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PLANT MICROBE INTERACTIONS

Effects of Monospecific Banks of Salt Marsh Vegetation on Sediment Bacterial Communities

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Abstract The aim of this study was to understand if two species of salt marsh plants, widely distributed in European estuaries (*Spartina maritima* and *Halimione portulacoides*) differently influence the distribution, activity, and metabolic physiology of sediment bacterial communities in monospecific banks, in comparison with uncolonized sediment (control). Microbiological descriptors of abundance and activity were assessed along vertical profiles of sediments. Rates of activity of the extracellular enzymes β-glucosidase, α-glucosidase, aminopeptidase, arylsulfatase, and phosphatase were generally higher in the vegetation banks in relation to control sediments where they were also less variable with depth. This is interpreted as an indirect effect related to supply of plant-derived polymeric substrates for bacterial growth. Parameters related to sediment texture (grain size, percent of fines or water content) showed significant relations with cell abundance or maximum hydrolysis rates, pointing to an indirect effect of plant colonization exerted through the modification of sediment physical properties. The profiles of utilization of sole-carbon-source (Biolog Ecoplates) showed that only the communities from the upper sediment layer of the *S. maritima* and the *H. portulacoides* banks exhibit consistent differences in terms of physiological profiles. Bacterial communities in control sediments exhibited the lowest physiological variability between surface and sub-surface communities. The results indicate that microbial colonization and organic matter decomposition are enhanced under the influence of salt marsh plants and confirm that plant coverage is a major determinant of the processes of organic matter recycling in intertidal estuarine sediments.

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Introduction

Salt marshes are complex systems that import, process and export organic matter, nutrients, and pollutants from the water column. Due to their high productivity and location at intertidal zones, they represent important sources of organic matter for the system where they are located [40]. Considering that the major portion of the total energy flow in these environments is through decomposition, much of the organic matter produced in the salt marsh will be locally transformed or remineralized by heterotrophic bacteria [37].

In salt marsh ecosystems, below-ground biomass of macrophytes can reach values up to tenfold higher than above-ground biomass, making the bacterial communities in the sediments important consumers of autochthonous primary production [56]. The rhizospheres are usually defined as the sediment immediately in contact with the roots or under the influence of root-derived compounds. The presence of plant species with distinct patterns of growth and resource allocation can lead to differences in the proportion of modified bulk sediment present and thus result in different populations and degrees of activity for sediment microorganisms in the rhizospheres [52] and in different parts of the root system [26]. Actively growing roots release organic compounds into the rhizosphere such as sloughed off cells, secretions, lysates, and exudates [55]. These compounds support the growth of the microbial community and may result not only in an increased cell density, but also in a community structure distinct from that in the bulk sediment [55].

Interactions between plants and rhizosphere bacteria have been studied mostly in cultivated plants, and information on interactions with wild plants, namely in salt marshes is more scarce. However, some studies show host specificities of bacterial populations from the rhizosphere of salt marsh vegetation in terms of composition [8] as well as in terms of abundance and heterotrophic activity rates [8, 13]. Exudates from the roots of salt marsh vegetation provide bacteria with high-quality sources of carbon and energy and enhance diazotrophy in the rhizosphere [1]. Considering that plant species, type of metabolism, and plant life stage [25] are some of the factors that affect the quantity and quality of organic matter released through the roots, heterotrophic bacteria in the rhizospheres may adapt and develop particular physiological features in response to changes in the nutritional environment [20, 25, 59]. The balance between bacterial and root activity will greatly influence the availability of oxidized and reduced forms of organic and inorganic nutrients [28]. Tidal water movements export some of the salt marsh organic matter to coastal waters as flocks or organic detritus and bacteria, but most of this material decomposes in situ by fermentation and anaerobic respiration [2].

Salt marsh vegetation is determinant to the dynamics of the estuarine processes and strongly influences the accumulation of heavy metals with recognized interest as bioindicators of metal contamination in coastal regions and applications in phytoremedition approaches [45, 46]. The present work is focused on sediment bacterial communities associated with uncolonized intertidal banks and with monospecific banks of two widely distributed salt marsh plant species (*Spartina maritima* and *Halimione portulacoides*). *H. portulacoides* (Chenopodiaceae) is perennial and is widely distributed in European salt marshes. This species is anemophilic and flowering occurs during late summer and autumn, depending on temperature [61]. *S. maritima* (Gramineae) is a primary colonist of intertidal mud flats well represented in European and African Atlantic coasts [60]. It is a perennial plant with an extensive, deep and well-aerated anchoring root system [61]. The hypothesis underlying this work was that salt marsh plant species may impose particular features upon the rhizosphere thus modulating the abundance, activity, and physiological profile of the associated bacterial communities and ultimately shape the profiles of organic matter diagenesis in estuarine sediments.

Materials and Methods

Study Area and Sample Collection

Ria de Aveiro is a shallow coastal lagoon located at Northwest Atlantic coast of Portugal (40°38′N. 8°44′W). It is a complex system characterized by narrow channels and extensive intertidal zones. The study area is a salt marsh at the east margin of Mira channel (Gafanha da Encarnação), one of the main channels of the estuarine system (Fig. 1).

Sediment samples were collected in November 2007, February and September 2008, 1 h before low tide with a steel cylindrical corer (8 cm diameter, 55.5 cm length), at monospecific banks of *H. portulacoides*, *S. maritima* and also at an unvegetated sediment bank at the lower limit of

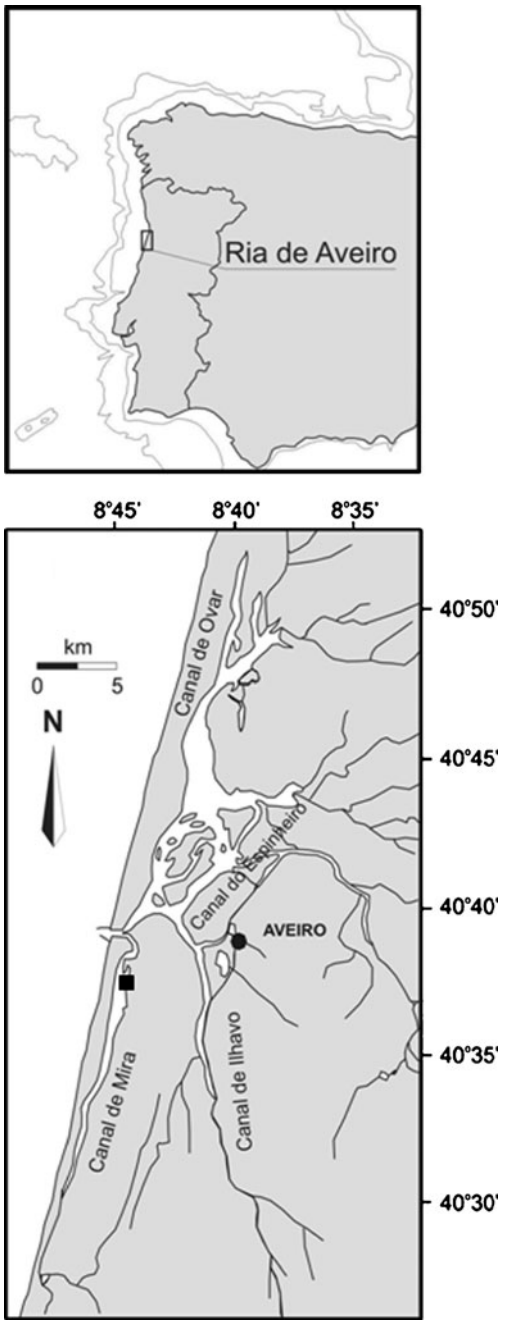

Figure 1 Ria de Aveiro (Portugal) with the study area marked with a *solid square*

the intertidal zone. The maximum values of below-ground biomass are typically found in April (6,620 gdw m^{-2}) at *S. maritima* bank, and in June (5,600 gdw m^{-2}) at the *H. portulacoides* bank [9]. Sediment cores (three replicates) were horizontally sectioned with a 2-cm pace. The corresponding depth layers from the replicate cores were pooled in order to obtain composed samples. Coarse debris and root parts, more abundant between surface and 10 cm depth, were manually removed. Sediments were transported to the laboratory and processed within 2-h after collection.

Sediment Characterization

The water content was determined by weight loss after drying at 60 °C for 24 h and was expressed as the percentage of sediment fresh weight. Grain size was analyzed by wet and dry sieving [44]. The silt and clay fraction (particles with diameter below 0.063 mm) was expressed as the percentage of dry weight of total sediment. The sand fraction (0.063-4.000 mm) was dry sieved through a battery of sieves spaced at 1 phi (φ) unit ($\varphi=-\log^2$ of the particle diameter expressed in mm). The sediments were classified according to the median value (P_{50}), following the Wentworth scale [21] with adaptations [34].

Total Prokaryote Abundance

The total abundance of prokaryote cells was only determined in the samples collected in November 2007, after 4',6-diamidino-2-phenylindole (DAPI) staining. Samples were fixed by the method already described [35] with minor modifications. Samples (0.5 g of fresh sediment) were fixed in 2% formaldehyde (5.56 ml of 37% formaldehyde and 100 ml of filtered seawater) for 4 h at 4 °C. Fixed samples were washed twice with 1× phosphate buffer saline (PBS), with centrifugation at 12,000×*g* for 2 min between washes, and stored in PBS/ethanol (1:1) at −20 °C. Five microliters of sediment suspension were diluted with 10 mL of 1× PBS. Cells were collected by filtration onto the surface of 0.2 μm-pore-size polycarbonate membrane (GE Osmonics Labstore) and stained with DAPI (2 μg mL^{-1}) for 3 min [42]. The membranes were mounted in a glass slide with Citifluor immersion oil as mounting medium and examined by epifluorescence microscopy (LEICA DMLS) with a mercury bulb and filter Chroma 31000 for DAPI detection. Microorganisms were counted at ×1,000 magnification. A minimum of 10 optical fields were enumerated in each replicate.

Activity of Extracellular Hydrolytic Enzymes

The activity of five ectoenzymes was analyzed fluorimetrically (Jasco FP-777 fluorometer) [6]. The following solutions of fluorogenic methylumbelliferone (MUF) or 4-methylcoumarinyl-7-amide (MCA)-labeled substrates were used: MUF-β-glucoside as a substrate for β-glucosidase, MUF-α-glucoside for α-glucosidase, MUF-phosphate for phosphatase, MCA-leucine for aminopeptidase, and MUF-sulfate for arylsulfatase. All substrates were obtained from Sigma Co. Sediment suspensions were prepared by adding 100 ml of sterile seawater to 1 g of fresh sediment and stirring in order to obtain homogeneous sediment suspensions. For the analysis of the activity of each enzyme, six aliquots of 1.5 mL were transferred to 2 mL microtubes and added of 50 μL of the stock substrate MUF solution and 25 μL of MCA substrate solution. Final saturating concentrations, established by previous kinetic assays, were 10 mM for β-glucosidase, 5 mM for α-glucosidase, 10 mM for acid phosphatase, 20 mM for aminopeptidase, and 2 mM for arylsulfatase. The initial fluorescence (λ_{ext}= 365 nm and λ_{em}=450 nm for MUF substrate and λ_{ext}= 380 nm and λ_{em}=440 nm for the MCA substrate) was read in three of the replicates after centrifugation (12,000×*g*) for removal of particles and addition of 100 μL of buffer solution (1.384 ml of ammonium, 0.375 g glycin and distillate water to 100 ml, pH 10.5) in order to enhance MUF fluorescence. The remaining three aliquots were incubated at in situ temperature for 3-4 h, after which particles were removed by centrifugation, the buffer solution (100 μL) was added and the final fluorescence was read. For the determination of aminopeptidase activity, the procedure was similar to that described for enzymes acting on MUF substrates but without the addition of the buffer solution and 2 h of incubation.

The rate of substrate hydrolysis was estimated from the increased variation of fluorescence, standardized to 1 h incubation, and converted to concentration units by means of a calibration curve prepared for each of the fluorescence products, MUF, and MCA, by the internal standard approach.

Sole Carbon Source Utilization Profiles

Biolog Ecoplates®, consisting in three replicates of 31 wells with different carbon sources and three control wells without any carbon source, were used to characterize the profiles of sole carbon source utilization of distinct bacterial assemblages. In addition to the specific carbon source, each well contains a minimal growth medium and tetrazolium salt which turns purple in the presence of an active electron transfer system, indicating that the substrate is being utilized by the microbes in the inocula [24].

The utilization of different sole carbon sources was analyzed only in three depth horizons (0-1, 5-6, and 9-10 cm) of each rhizosphere and in unvegetated sediment.

Cell suspensions were obtained by incubating 2.5 g fresh sediment in 20 ml of sterile Ringer solution with glass beads for 2 h at 4 °C, with shaking. The resulting suspension was centrifuged at 1,000×*g* (IECB-22M centrifuge) for 5 min. Approximately 130 μL aliquots of the resultant supernatant were inoculated in each well of the Biolog Ecoplates® with a multipipettor. For each sample, two replicate microplates were inoculated and one microplate inoculated with sterile Ringer solution was used as a negative control. All plates were incubated at room temperature, without agitation, in the dark, for 36 h, which was previously defined as the time necessary to achieve an average well color development (AWCD) >0.7 in all samples. The optical density (λ=590 nm) of each well was determined in a microplate reader (TECAN Sunrise) immediately after inoculation (0 h) and at the end of the incubation (36 h). The utilization of the carbon sources was estimated from the average of the OD_{590} in the three replicates of each substrate, subtracted of the average OD_{590} of the blank wells. The AWCD for each sample was calculated as the mean value of corrected absorbance in the 93 wells containing carbon sources, corrected for the absorbance of the blank wells.

Statistical Analysis

The statistical analyses were performed with SPSSWIN 12.0 software. Significant differences in ectoenzymatic activities at different sediment horizons were assessed using one-way analysis of variance (ANOVA). The normality of the data set was confirmed by the Kolmogorov-Smirnov test. Pearson's coefficient was calculated in order to assess correlation between sediment properties and microbiological descriptors. A multiple stepwise linear regression analysis was used to identify the major sources of variability of microbiological descriptors (dependent variables). Physical and chemical parameters were used as independent variables for which autocorrelation were checked.

Sole carbon source utilization data provided by the Biolog Ecoplate® approach were used in an ordination analysis. The bi-dimensional representation of the similarity between selected samples, assessed by a Euclidean distance

Table 1 Water content and grain-size analysis of the different depth layers at the control unvegetated site and at the monospecific vegetation banks

Sample (cm)		Water content (%)			Grain-size analysis		
		Nov.	Feb.	Sep.	% Fines	Median (φ)	Sediment classification
Sediment without vegetation	0-1	13.3	9.5	17.3	35.2	2.24	Very fine silt sand
	3-4	15.1	11.1	14.2	26.0	1.91	Very medium silt sand
	5-6	13.0	10.7	18.0	25.2	1.92	Very medium silt sand
	7-8	10.8	8.1	10.9	21.0	1.82	Silt medium sand
	9-10	18.6	14.7	14.1	19.5	1.80	Silt medium sand
	11-12	13.7	17.9	10.3	17.8	1.73	Silt medium sand
	13-14	12.1	7.2	12.1	13.5	1.77	Silt medium sand
	15-16	10.3	11.9	11.8	11.4	1.74	Silt medium sand
Spartina maritima bank	0-1	11.4	9.1	19.1	85.3	4.42	Mud
	3-4	20.6	13.7	25.4	71.1	4.30	Mud
	5-6	17.0	10.4	25.3	83.6	4.41	Mud
	7-8	26.8	15.1	20.4	92.8	4.46	Mud
	9-10	24.7	18.0	30.9	93.6	4.47	Mud
	11-12	11.9	12.8	22.2	88.4	4.43	Mud
	13-14	17.7	9.4	20.4	83.3	4.40	Mud
	15-16	13.6	9.4	28.9	76.7	4.35	Mud
Halimione portulacoides bank	0-1	24.9	10.1	33.9	93.4	4.47	Mud
	3-4	24.7	16.2	22.2	94.8	4.47	Mud
	5-6	25.2	12.9	26.3	96.0	4.48	Mud
	7-8	26.1	13.0	23.5	97.5	4.48	Mud
	9-10	28.0	13.8	21.7	94.3	4.48	Mud
	11-12	34.4	16.9	13.5	95.8	4.48	Mud
	13-14	33.1	11.9	26.4	92.5	4.46	Mud
	15-16	18.0	14.2	18.0	69.0	4.27	Mud

model, using the corrected OD_{590} values for each substrate as a measure of metabolic activity, was obtained by the multidimensional scaling (MDS) method.

Results

Water content in relation to sediment fresh weight ranged from 7.2% at control sediments to 34.4% at the *H. portulacoides* bank. In general, colonized sediments showed higher relative water content and finer texture than the control sediment. The granulometry of control sediment was characterized by a variety of particle sizes, ranging from very fine to medium silty sand (Table 1). The highest content in fine particles was found in sediments of the vegetation banks, with the fraction of fine particles varying between 69.0% and 97.5% of the sediment dry weight.

The average prokaryote abundance for each sediment type was 6.9×10^8 cells gdw^{-1}, 9.9×10^8 cells gdw^{-1} and 8.3×10^8 cells gdw^{-1} for control sediments, sediments of the *S. maritima* bank, and sediments of the *H. portulacoides* bank, respectively, being the differences statistically significant (ANOVA, $p<0.05$) The vertical distribution of prokaryotes was characterized by maxima at the surface (Fig. 2) and a general decrease of abundance with increasing depth. Unvegetated sediments were vertically more homogeneous as to cell abundance. The highest cell densities (1.7×10^9 cells gdw^{-1}) were observed in the upper sediment layer at the *S. maritima* bank (Fig. 2).

The vertical profiles of variation of potential maximum activity of β-glucosidase, α-glucosidase, aminopeptidase, arylsulfatase, and phosphatase are presented in Fig. 3. As a general trend, the rates of polymer hydrolysis were significantly higher ($p<0.05$) in surface sediments and decreased along the following 4-6 cm. Below this level, a background of low hydrolytic activity was reached. The gradient was steeper from the first (November 2007) to the third (September 2008) sampling campaign. In the profiles corresponding to the month of September, there was also a slight increase in activity at the 13-14 cm depth layer, for all tested enzymes.

The decrease of activity between surface and sub-surface sediments was in most cases, sharper in the vegetation banks than in unvegetated sediment, which were vertically more homogeneous. Globally, unvegetated sediments showed lower activity rates. The highest rates were more often observed in sediments of the *H. portulacoides* bank. Aminopeptidase showed the highest activity rates (0-3,995 nmol $gdw^{-1}h^{-1}$), and α-glucosidase presented the lowest (0-80 nmol $gdw^{-1}h^{-1}$).

The intensity of the utilization of sole carbon sources, expressed by the AWCD in each sediment type varied with depth and with different types of sediments (Fig. 4). The

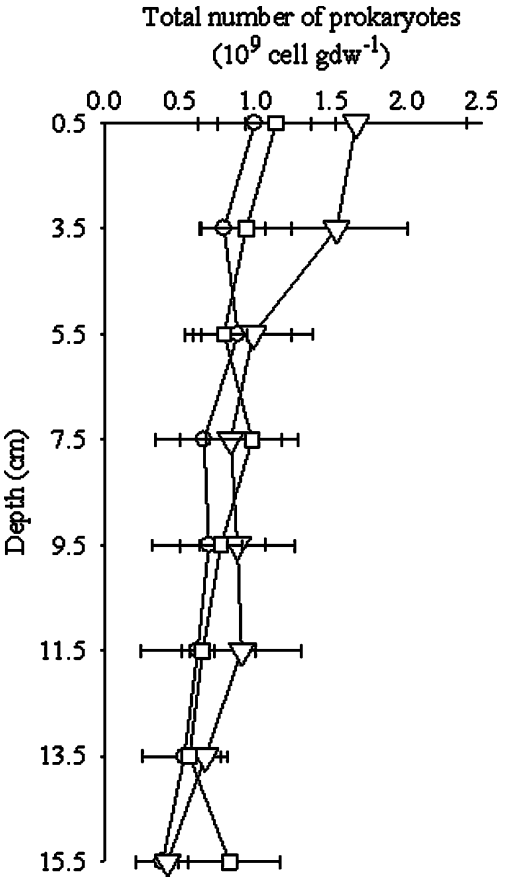

Figure 2 Vertical abundance of prokaryote cells after DAPI staining in unvegetated sediments (○); sediments from *Spartina maritima* bank (▽) and sediments from *Halimione portulacoides* bank (□). *Error bars* represent the standard deviation of the three replicates

average rate of substrate respiration was highest at the 0-1 cm layer of sediment, at all sites. The highest value of AWCD corresponded to the *H. portulacoides* bank and the lowest to the unvegetated sediment. The average utilization of the carbon sources of the Biolog Ecoplate® decreased with depth. The decline was steeper in sediments of the *H. portulacoides* bank.

The utilization of the individual sole carbon source of the Biolog Ecoplate® in all sediment types, expressed as the corrected average values of color development for each substrate after 36 h of incubation, are summarized in Fig. 5. The sugars D-mannitol, N-acetyl-D-glucosamine and glycogen were the most utilized substrates in salt marsh sediments. 2-Hydroxy-benzoic acid was the least used carbon source in all samples.

The analysis of the utilization of sole carbon sources grouped according to their chemical nature indicates that carbohydrates and polymers were the preferred substrates in unvegetated sediments and in sediments of the two vegetation banks, followed by carboxylic acids (Fig. 6). In contrast, phenolic and phosphorilated substrates were the least used substrates. The pattern of vertical variation of the

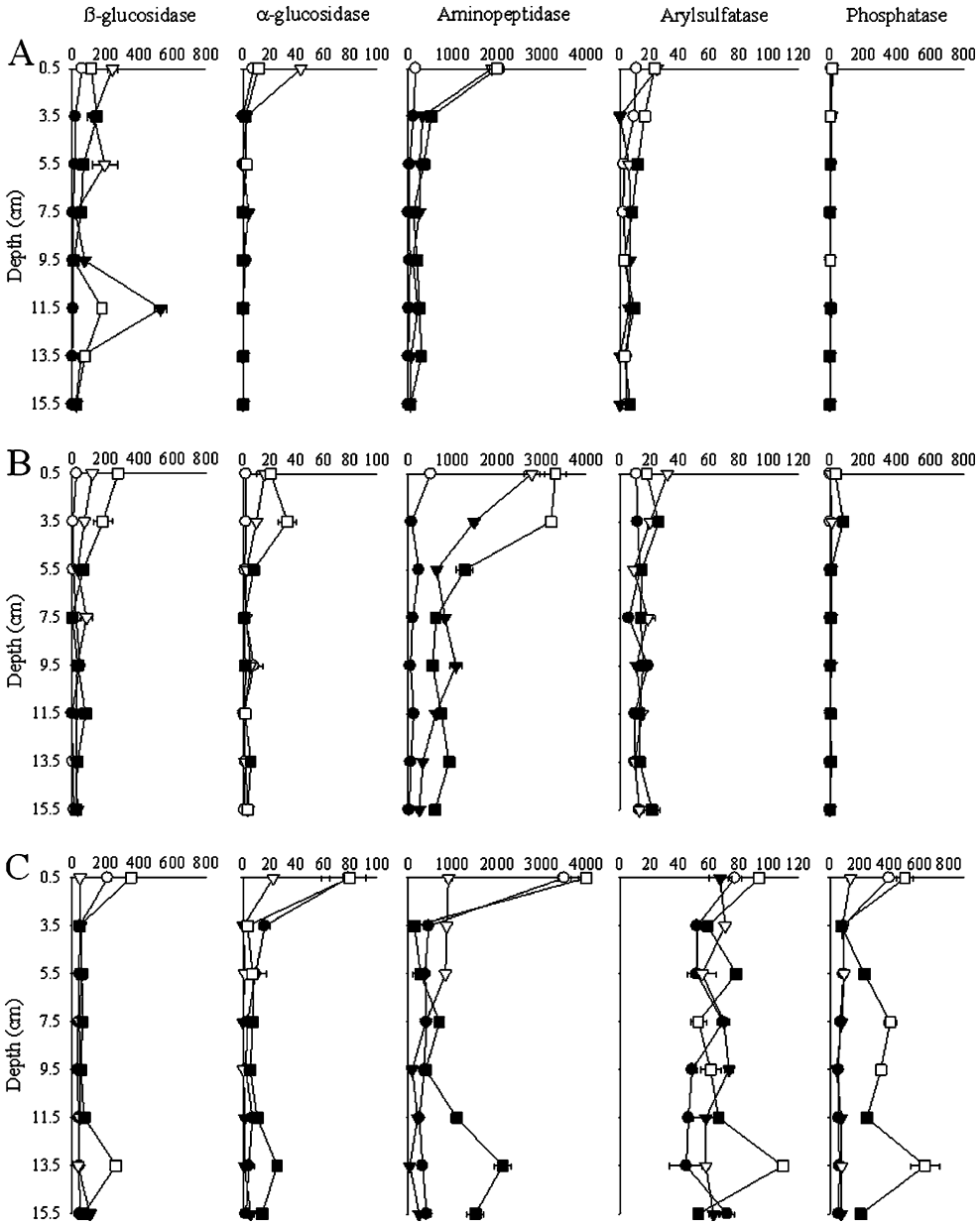

Figure 3 Profiles of extracellular enzymatic activity of β-glucosidase, α-glucosidase, aminopeptidase, arylsulfatase, and phosphatase (nmol $gdw^{-1}h^{-1}$) in unvegetated sediments (○); sediments from *Spartina maritima* bank (▽) and sediments from *Halimione portulacoides* bank (□) in November 2007 (**a**), February 2008 (**b**) and September 2008 (**c**). *Error bars* represent standard deviation of three replicates. *Solid symbols* correspond to values that are significantly different from the value of the upper sediment layer, in the same sediment column (one-way ANOVA, $p<0.05$)

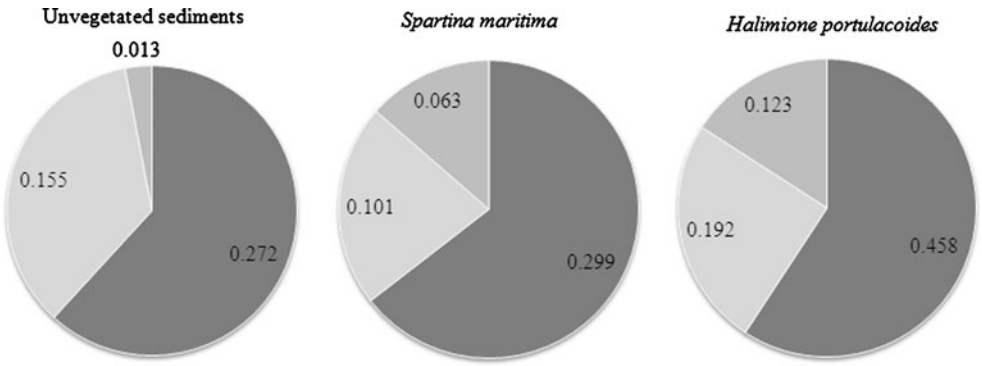

Figure 4 Average of values of AWDC (OD_{590}) on different depth horizons of the studied sediment types: 0-1, 5-6, 9-10 cm

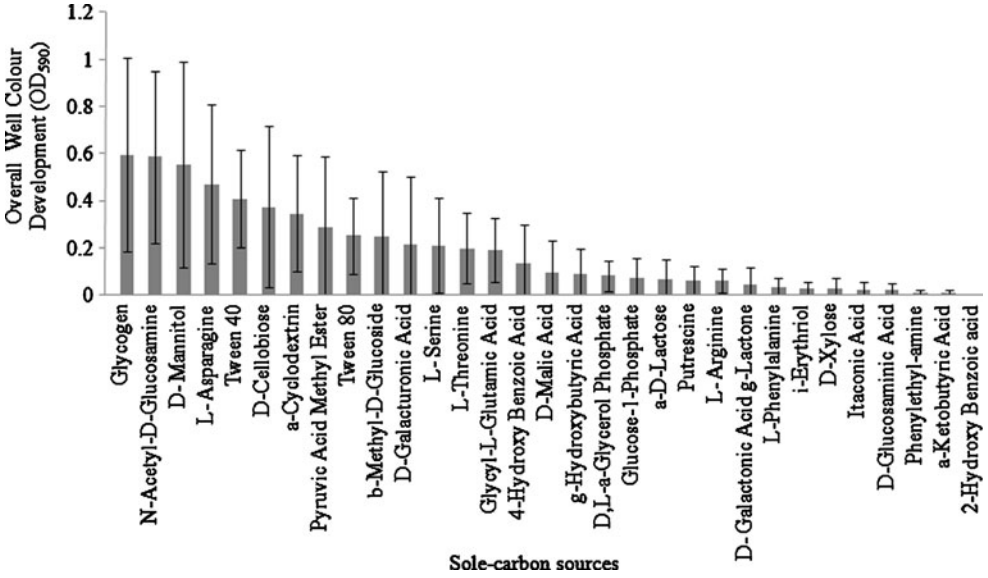

Figure 5 Values of overall well color development (OD_{590}) of the 31 different substrates of Biolog Ecoplates® after 36 h of incubation averaged for all sediment samples

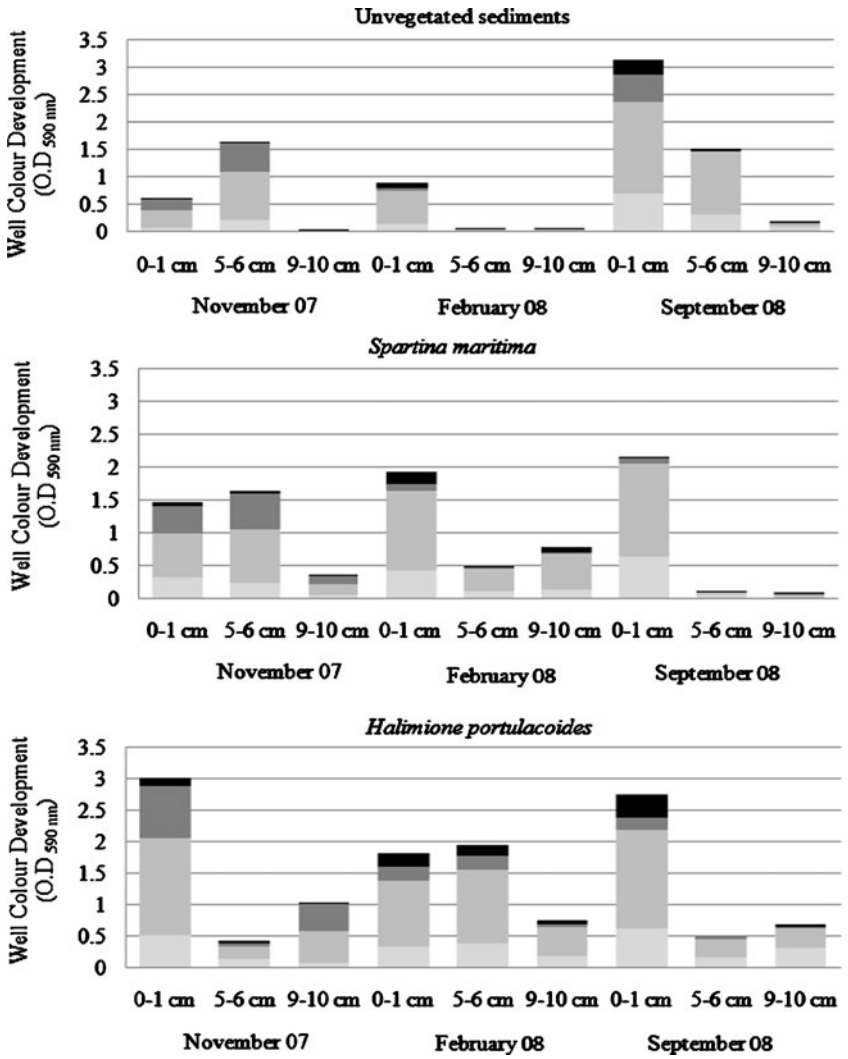

Figure 6 Utilization sole carbon sources grouped according to their chemical classification in the different types and depth layers: Amino acids and amines; carbohydrates and polymers; carboxylic acids; phenolic and phosphorylated compounds

rate of utilization of the different groups of substrates shows the same trend as described for the values of AWCD, and corresponds to a decrease of activity with increasing depth (Fig. 6). The rates of sole carbon source utilization showed signs of seasonal variation with higher values of AWCD being obtained in later summer (September 2008).

The bidimensional plots obtained by MDS analysis of the Biolog Ecoplate® data using Eucledian distance as a similarity index are presented in Fig. 7. The communities developing in the 0-1 cm layer of sediment at the vegetation banks were always physiologically distinct from the communities found in deeper sediment layers. In unvegetated sediments, the difference between the upper layer and the rest of the sediment column was only found in September 2008. Below the 5-6 cm layer, bacterial communities in colonized and unvegetated sediments were physiologically more similar.

The results of the analysis of correlation between sediment properties and descriptors of bacterial abundance and activity are presented in Table 2. Total prokaryote abundance, β-glucosidase, α-glucosidase, aminopeptidase, and the average rate of utilization of the 31 sole carbon sources of Biolog Ecoplates® (AWCD) correlated negatively with sediment depth. Arylsulfatase and phosphatase did not show significant correlation with depth but rather a positive correlation with water content. The abundance of cells and the activity of β-glucosidase also showed positive correlation with the percentage of fine particles. Total prokaryote abundance was not significantly correlated with AWCD, but was positively correlated with the activity of all the extracellular enzymes tested (Table 2).

The results of stepwise multiple regression analysis is presented in Table 3. The independent variables related to depth and sediment texture explained 80% of variability of total prokaryote abundance. However, these descriptors explained only 23% (α-glucosidase) to 57% (phosphatase) of the variability of the activity of ectoenzymes. Depth was included in the regression models with a negative relation with all the enzymes with the exception of β-glucosidase, which, in turn, was negatively affected by the sediment water content.

Discussion

This work was structured around the hypothesis that sediment colonization by salt marsh plants has direct effects on bacterial heterotrophic processes (organic matter decomposition and mineralization) through the release of root exudates and deposition of plant material, and indirect effects exerted by changes in the erosion/deposition rates. For the assessment of direct effects, the rates of extracellular enzymatic activity and the patterns of utilization of sole carbon sources were analyzed. Indirect effects were inferred from the relations between bacterial distribution and activity and sediment textural properties. In addition, we tried to demonstrate that different species of salt marsh

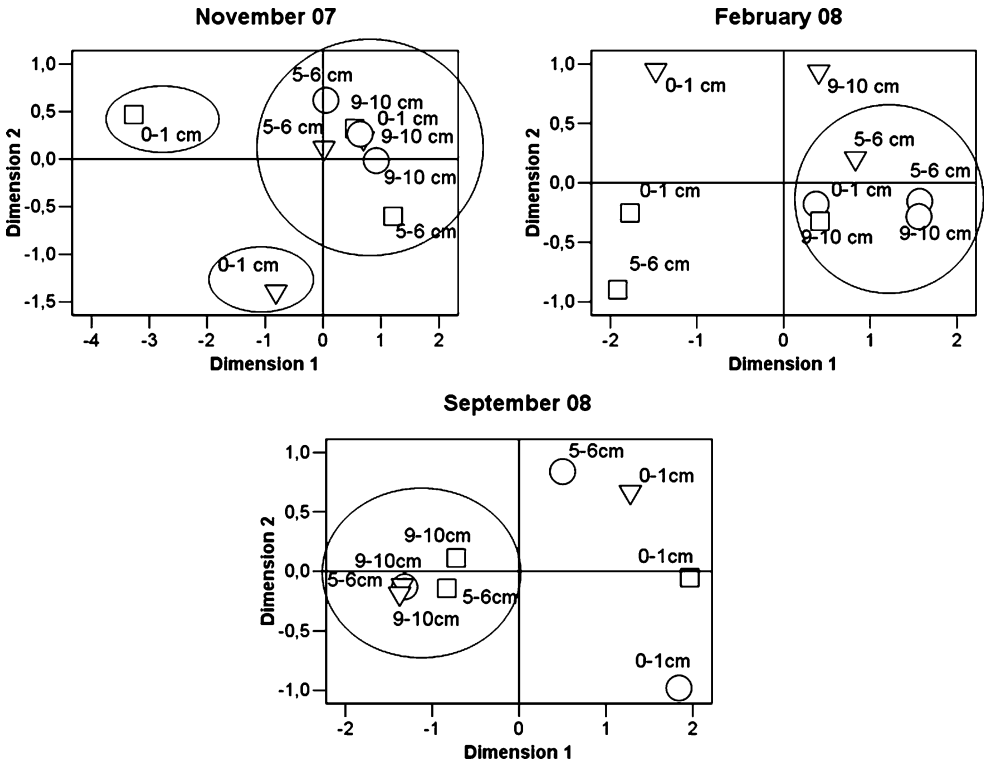

Figure 7 Bi-dimensional plot of similarity between the sole carbon source utilization profiles of bacterial communities in control sediments (○); sediments from *Spartina maritima* bank (▽) and sediments from *Halimione portulacoides* bank (□)

Table 2 Pearson's product–moment correlation coefficients (r) between sediment characteristics and microbiological variables

Parameters	Depth	Water content	% fines	Median (φ)	Total prokaryote abundance	β-glucosidase	α-glucosidase	Aminopeptidase	Arylsulfatase	Phosphatase	AWCD
Depth	1.000										
Water content	−0.056	1.000									
% fines	−0.118	0.690**	1.000								
Median (φ)	−0.047	0.488**	0.982**	1.000							
Total Prokaryote Abundance	−0.758**	0.005	0.341	0.361	1.000						
β-glucosidase	−0.266*	0.214	0.446*	0.348**	0.427*	1.000					
α-glucosidase	−0.433**	0.129	0.160	0.073	0.677**	0.541**	1.000				
Aminopeptidase	−0.469**	0.073	0.380	0.296*	0.645**	0.576**	0.833**	1.000			
Arylsulfatase	−0.106	0.337**	0.281	0.110	0.538**	0.214	0.455**	0.368**	1.000		
Phosphatase	−0.094	0.353**	−0.150	0.152	0.665**	0.368**	0.617**	0.499**	0.766**	1.000	
AWCD	−0.688**	0.007	0.301	0.201	0.490	0.644**	0.718**	0.813**	0.337	0.469*	1.000

r values lacking an asterisk were not significant

*$p<0.05$; **$p<0.01$ level

plants impose special features on the bacterial communities such as observed for several species of cultivated plants [3].

The results show that prokaryotes in plant-colonized sediment banks are more abundant than in unvegetated sites and that this trend is enhanced in the upper sediment layer especially in the *S. maritima* bank. The availability of substrates for growth, in addition to temperature, is reported in the literature as the most relevant factor of regulation of bacterial dynamics in the coastal environments [5, 6, 31]. Highest cell abundance in surface sediments is a common feature of the vertical patterns of bacterial distribution in sediments [30, 39] and it develops as a consequence of higher oxygen availability, sedimentation of organic particles from the water column, and inputs of organic carbon derived from benthic primary production [14, 18]. Although organic carbon was not analyzed in this work, the vertical decline of abundance was steeper in plant-colonized sediments and may indicate that plant-derived materials influence the abundance of prokaryotes in the surface sediment. The enrichment of surface sediments may result from the combination of inputs from root-derived products and also from the deposition of detrital material from the aerial portion of the plants [7]. Moreover, even if free oxygen leaks from plant roots creating an oxic surrounding [3] some dependence on alternative inorganic electron acceptors may induce competition with the plants that use them as nutrients. The outcome of the competition for inorganic compounds may contribute to the decrease in bacterial abundance in the deeper sediment layers.

In addition to the direct release of plant-derived substrates suitable for bacterial growth, salt marsh vegetation may also enhance sediment microbial colonization by modifying the sediment physical structure. Sediment properties and plant attributes cooperate in the shaping of bacterial communities and the relative contribution of both interplayers is difficult to assess [3]. Changes in microbial abundance within scales of a few centimeters in coastal sediments are related to combined effects of the chemical environment and of the physical properties of the sediments [29]. The establishment of plant communities affects sediment properties by inducing shifts in the rates of erosion and sedimentation [53]. In this work, a high proportion (80%) of the variability of bacterial abundance could be explained by physical properties of the sediment and prokaryote abundance was directly related with the median φ and with the percentage of fines. This effect may also correspond to an indirect relation with the content in organic matter. Sediments from the vegetation banks were classified as mud whereas unvegetated sediments, collected at a lower level of the intertidal zone and consequently more exposed to inundation and tidal forcing, were composed by sand with variable silt content. At the unvegetated site, the sediment texture was characterized

Table 3 Regression equations for the variation of microbiological parameters obtained from stepwise multiple regression analysis

Dependent variable	Independent variables	Regression equation	Adj. R^2
TPA	Depth (β=-0.897; p=0.000) Median φ (β = 2.430; p=0.000) % fines (β=-2.151; p=0.001)	TBN=4.7E+08 – 5.5E+07 Depth+5.9E+08 Median φ – 1.9E+07 % fines	0.796
β-glucosidase	Median φ (β=0.754; p=0.003) Water content (β=-0.490; p=0.039)	β-glucosidase=-13.979+70.544 Median φ - 7.834 Water content	0.291
α-glucosidase	Depth (β=-0.516; p=0.010)	α-glucosidase = 11.120 - 0.948 Depth	0.233
Aminopeptidase	Depth (β=-0.567; p=0.002) Median φ (β=0.340; p=0.047)	Aminopeptidase = 297.682 - 60.402 Depth + 142.829 Median φ	0.403
Arylsulfatase	Depth (β=-0.636; p=0.001)	Arylsulfatase = 14.488 - 0.849 Depth	0.377
Phosphatase	Depth (β=-0.770; p=0.000)	Phosphatase = 10.996 - 0.725 Depth	0.574

Dependent variables=total prokaryote abundance (TPA), β-glucosidase, α-glucosidase, aminopeptidase, arylsulfatase, and phosphatase; independent variables=depth, water content, median φ, % fines

by a lower percentage of fines and median φ, establishing a clear relation between sediment texture and microbial colonization. At the vegetation banks, sediments were more homogeneous with only a slight change in texture at the 15-16 cm layer which corresponds approximately to the limit of root length. The colonization of intertidal sand or mud banks with salt marsh vegetation enhances the retention of fine particles from the water column [57].

Microbial communities have access to an elevated supply of carbon and energy rich materials from plant roots [33, 43]. The patterns of extracellular enzymatic activity in the salt marsh in which this study was conducted, confirm the development of more active heterotrophic microbial communities in sediments of *H. portulacoides* and *S. maritima* banks than in unvegetated sediments.

Extracellular enzymatic hydrolysis is often the first and limiting step in the process of bacterial organic matter degradation [6, 38, 54]. Depending on the particular sediment bank and on the type of extracellular enzyme, the potential for polymer hydrolysis was up to three times higher under the influence of roots than in unvegetated sediments denoting an adaptation towards the quantity and also the quality of the available organic sources.

Aminopeptidase and β-glucosidase were the most active enzymes. Aminopeptidase is an essentially periplasmic activity widespread in aquatic bacteria [36] related to heterotrophic activity. High rates of protein degradation occur in systems that are impacted by raw or treated domestic wastewater [10, 22]. The α-glucosidase and β-glucosidase activities relate to primary production [54] and reflect the origin of the polysaccharides being used by bacteria [58]. High rates of activity indicate that plant-derived detritus may be an important source of carbon in the upper sediment layers. Phosphatase is produced by bacteria and plants in sediments and it is related with P acquisition in nutrient-deficient environments [47]. Arylsulfatases are widespread in soils and sediments being produced by bacteria, fungi, and animals and are related to the initiation of breakdown of arylsulfate esters for the microbial community providing free sulfate for organisms to grow [15, 41]. Salt marsh sediments are usually not sulfate limited which explains the low activity of arylsulfatase. However, the marked increase of arylsulfatase and phosphatase activities in September 2008 may reveal nutrient limitation or competition with salt marsh vegetation or with benthic primary producers in late summer. Also, some phosphatase activity may not be related to bacterial activity but correspond to root-derived dissolved enzymes since exudation of phosphatases from the roots is one of the strategies used by plants to mitigate phosphate deficiency [48]. The life cycle of plants also explains the seasonal differences observed in enzyme activities. In September, both the plants are in the flowering stage when nutrient demand is enhanced [17], with enhanced needs in terms of nutrients [23, 27] thus competing with microorganisms for the available mineral resources [32].

The results suggest that different species of salt marsh plants impose different levels of effects upon bacterial activity in the sediment banks. The ranges of activity of different extracellular enzymes can be used as an indicator of the spectra of biodegradable polymeric substrates [38]. In general, *S. maritima* banks showed denser bacterial colonization but the highest rates of extracellular enzymatic activity were more often found at the *H. portulacoides* bank indicating that cells in sediments of this vegetation bank are, on average, individually more active in the processes of polymer extracellular hydrolysis.

Biolog Ecoplates® are frequently used as a culture-dependent approach to distinguish the catabolic potential among different types of sediments [16] and with the appropriate adaptations and standardizations it is considered to give useful information even in anaerobic habitats [11,

12]. In this study, information on the total cell abundance was available from the epifluorescence cell counts and standardization of inoculum density was not considered necessary because values varied onlythreefold and this variation is meaningful in terms of differences between samples. Also, taking into consideration that colonized sediments receive free oxygen from plant roots, incubation of cell suspensions was conducted in the presence of oxygen, and therefore, the physiological profiles correspond to the fraction of the community involved in aerobic respiration. However, the analysis of the profiles of utilization of the sole carbon sources provided by the Biolog Ecoplates®, could not establish clear differences in the physiological diversity of the bacterial communities inhabiting deeper layers of the sediment banks colonized by different plant species. The only consistent physiological differences are restricted to surface sediments of the vegetation banks, which at this site generally correspond to a sub-oxic layer [19, 49], indicating that organic matter derived from the above ground portion of the plant may be considerably distinct in composition and nutritional quality. In fact, the higher bacterial activity in the surface of the plant-colonized sediment banks can be explained by the accumulation of more biodegradable polymeric material at surface, originated from the water column and from plant detritus. This hypothesis was supported by the pattern of utilization of different groups of substrates. Our results show that polymers and carbohydrates were the most used carbon sources in the surface sediments, such as observed in other studies [50, 51]. These compounds represent a significant proportion of root released DOM [4] and are considered as the more bioavailable carbon sources in sediments influenced by root exudates [55].

Considering that (1) bacterial communities associated to sediments colonized by different plant species show distinct vertical profiles of abundance and extracellular enzymatic activity; (2) that with the exception of the uppermost sediment layer, bacterial communities are similar in terms of sole-carbon-source utilization profiles; (3) and that the content in organic matter (loss by ignition) within the layer that is influenced by plant roots (0-10 cm) is approximately 10% of sediment dry weight in both vegetation banks (data not shown) we can further hypothesize that the nature and the relative abundance of polymeric substrates, rather than the total amount of available organic matter, may be major factors underlying the specific imprint of each plant on the associated sediment bacterial assemblages.

Conclusion

The results from this work support the hypothesis that the salt marsh plant colonization of sediment banks in intertidal zones has effects on the activity of prokaryote communities and enhances heterotrophic processes of carbon recycling. Plant-specific effects were in general detectable on the profiles of polymer hydrolysis but not on community level physiological profiles. The differences between the bacterial communities of the sediments of monospecific banks of different salt marsh plants in terms of cell abundance and extracellular enzymatic activity demonstrate the existence of plant species-specific effects on the processes of diagenesis of complex organic substrates. As a consequence, it is predictable that changes in the pattern of plant colonization may significantly affect the biogeochemical cycles in estuarine systems. However, in terms of the utilization of sole carbon sources for respiration, sub-surface microbial communities were not significantly distinct neither between different sediments of distinct vegetation banks nor between salt marsh and unvegetated sediments. Mixed assemblages preserve the capacity for the utilization of a broad spectrum of simple substrates making them extremely adapted to mineralize under the high temporal and spatial variability that characterizes estuarine sediments, particularly under the influence of plant roots.

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