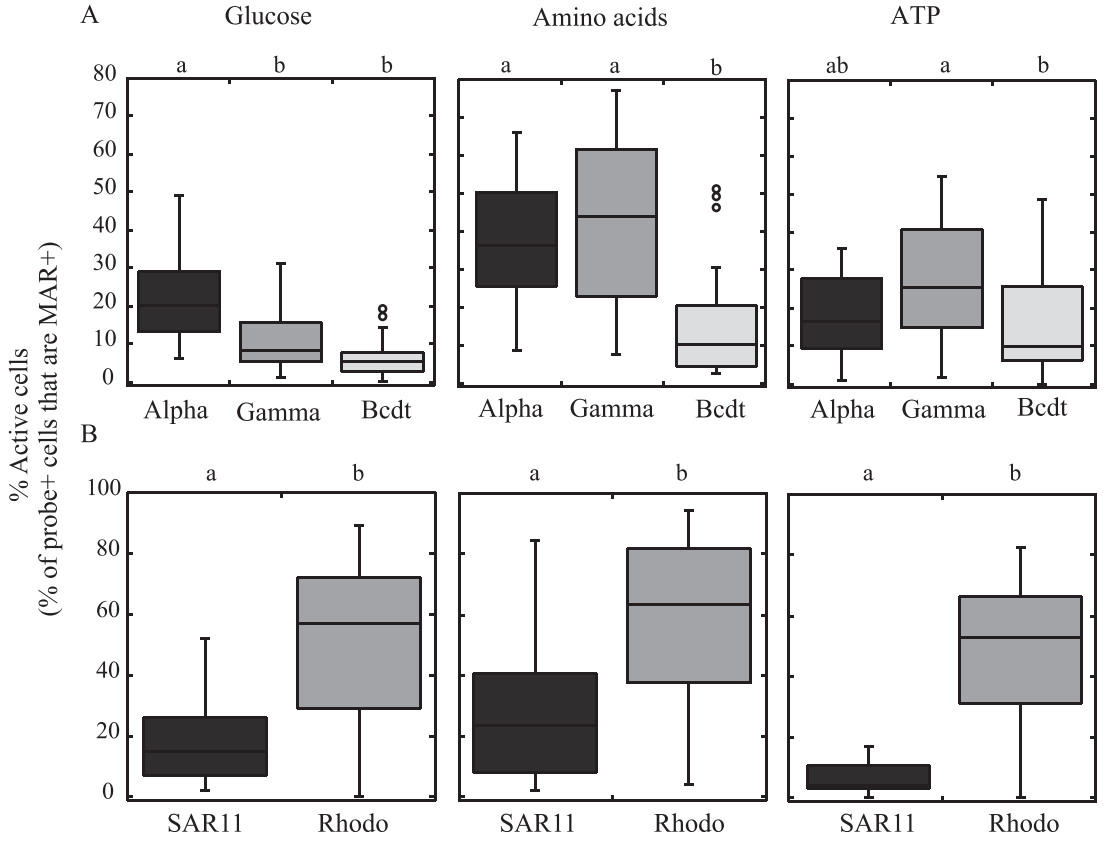

Fig. 6. Box plots representing the percentage of cells affiliated to (A) Alphaproteobacteria (Alpha), Gammaproteobacteria (Gamma), and Bacteroidetes (Bcdt), and (B) *Rhodobacteraceae* (Rhodo) and SAR11 active in the uptake of glucose, amino acids, and ATP. Data collected in comparable studies from other marine regions were also included in the analysis (*see* Fig. 5 legend). Significant differences were found for all substrates according to a Kruskal–Wallis test ($p < 0.05$, n ranges from 20 to 36). The Tukey's HSD test was used to determine which groups differed from each other. Within each panel, groups sharing the same letters were not significantly different.

oceanic upwelling systems are scarce (but *see* Malmstrom et al. 2004, 2005; Longnecker et al. 2006). Thus, our data are among the first reports of single-cell activity of groups of oceanic bacteria in the uptake of different organics. We combined our results with data from previous studies to explore large-scale patterns of bacterial substrate specialization at the level of three labile organic monomers commonly found in seawater: glucose, amino acids, and ATP.

In a previous review of the distribution of activity across major phylogenetic groups, del Giorgio and Gasol (2008) detected a wide range in the proportion of Alpha-, Gammaproteobacteria, and Bacteroidetes active in the uptake of different substrates, including thymidine, leucine, amino acids, protein, and glucose. For example, the percentage of active Alphaproteobacteria or Bacteroidetes cells in amino acid uptake spanned from $< 10\%$ to $> 60\%$. Yet, it should be taken into account that the latter analysis included results with substrates added at very different concentrations, while a strong effect of concentration on substrate uptake activity for different bacterial groups has been demonstrated (Alonso and Pernthaler 2006). Indeed, results from the addition of leucine at 20–40 nmol L^{-1} are usually taken as a proxy for biomass production, rather than substrate uptake activity. For instance, Bacteroidetes have usually low activity in leucine uptake at tracer concentration (Cottrell and Kirchman 2000), but are substantially more active at concentrations of about 20 nmol L^{-1} (Cottrell and Kirchman 2003; Longnecker et al. 2006). In the present analysis, we only included studies that targeted the same model compounds added at tracer concentration (< 1 nmol L^{-1}). Thus, we aimed at analyzing the use of different LMW substrates by distinct bacterial groups rather than assessing their general metabolic activity.

By contrast to the degradation of high-molecular-weight compounds, which requires more specialized enzyme systems (Arnosti 2011), the uptake of LMW substrates can be considered a prevalent trait among heterotrophic groups because they are directly assimilated by bacteria and represent essential resources for their growth and energy requirements. For instance, the compound ATP, which is analyzed here, represents a model substrate of the organic-P pool (Azam and Hodson 1977). Yet, we found that major taxonomic bacterial groups significantly differed in the mean values and ranges of percent active cells using glucose, amino acids, or ATP (Fig. 6). These results are remarkable if we consider the great variability of environmental conditions included in the analysis, ranging from

coastal to offshore waters from Mediterranean, Atlantic, and marine polar environments (Fig. 6). Coastal and open-sea systems are very different in terms of DOM production mechanisms (i.e., phytoplanktonic primary production vs. grazing-related processes) and the frequency of pulses of organic matter (Nagata 2000). Although contrasting results were found for the single-cell activity of certain taxa between both environments (i.e., differences in the activity of *Rhodobacteraceae*; Fig. 6), our results suggest that broad phylogenetic bacterial groups show some level of functional coherence in the use of these model compounds independently of the type of environment.

Bacteroidetes had the lowest activities in the uptake of the three LMW compounds, in agreement with their previously reported preference for HMW substrates (Cottrell and Kirchman 2000). This observation is also supported by genomic and transcriptomic data; few Bacteroidetes-like transporters for organic monomers were identified in a transcript library retrieved from coastal seawater (Poretsky et al. 2010), and members of this group contain numerous glycolytic and proteolytic enzymes in their genomes (Cottrell et al. 2005). Alpha- and Gammaproteobacteria showed similar high activity in the uptake of amino acids, which supported the view that most bacterial taxa use these compounds as sources of C and N (Poretsky et al. 2010).

The average values of Alpha- and Gammaproteobacteria active in amino acid uptake were not significantly different between coastal and offshore samples (Fig. 5), which suggests that these substrates represent an essential substrate for proteobacterial metabolism in a wide range of marine environments. However, a wide range of variation in the percentage of Gammaproteobacteria active in amino acid uptake was found for both coastal and offshore environments. Such large variation was due to the fact that their activity was significantly higher in polar than in temperate waters (average of 51% and 27% active Gammaproteobacteria, respectively), and data from both systems were combined in the analysis. Differences in the composition of Gammaproteobacteria, which might include more active taxa in polar waters, could explain these results. Yet, when pooling data from all environments, the activity of Gammaproteobacteria in amino acid uptake was still significantly higher as compared with Bacteroidetes (Fig. 6).

By contrast to the amino acid results, Alpha- and Gammaproteobacteria showed differences in the uptake of glucose and ATP (Fig. 6A). The higher uptake activity of ATP by Gammaproteobacteria could reflect a specific high requirement of phosphorous for growth, because this group has also been detected as highly active in the uptake of inorganic P (Sebastian et al. in press). It is remarkable that while the percentage of active Alphaproteobacteria and Bacteroidetes taking up ATP was higher in a marine region characterized by strong P limitation (i.e., Northwest Mediterranean, generally $> 20\%$ of active cells; Alonso-Sáez and Gasol 2007) when compared with the results presented here, the percentage of Gammaproteobacteria active in ATP incorporation was consistently high in both regions. Thus, this suggests that the high P requirement for this group is independent of the nutrient-limitation regime of the ecosystem. In turn, the higher activity of Alphaproteobacteria in the uptake of glucose could indicate their preference for sugars as a C source for building biomass. Even if glucose uptake is usually assumed to be a ubiquitous trait among aquatic bacteria, MAR–FISH studies have shown that this substrate is actually used by a rather limited fraction of prokaryotes (Alonso and Pernthaler 2005; Elifantz et al. 2005; Alonso-Sáez and Gasol 2007).

The most contrasting results in terms of activity were found between both alphaproteobacterial groups, with *Rhodobacteraceae* showing significantly higher activities as compared with SAR11 (Fig. 5B). This indicates that the two groups perform differently in the turnover of these LMW compounds and, thus, could be regarded as different boxes for this functional trait. Members of *Rhodobacteraceae* have been described as generalists based on their genome characteristics (Moran et al. 2004) and high in situ activities have been generally reported for the group (Alonso and Pernthaler 2006; Alonso-Sáez and Gasol 2007; Alonso-Sáez et al. 2008). Despite the general versatility in their metabolism, *Rhodobacteraceae* were not active under oligotrophic open-ocean conditions (Fig. 4). This agrees with the view that this group largely depends on pulses of organic matter and takes advantage of transient high-nutrient niches, which are more readily available in coastal systems and upwelling areas (Moran et al. 2004; Poretsky et al. 2010). Accordingly, the biosynthetic single-cell activity of the *Roseobacter* clade-affiliated (RCA) cluster (taking up leucine at 20 nmol L^{-1}) was observed to be higher at the coastal shelf waters as compared with waters from the basin in the highly productive Arctic region of the Chukchi Sea (Malmstrom et al. 2007). However, the fact that our single-cell measurements with substrates at tracer concentrations are among the only ones reported for *Rhodobacteraceae* in oligotrophic oceanic waters could be biasing these results, and more studies are needed in order to test whether this pattern is maintained in other similar low-nutrient marine regions.

Pelagibacter ubique, an isolate of the other alphaproteobacterial group studied (i.e., SAR11), has a streamlined genome and, therefore, shows less versatility in their metabolism (Giovanonni et al. 2005). *P. ubique* is adapted to low-nutrient conditions and, indeed, high activities of SAR11 have been detected in oligotrophic oceanic waters (i.e., $> 70\%$ of active cells taking up amino acids in the Sargasso Sea; Malmstrom et al. 2004, 2005). However, some studies suggested that the marine SAR11 clade is highly diverse (Venter et al. 2004) and includes ecotypes with different metabolic features (Schwalbach et al. 2010). Along the Atlantic oceanic gradient analyzed here, we found that the activity of SAR11 was relatively high in the upwelling area (Fig. 4), which suggested that certain members of this clade can successfully thrive under nutrient-rich open-ocean conditions.

Interestingly, the percentages of active SAR11 cells previously found by MAR-FISH for a wide range of coastal environments (Alonso-Sáez and Gasol 2007; Alonso-Sáez et al. 2008; Straza et al. 2010) were usually lower than those first reported in samples from the Sargasso Sea (Malmstrom et al. 2004, 2005), which suggests that different SAR11 phylotypes may be present in coastal

vs. open-ocean environments. In this regard, contrasting results have been found by comparative genomic analysis. While substantial evolutionary divergence was found between the genome of a coastal SAR11 isolate (HTCC1062) and SAR11 metagenomic fragments retrieved from the Sargasso Sea, core genomic features seemed to be conserved between genomes from both regions (Wilhelm et al. 2007). However, it is remarkable that differences in the presence of a glycolysis operon between an open-ocean SAR11 (HTCC7211) and coastal isolates have been demonstrated, which explains why the SAR11 HTCC7211 was not able to incorporate glucose while coastal isolates efficiently used this substrate (Schwalbach et al. 2010). Even though metagenomic data suggest that glycolytic SAR11 are more commonly found in coastal environments (Schwalbach et al. 2010), we did not detect significant differences in the activity of SAR11 cells in glucose uptake from coastal to offshore environments (Fig. 5). This suggests that at least some open-ocean SAR11 phylotypes are also capable of taking up glucose.

A few previous studies have also analyzed changes in single-cell activity of different bacterial groups across trophic marine gradients. These earlier studies typically included samples from coastal or estuarine systems to open waters, and assessed the biosynthetically active cells (taking up leucine or thymidine at saturating concentration), rather than substrate specialization. Contrasting results were found among these studies. For example, Longnecker et al. (2006) did not find substantial differences in the abundance of biosynthetically active cells of different bacterial groups across the Oregon upwelling system and, instead, a large variability in the percentage of active cells for each group was detected. By contrast, Cottrell and Kirchman (2003) detected differences in the specific activity of the bacterial groups (particularly Alpha-, Betaproteobacteria, and Bacteroidetes) along a salinity gradient in the Delaware estuary.

Other studies measuring biosynthetically active cells (taking up leucine at 20 nmol L^{-1}) in polar environments found similar results to the patterns reported here along the Atlantic trophic gradient. For example, the biosynthetic activity of a major member of Bacteroidetes (*Polaribacter*) increased from the Western Arctic basin ($<$ 10% of active cells) to shelf nutrient-rich waters ($\sim$ 50% active cells; Malmstrom et al. 2007). Similarly, the biosynthetic activity of SAR11 and a group of *Rhodobacteraceae* (RCA cluster) increased toward shelf waters in the same system (Malmstrom et al. 2007). In a study along the West Antarctic Peninsula that analyzed the uptake of amino acids at tracer concentrations, *Polaribacter* also increased their activity from offshore to coastal waters and no significant differences were found for the activity of SAR11 across that marine gradient (Straza et al. 2010).

In conclusion, pooling data from different marine environments, we found that the groups Bacteroidetes and *Rhodobacteraceae* had higher activities in nutrient-rich environments such as coastal or upwelling systems. By contrast, members of Gammaproteobacteria and the SAR11 clade were adapted to a wide range of environmental marine conditions. The broad phylogenetic groups of bacteria showed significant differences in the uptake of three labile LMW organics. The functional traits analyzed here (i.e., uptake activity of glucose, amino acids, and ATP in terms of percentage of active cells per group) is indicative of the relative activity of each group in the turnover of these compounds. Yet, it should be taken into account that a higher percentage of active cells does not always imply a higher bulk uptake, and the contribution of a bacterial group to bulk substrate uptake is also determined by its in situ abundance. Substrate bulk uptake rates could also reflect differences in the in situ availability of the substrates and/or the limiting factors in the system. For instance, the bulk ATP uptake rates measured in this study were substantially lower than those measured in a previous study in oligotrophic Mediterranean coastal waters, which are strongly limited by phosphorous (Alonso-Sáez and Gasol 2007).

It is also remarkable that only a relatively low fraction of total cells were active in the uptake of these labile substrates even in the upwelling area ($<$ 50% active bacteria). Yet, the fact that significant differences in the uptake of these essential compounds could be detected among broad bacterial taxa in such a wide range of marine environments supports the idea that the bacterioplankton 'black box' could be opened in several phylogenetically coherent boxes in terms of carbon use. If this is confirmed, bacterial community composition could help us explain and possibly predict patterns of DOM utilization. Improving our knowledge about the substrates that are most widely used as C sources for marine bacteria, and how different groups participate in their turnover, will be crucial in order to obtain a realistic view of the role of bacteria in DOM degradation, and to determine whether the inclusion of different functional boxes could help us improve models of oceanic carbon cycling.

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