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Graphical Abstract

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Use of semi-quantitative kit methods to study the heterotrophic bacterial community of *Posidonia oceanica* meadows: Limits and possible applications

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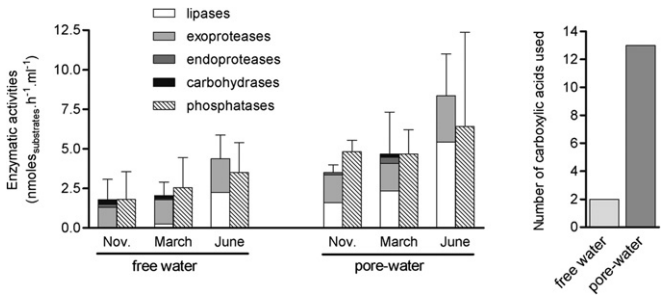

Highlights

► We study the heterotrophic bacterial community of *Posidonia oceanica* meadows. ► We investigate ecological key processes with semi-quantitative kit methods. ► Results are consistent with studies applying commonly used methods in Microbiology.

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ABSTRACT

Rapid, easy and low cost semi-quantitative methods were tested to study the heterotrophic bacterial community of *Posidonia oceanica* meadows and were compared to techniques commonly used in microbial ecology. Free and pore-water bacterial densities were estimated by luminescence, and principal enzymatic activities, metabolic capabilities and benthic mineralisation processes were studied with microtitration methods: ApiZym galleries, Biolog microplates and BART™ tests. Bacterial densities varied little throughout the year and were around $5.0 \cdot 10^5$ and $6.0 \cdot 10^6$ cells ml^{-1} of free and pore-water, respectively. The combined use of the ApiZym gallery and the Biolog microtitration plate permitted highlighting bacterial enzymatic activities susceptible to degrade principal organic polymers present in the *Posidonia* meadow, and to correlate these enzymatic activities to the subsequent potential utilization of resulting monomeric products. Levels of enzymatic activities (1.80–8.36 $nmoles_{substrates}\ h^{-1}\ ml^{-1}$) and energetic bacterial metabolism (1.80–6.42 $nmoles_{substrates}\ h^{-1}\ ml^{-1}$) presented seasonality relying on the temperature regime and on the primary production (*Posidonia* and phytoplankton). Main mineralization processes of buried organic matter through sulfate and iron reduction activities were successfully detected. Despite the complexity of the studied ecosystem, results obtained by this semi-quantitative approach, compared to studies applying commonly used methods in microbial ecology, highlighted the same bacterial dominant key processes. Their low cost, rapid and easy use, and the low level of expertise and sophistication they require means that these techniques are of use to many employed in environmental surveys.

1. Introduction

Posidonia oceanica (L.) Delile, the endemic marine magnoliophyte of the Mediterranean Sea, forms highly productive meadows from the surface down to 40 m depth (Boudouresque and Meinesz, 1982; Duarte and Chiscano, 1999; Gobert et al., 2006a). In coastal ecosystems dominated by macrophytes, most of the primary production is not immediately used by macro-consumers (Mann, 1988). It is fragmented and chemically modified before entering the food chain, or is transferred, via bacterivores, to higher trophic levels after its transformation into bacterial biomass (Velimirov and Walenta-Simon, 1992). Moreover, the final decomposition of most *Posidonia* detritus takes place in sediments (Marbà et al., 2006; García-Martínez et al., 2009). Dissolved organic carbon (DOC) and organic exudates represent another source of organic matter (OM) more easily mobilisable for bacterial growth (Murray and Wetzel, 1987; Navarro et al., 2004). The productivity of the heterotrophic bacterial community of the *Posidonia* bed water column also depends on temperature: higher under summer (above 16 °C) and lower under winter (bellow 16 °C) temperature regimes (Velimirov and Walenta-Simon, 1992, 1993). Benthic mineralization rates also increase with increasing temperatures (Pedersen et al., 2011).

Bacterial densities, microbial metabolic capabilities or enzymatic activities, and detrital organic matter mineralization have been already extensively studied in *Posidonia* meadows. Classic methods used for these purposes give quantitative and relevant results, but require a high level of expertise and sophistication, are expensive and time-consuming, and not easily applicable to large environmental surveys by concerned field workers. Semi-quantitative kit assays can be performed as an alternative to these methods. Holm-Hansen and Booth (1966) proposed to use ATP as an indicator of micro-organism biomass in the environment.

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This method relies on the endergonic reaction between firefly luciferase and its substrate, luciferin; it consumes bacterial ATP and liberates photons proportionally to the bacterial density (Poulicek et al., 2000). The environmental community-level carbon utilization Biolog assay has been used in many studies both in terrestrial and aquatic environments (e.g. Matsui et al., 2001; Antony et al., 2011). The possible utilization of the 95 carbon substrates proposed by this microtitration plate allows characterizing the metabolic fingerprint of a natural microbial community (van Heerden et al., 2002; Stefanowicz, 2006). Techniques based on the use of chromogenic substrates allow to detect specific enzymatic activities of the microbial community of environmental samples (Humble et al., 1977). Relative activities of hydrolase exoenzymes against amino acids, carbohydrates, fatty acids, lipids, proteins and phosphorus compounds can be easily estimated using Apizym galleries (Chisholm et al., 1997; Fernex et al., 2001). Biological activity reaction tests (BART™), originally developed to assess biological activity associated with nuisance bacteria in water wells, were successively used to detect the presence and activity of indigenous denitrifying, iron-related and sulfate-reducing bacteria in oil contaminated groundwater samples (Cross et al., 2006).

There is a growing demand of environment managers to have at disposal easy, cheap but reliable methods to characterize the functioning and the health status of ecosystems (e.g. Gobert et al., 2010). A panel of existing techniques developed in microbiology was thus tested to highlight ecological key processes of the heterotrophic bacterial community of *Posidonia oceanica* meadows. Observed trends are compared to results obtained with methods commonly used in microbial ecology in order to find out whether the applied methodology has potential for future applications.

2. Material and methods

2.1. Sites and sampling procedures

The Calvi Bay is situated in the North-West of Corsica. The benthic ecosystem is dominated by a dense *Posidonia oceanica* bed from the surface down to 40 m depth. Free water, at different depths, and pore-water samples were collected using scuba in November 2006, March and May 2007 (Table 1) in 5 sites of the Calvi area (Fig. 1): Punta Bianca (16 m depth), outside of the Calvi Bay influence; Calvi city (13 m depth) in the vicinity of the pipe discharging at 36 m depth the physicochemically-treated domestic wastewaters of Calvi city (Vermeulen et al., 2011); Stareso-1 (12m depth), close to the STARESO oceanographic station; Stareso-2 (15m depth), on a bare banana-shaped sand patch enclosed in the *Posidonia* bed; Stareso-3 deeper site (30m depth), distant from the STARESO. All sites are colonized by a healthy *Posidonia* bed, except for Stareso-2 which is a bare site. Free water was sampled with 60 ml sterile plastic syringes (1 syringe by sample); pore-water was sampled with a 4 interconnected stainless steel needles (1 syringe by sample), linked up to syringes with a fine plastic tube (Gobert et al., 2006b). Syringes were immediately returned to the laboratory of the STARESO for sample analyses and incubations (within 1 h). Benches and materials were previously carefully cleaned with alcohol.

2.2. Environmental parameters

Seawater temperature (°C) was recorded with a minilog probe placed at 3 m depth in the STARESO harbour. Chlorophyll *a* concentrations (μg L^{-1}) were measured by HPLC according to Williams and Claustre (1991) on samples collected regularly (every 6 days in average) at 1 m depth close to the STARESO. Rainfall (mm day^{-1}), wind speed (m s^{-1}) and wind direction (Â°) data were

Table 1
Mean (±SD) bacterial densities (10^5 cells ml^{-1}) ($n = 4$) measured in the Punta Bianca, the Calvi city, Stareso-1 *P. oceanica* bed 12 m depth, Stareso-2 bare sand patch and Stareso-3 *P. oceanica* bed 30 m depth, in pore-water samples and at different depths in the water column (bot. = from bottom) in November, March and May. nd = no data.

Site (depth m)	Punta Bianca (16 m)		Calvi city (13 m)		Stareso-1 (12 m)			Stareso-2 (15 m)			Site (depth m)	Stareso-3 (30 m)		
Sampling date	11/14 (2006)	nd	11/14 (2006)	nd	11/11 (2006)	03/21 (2007)	05/30 (2007)	11/12 (2006)	03/21 (2007)	05/30 (2007)	Sampling date	nd	03/23 (2007)	05/29 (2007)
Sampling depths of vertical profiles and corresponding bacterial densities														
Surface layer	nd	nd	nd	nd	nd	7.67 ± 0.78	3.79 ± 0.39	nd	8.71 ± 0.88	3.70 ± 0.41	Surface layer	nd	4.17 ± 0.23	5.66 ± 0.16
1 m depth	1.10 ± 0.14	nd	1.93 ± 0.08	nd	2.12 ± 0.58	6.69 ± 0.59	3.92 ± 0.39	1.95 ± 0.52	9.21 ± 1.46	3.30 ± 0.41	1 m depth	nd	7.18 ± 1.45	5.51 ± 0.46
3 m bot.	1.44 ± 0.19	nd	3.61 ± 0.55	nd	2.32 ± 0.11	6.94 ± 0.24	3.60 ± 0.25	1.93 ± 0.48	8.23 ± 0.90	4.29 ± 0.25	10 m depth	nd	7.38 ± 2.57	6.90 ± 0.44
											20 m depth	nd	4.63 ± 0.98	8.27 ± 0.22
Canopy/20 cm bot.	1.17 ± 0.30	nd	2.28 ± 0.31	nd	5.35 ± 2.79	3.99 ± 0.26	4.44 ± 0.45	1.85 ± 0.14	6.45 ± 0.79	3.58 ± 0.18	Canopy	nd	3.50 ± 0.07	5.58 ± 0.40
Sediment interface	nd	nd	nd	nd	nd	4.67 ± 0.12	4.99 ± 0.76	nd	6.66 ± 0.59	4.28 ± 0.22	Sediment interface	nd	6.38 ± 0.26	13.50 ± 2.15
Pore-water	108.67 ± 7.88	nd	85.69 ± 11.78	nd	68.71 ± 15.55	56.94 ± 5.65	111.67 ± 14.01	52.04 ± 3.08	39.27 ± 3.11	40.25 ± 2.88	Pore-water	nd	31.01 ± 0.50	15.12 ± 0.70

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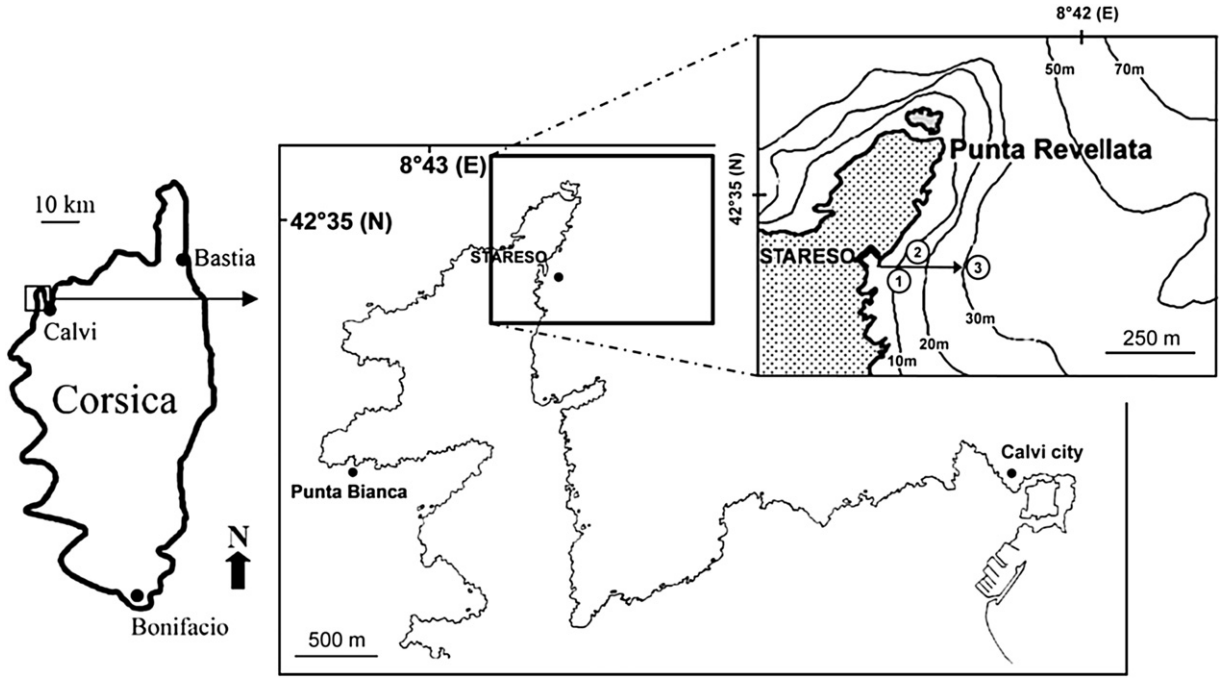

Fig. 1. Location of sampling sites. Punta Bianca, Calvi city and 3 sites distant from the STARESO oceanographic station (numbered circles in the STARESO area zoom) along a West-East axe from the coast (1 = Stareso-1 *P. oceanica* bed 12 m depth; 2 = Stareso-2 sand patch; 3 = Stareso-3 *P. oceanica* bed 30 m depth).

purchased from the meteorological station of the Calvi airport. Orthophosphates (PO_4^{3-}), nitrates (NO_3^-) and ammonium (NH_4^+) were measured in water samples (excepted Stareso-2 sand patch) with a nutrient automatic chain analyzer (SAN-Skalar; method described by Strickland and Parsons (1972) for an automatic system (Grasshoff et al., 1999) adapted for oligotrophic environments).

2.3. Bacterial community study

Bacterial densities of free and pore-water samples were quantified with a Lumac Biocounter M1500 luminometer. RapidScreen™ H&B kits were purchased from Celsis International B.V. 100 µl of Celsis LuminEX™ (a specific detergent for bacterial cells) are added in 5 reading cells (the fifth being a blank), each cell containing 100 µl of water sample. The ATP extraction takes 30 s. 100 µl of Celsis LuminATE™ (lyophylised powder containing luciferase and luciferin) are then added in the 4 sample cells (100 µl of MilliQ water in the blank), and photon counting is immediately done. Photon emissions are converted into ATP quantities, secondly expressed as equivalent bacterial densities from a pre-established linear regression for the Calvi Bay.

Metabolic fingerprints of bacterial communities were characterized with the microtitration plate Biolog GN2 (Biolog MicroPlate™, Biolog, Inc.), consisting of 95 different carbon substrates as sole-carbon source incorporated to a basal medium: 25 carbohydrates, 24 carboxylic acids, 20 amino acids, 6 amines/amides, 2 aminated sugars, 3 methylated derivatives, 3 phosphorylated derivatives, 2 nucleic acids, 5 polymers and 5 miscellaneous (Appendix A). After inoculation of the 96 wells, microtitration plates were incubated during 7 days at room temperature, then dried in an oven and hermetically stocked at 4 °C. Dried wells were afterwards filled with a mixture of milliQ water containing octanol and glycerol in order to disclose all the substrates used. Octanol dissolves formazan crystals, the purple chromogenic products of the reduction of tetrazolium salts revealing the oxydoreduction of carbon substrates; glycerol secondly intensifies the purple colouration off wells. Microplates were then read with a Multiskan EX at 550 and 660 nm, and all the metabolised substrates were listed as an on-off signal.

The activity of constitutive bacterial enzymes was determined with the Api20E gallery micro-method (ApiZym, API Laboratory Products, Ltd.). The 20 gallery wells permit to detect the presence of 3 lipases (esterase (C 4), esterase lipase (C 8) and lipase (C 14)), 3 exoproteases (leucine, valine and cystine arylamidase), 2 endoproteases (trypsin and α-chymotripsin), 8 carbohydrases (α- and β-galactosidase, β-glucuronidase, α- and β-glucosidase, N-acetyl-β-glucosamidase, α-manosidase and α-fucosidase) and 3 phosphatases (alkaline phosphatase, acid phosphatase and naphtol-AS-BI-phosphohydrolase). 100 µl of samples were added in each of the 20 gallery wells, and galleries were incubated at ambient temperature for 48 h. Afterwards, API reagents ZYM A and ZYM B were applied. Results were compared with the colour chart provided by the kit manufacturer after 6 h of incubation. Enzyme activities are expressed in nanomoles of hydrolysed substrate by hour and sample ml, or by hour and bacteria when divided by their corresponding bacterial densities. Carbohydrase, lipase and protease activities are analysed independently of phosphatase activities. Phosphatase activities reflect the metabolism of phosphate radicals, mobilized under the form of highly energized molecules (e.g. ATP, glucose-6-phosphate ...), and usable afterwards in endoenergetic metabolic processes. It is therefore of value to consider the energetic status of the microbial community independently of its potential to hydrolyse complex polymers such as proteins, carbohydrates or fatty acids.

Bacterial denitrifying (DN-BART™), iron- (IRB-BART™) and sulfate-reducing (SRB-BART™) biological activities (Droycon Bioconcepts, Inc.) were measured in pore-water. Each BART consists of a tube containing a ball which floats once the tube is filled with 15 ml of pore-water; bacterial activities are highlighted by the appearance of colour or foam around the ball or in the tube bottom, depending on the test. BARTs were incubated at room temperature and daily checked for bacterial activities. Results are expressed as colony formatting units (cfu) per sample ml.

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2.4. Statistical analysis

Mathematical and statistical data treatment was performed with Excel (Microsoft Inc.) and Statistica (Statsoft Inc.) software. Significant differences between mean bacterial densities of collected samples were highlighted with parametric (Student test ($n = 2$) and ANOVA ($n > 2$)) or non-parametric (Mann Whitney test ($n = 2$) and Kruskal–Wallis test ($n > 2$)) analyses of variance, depending on data distribution. Multiple comparisons of means (ANOVA and Kruskal–Wallis test) were followed by their respective post-hoc test of Scheffé or Dunn. In order to visualize functional and hydrolytic affinities between micro-organisms communities, 2 grouping methods permitting the information synthesis contained in all variables were performed: principal component analysis (PCA) and ascendant hierarchical classification (cluster analysis). Samples were clustered using the nearest neighbour method (Zar, 1984).

3. Results

3.1. Environmental parameters

The data show that from December to April, temperature was under the 16 °C barrier; from May to November, temperature was above this 16 °C barrier (Fig. 2A); Chl.*a* concentrations were low and a noticeable phytoplanktonic spring bloom occurred in March 2006 only but not in spring 2007 (Fig. 2B). Rainfall was low (daily mean on 3 years = 1.8 mm day^{-1}), and more frequent during the winter regime (Fig. 2C). Rainfall events were recorded from 03/19–21/2007 (4.4–28.8 mm day^{-1}) and from 05/27–28/2007 (4.6–10.8 mm day^{-1}), i.e. just before sampling at Stareso-1,2 or Stareso-3 sites, respectively (sampling dates in Table 1). Stronger winds blowing from North–North East (daily mean on 3 years = 3.5 m s^{-1}) were recorded from 03/18–21/2007 (2.1–7.9 m s^{-1}) and from 05/27–29/2007 (3.6–8.4 m s^{-1}). In contrast, no rain nor stronger winds were recorded during November 2006 field campaign. Nutrient concentrations profiles are shown in Fig. 3. Free water NO_3^- and NH_4^+ concentrations are low and similar between sites (below detection limit (DL) for PO_4^{3-}; Fig. 3A). A winter NO^{3-} enrichment of the water column is observable between 01–03/2006 and 01–03/2008. Nutrient very low concentrations reflect the oligotrophic status of Calvi coastal waters (Gobert et al., 2002). NO_3^- and NH_4^+ are in average 3 and 41 times more concentrated in pore-water than in the water column, respectively (Fig. 3B). The variability of pore-water nutrient concentrations indicates the important heterogeneity of the sedimentary compartment (Gobert et al., 2003). There is no enrichment attributable to the wastewater discharge in the vicinity of the Calvi city site, no seasonal impact due to summer tourism and no confinement effect within the Bay.

3.2. Bacterial densities

Bacterial densities (cells ml^{-1}) measured in free and pore-water in the different sampled sites and periods are summarized in Table 1. Pore-water bacterial densities were significantly ($p < 0.05$) higher (10^6–10^7) than their corresponding water column densities (10^5) by 1–2 orders of magnitude. In November, free water bacterial densities were similar between all sampled sites and depths; mean density equals 2.25 ± 1.17 10^5 cells ml^{-1} (min. = 1.10 and max. = 5.35 10^5 cells ml^{-1}). Pore-water mean density equals 78.78 ± 24.20 10^5 cells ml^{-1} and is the lowest in Stareso-2 bare sand patch (52.04 ± 3.08 10^5 cells ml^{-1}), when compared to the 3 other sites colonized by *Posidonia* shoots. In March and May, mean bacterial densities measured in Stareso-1,2,3 sites together were

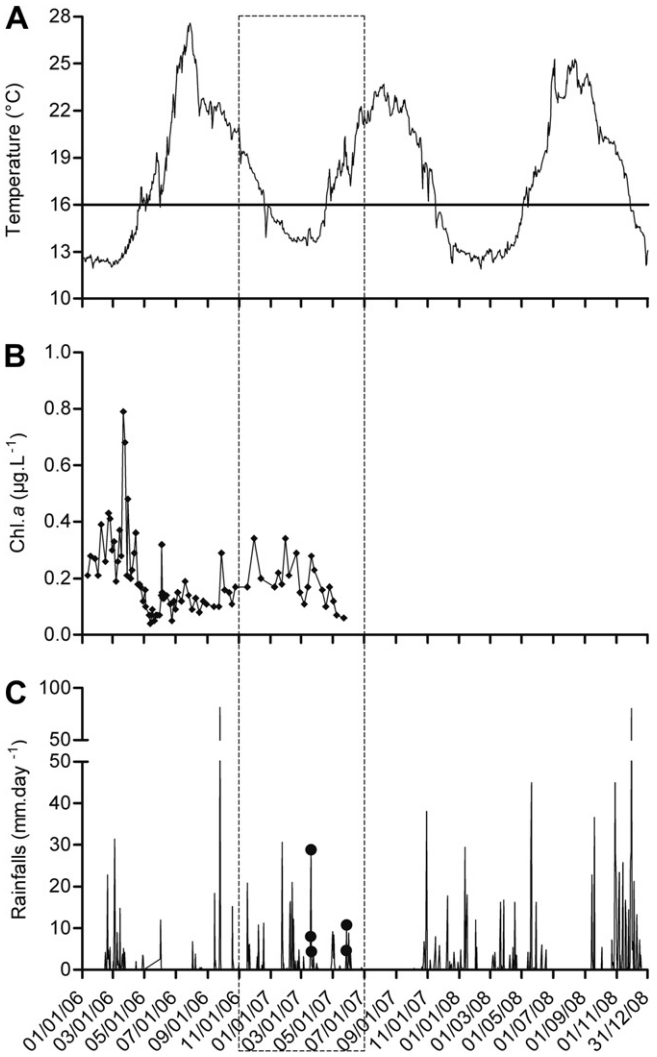

Fig. 2. A) Mean daily temperature (°C; 01/01/2006–12/31/2008), B) Chl.*a* concentration (µg L^{-1}; 01/01/2006–31/05/2007) and C) Mean daily rainfall (mm day^{-1}; 02/01/2006–12/31/2008) profiles. The dotted rectangle frames the 3 sampling campaigns. The 16 °C cut off symbolizes the border between the winter and the summer temperature regimes. Black circles on the rainfall profile highlight rainfalls recorded from 03/19–21/2007 (4.4–28.8 mm day^{-1}) and from 05/27–28/2007 (4.6–10.8 mm day^{-1}), respectively.

6.40 ± 1.74 10^5 and 5.33 ± 2.56 10^5 cells ml^{-1}, respectively. However, bacterial densities of Stareso-3 *Posidonia* bed 30 m depth tend to be lower in March (5.54 ± 1.65 10^5), and were significantly higher ($p < 0.05$) in May (7.57 ± 3.10 10^5). Pore-water bacterial densities decrease between Stareso-1 (12 m depth, high *Posidonia* density), Stareso-3 (30 m depth, low *Posidonia* density) and Stareso-2 (15 m depth bare sand patch, no *Posidonia*) sites, in March and June.

3.3. Oxidative metabolism

A detailed view of carbon substrates metabolized by free and pore-water heterotrophic bacterial communities of the different sites, for the 3 sampled periods, is given in Appendix A. The diversity of carbon substrates potentially usable by heterotroph bacteria as sole source of carbon is quite large (Table 2). Free and pore-water bacteria commonly consume glycogen, amino sugars, some carbohydrates, acetic and succinic acids, some amino acids, thymidine, putrescine, glycerol and phosphorylated glucose. Bacterial communities of pore-water samples are furthermore able

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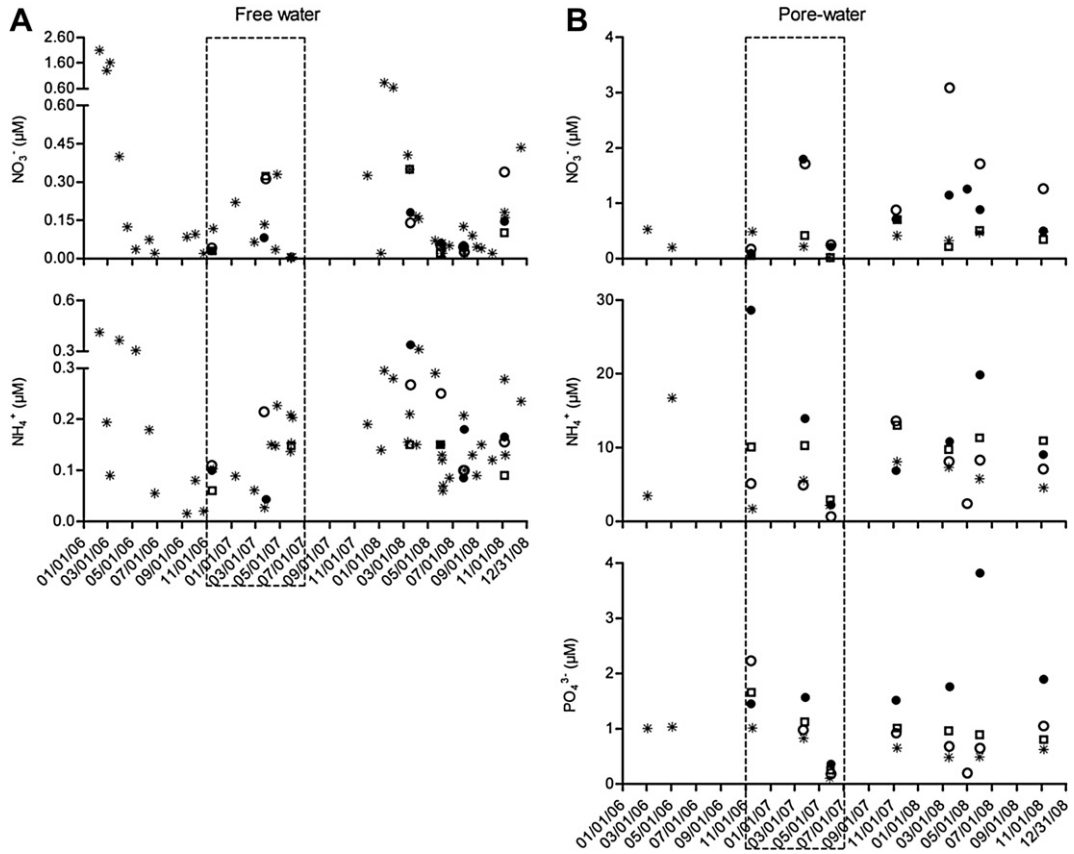

Fig. 3. NO_3^-, NH_4^+ and PO_4^{3-} (bellow DL in free water) concentrations in A) free water and B) pore-water samples from 2006 to 2008. Black circles = Punta Bianca; empty circles = Calvi city; stars = Stareso-1 *P. oceanica* bed 12 m depth; empty squares = Stareso-3 *P. oceanica* bed 30 m depth. Dotted rectangles frame the 3 sampling campaigns.

to use α-cyclodextrin, D-trehalose and D-mannitol, pyruvic acid methyl ester, inosine, L-asparagine, and more specifically most of the carboxylic acids proposed. Cluster analysis (Fig. 4A) shows that bacterial populations of free water are characterized by their relatively homogenous metabolic fingerprint (medium to low dissimilarities, in average). Bacterial communities of pore-water differ from the ones of the water column mainly by their metabolic abilities to use the diversity of carboxylic acids proposed. There are notable differences between metabolic fingerprints of pore-water bacterial communities (dissimilarities higher than 70% of the maximum linkage distance). The PCA gives similar results.

3.4. Enzymatic activities

A detailed view of enzymatic activities sustained by free and pore-water heterotrophic bacterial communities of the different sites, for the 3 sampled periods, is given in Appendix B. Highest hydrolytic activities in decreasing order are, in free and pore-water, phosphatase (naphtol-AS-BI-phosphohydrolase > alkaline phosphatase > acid phosphatase) and exoprotease (valine > cystine > leucine arylamidase), followed by lipase activities (esterase lipase (C8) > lipase (C14) > esterase (C4)). Low carbohydrase (N-acetyl-β-glucosaminidase > α-galactosidase = β-galactosidase > α-glucosidase = α-manosidase) and endoprotease (trypsin > chymotrypsin) activities are observed in November and March. Table 3 summarizes the mean contribution of each of the 4 enzymatic activities (carbohydrases, endo- and exoproteases and lipases) calculated in free and pore-water for each period, all sites together, and the corresponding mean phosphatasic activity. Principal differences are observed for lipolytic activity, well present in free water and more important in pore-water in May when compared to the 2 other periods. Ratios of mean enzymatic activities over mean phosphatasic activities, by season, are close to 1.00, and reflect the balance existing between levels of enzymatic activities and energetic metabolism. Ratios calculated for free water are 1.00, 0.80 and 1.25 in November, March and May, respectively; they are equal to 0.73, 1.00 and 1.30 in pore-water. The cluster analysis distinguishes 3 enzymatic facies (Fig. 4B). Mean enzymatic activity levels of free and pore-water in November and March might be differentiated and May pore-water samples differ highly from all other samples. May free water mean enzymatic activity is close to those measured in November and March in pore-water. The PCA gives similar results. Bacterial density ratios over enzymatic activity levels and energetic metabolism (Table 4) express the mean level of enzymatic activity and energetic metabolism sustained by bacteria, in free and pore-water, for each sampling period. Mean enzymatic activity of bacterial cells in November free water samples is similar to the one of May. It is lower in March. In sediment, a different tendency is observed: low bacterial enzymatic activity levels of November increases in March and are the highest in May. Values of energetic metabolism levels show similar profiles.

3.5. Pore-water biologic activities

Aerobic Sulfate-Reducing Bacteria (SRB) are well represented in pore-water of the different sites, their approximate populations ranging from 18 to 700×10^3 cfu ml^{-1} (all sites together). These values correspond to a detectable activity within 2 (700×10^3) to 4 (18×10^3) incubation days. Anaerobic SRB are slightly less abundant (from 5 to 700×10^3 cfu ml^{-1}, all sites together) than their aerobic homologous. Approximate densities of populations of pore-water

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Table 2
Main and common carbon substrates of the Biolog GN2 microtitration plate metabolized by heterotrophic bacterial communities from pore-water samples and from the water column.

Substrate families	Carbon substrates used	
	Common to free and pore-water samples	Specific to pore-water samples
Carbohydrates	L-arabinose	
	D-cellobiose	
	D-fructose	
	D-galactose	
	α-D-glucose	
	α-D-lactose	
	Sucrose	
Amino acids	L-alanine	L-asparagine
	L-alanyl-glycine	
	L-aspartic acid	
	L-glutamic acid	
	L-leucine	
	L-proline	
	L-serine	
	L-threonine	
Carboxylic acids	Acetic acid	Cis-aconitic acid
	Succinic acid	Citric acid
	–	Formic acid
	–	D-galacturonic acid
	–	D-gluconic acid
	–	D-glucosaminic acid
	–	D-glucuronic acid
	–	α-hydroxybutyric acid
	–	β-hydroxybutyric acid
	–	D,L-lactic acid
	–	Propionic acid
Amines/amides	Putrescine	
Phosphorylated derivatives	α-D-glucose-1-phosphate	
	D-glucose-6-phosphate	
Amino sugars	N-acetyl-D-galactosamine	
	N-acetyl-D-glucosamine	
Nucleic acids	Thymidine	
Polymers	Gly[illegible]	α-cyclodextrin
Methylated derivatives	–	Pyruvic acid methyl ester
Miscellaneous	Glycerol	Inosine

Iron-Reducing Bacteria (IRB) vary between sites from 2.3×10^3 to 9×10^3 cfu ml^{-1} (all sites together). These values correspond to a detectable activity within 4 (9×10^3) to 5 (2.3×10^3) days of incubation. Finally, Denitrifying Bacteria (DN) were not detected in this work.

4. Discussion

4.1. Bacterial densities

Bacterial densities measured by luminescence within the *Posidonia* bed water column (Table 1) are equivalent to the ones measured by epifluorescence for different seagrass meadows in the NW Mediterranean sea (Velimirov et al., 1984; Velimirov, 1986; Velimirov and Walenta-Simon, 1992, 1993). In marine environments, bacterial and phytoplanktonic productions are closely linked (Turley et al., 2000; Valencia et al., 2003; Rooney-Varga et al., 2005): spring blooms are followed by an increase of BSP, subsequently regulated by different actors of the microbial food-web (Nagata, 2000). The small phytoplankton bloom in spring 2007 (Fig. 2B) cannot alone explain the higher bacterial densities measured at Stareso-1 and -2 sites. Rains (4.4–28.8 mm days^{-1}) in March 19th to 21st (Fig. 2C), prior sampling at Stareso-1 and -2, have surely enriched the environment in nutrients (Lapointe et al., 2004). The stronger winds (2.1–7.9 m s^{-1}) from North–North East could impact nutrient availability through wave action inducing sediment resuspension (Moraleszamorano et al., 1991) and deep cold water upwellings in the Calvi Bay (decrease of 0.7 °C at 3 m depth; Gobert, 2002). The bacterioplankton response to these environmental forcings diminishes rapidly (a few tens of hours) once perturbations end (Wainright, 1987; Lapointe et al., 2004). Lower bacterial densities measured in Stareso-3 samples 2 days later reflect this return to the normal. Rains (4.6–10.8 mm days^{-1}) and stronger North–North East winds (3.6–8.4 m s^{-1}, decrease of 0.9 °C at 3 m depth) of May 27th to 29th can also explain higher densities measured at Stareso-3 site when compared to Stareo-1 and -2 sites.

The density of benthic bacteria relies on the provision of inorganic compounds and detrital OM to the sediment. In *Posidonia*

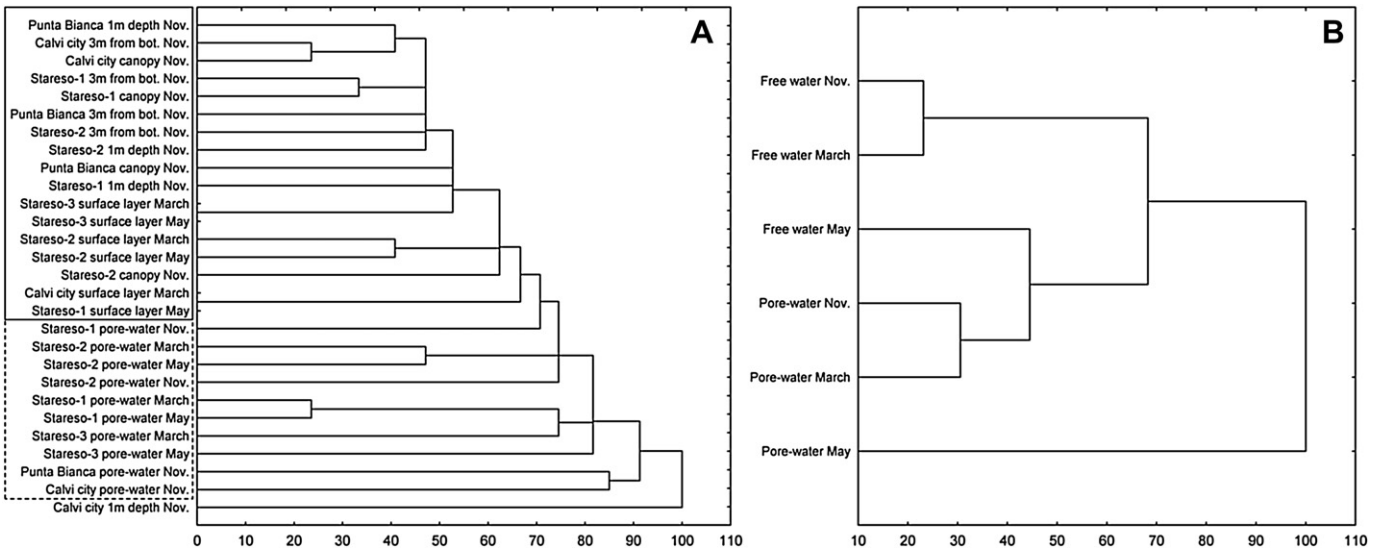

Fig. 4. A) Dendrogram of carbon source utilization by heterotrophic bacterial communities in November, March and May. Rectangles group samples from pore-water (dotted line) and from the water column (full line). B) Dendrogram of mean enzymatic activity levels sustained by heterotrophic bacterial communities from pore-water samples and from the water column in November, March and May. *X* axis show linkage distances between samples in % of the maximal distance.

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Table 3
Mean (±SD) lipase, exorprotease, endoprotease, carbohydrase and phosphatase activities (nmole of hydrolysed substrates by hour and ml) sustained by heterotrophic bacterial communities from pore-water samples and from the water column in November, March and May.

Sample	Free water			Pore-water		
Season	Nov.	March	May	Nov.	March	May
Lipases	0.05 ± 0.10	0.25 ± 0.24	2.25 ± 0.90	1.60 ± 0.88	2.33 ± 1.35	5.44 ± 2.57
Exoproteases	1.26 ± 0.80	1.54 ± 0.48	2.13 ± 0.61	1.75 ± 0.85	1.75 ± 1.59	2.92 ± 1.35
Endoprotease	0.19 ± 0.22	0.07 ± 0.06	–	0.15 ± 0.29	0.39 ± 0.67	–
Carbohydrases	0.29 ± 0.39	0.19 ± 0.26	–	–	0.19 ± 0.34	–
Phosphatases	1.80 ± 2.20	2.55 ± 1.30	3.50 ± 0.82	4.81 ± 2.97	4.67 ± 3.29	6.42 ± 6.07

meadows, this provision depends on the seagrass bed productivity (Gobert, 2002). Hence maximum densities are found in Stareso-1, Punta Bianca and Calvi city sites where the seagrass bed is dense and not deep. In contrast, densities are lower in the Stareso-2 bare sand patch (without *Posidonia*) and in the Stareso-3 deep *Posidonia* bed where shoots are scattered and the meadow less productive. Benthic bacterial abundances recorded by Canon et al. (1998) in the Calvi Bay *Posidonia* bed (14 m depth) varied between 2.5 and 7.5 10^8 cells g^{-1} of sediment. As we sampled pore-water with syringes (no sediment core), densities measured only correspond to free-living bacteria and little associated to sediments grains. Densities (10^6 to 10^7 cells ml^{-1} of pore-water; Table 1) are then underestimated when compared to other Mediterranean seagrass sedimentary environments (10^7 to 10^9 cells cm^{-3} or g^{-1}) studied by epifluorescence microscopy after separating bacteria from sediment grains via sonication (García-Martínez et al., 2009).

4.2. Bacterial metabolism

Phytoplankton exudes diverse biochemical compounds including carbohydrates, nitrogenous compounds, lipids and organic acids (Dafher and Wangersky, 2002; van Heerden et al., 2002). Data concerning exudate compounds by marine magnoliophytes are more scattered. *P. oceanica* exudes amino acids (Jørgensen et al., 1981), and *T. hemprichii* and *H. ovalis* exude glycolate, a product of photorespiration (Fogg, 1976). In aquatic ecosystems, extracellular bacterial enzym[illegible]ay an important role as bacteria are only able to ingest DOM [illegible] a molecular weight below 600 Da (Weiss et al., 1991; Amon and Benner, 1994). Somvile and Billen (1983) highlighted the narrow relationship existing between exoprotease activities, determined with fluorogenic aminoacyl-P-naphthylamide, and amino acid consumption. This trend was also observed for glucosidase, protease and glucosamidase activities in fjords, using fluorogenic methylumbelliferyl-substrates (Hoppe, 1983). Fenice et al. (2007) tested extracellular enzyme activities by plate-screening on bacterial strains isolated from various substrates (water, sediments, *Posidonia*, etc.) sampled in the Tyrrhenian Sea: the main enzymatic potentialities were directed against phosphate, lipid and peptide substrates, and were common to most strains; in contrast glucosidase activities were little represented. Zaccone et al. (2002) obtained similar results when they evaluated with fluorogenic substrates the metabolic potential of free-water heterotroph bacteria of the Adriatic Sea to degrade OM. We also observed higher phosphatase, lipase and exoprotease activities with ApiZym galleries. The diffuse colouration revealed that low carbohydrase and endoprotease activities in November and March were hidden in May by an excess of yellow coloured Zym B (used to reveal the colouration of Api20E gallery wells). The capability of released nitrogenous compounds (amino acids, proteins and polypeptides) to provide a source of both C and N can explain the greater protease activity with respect to carbohydrase (Zaccone et al., 2002). Extracellular phosphatases serve multiple ecological functions, as revised by Hoppe (2003), by supplying the pools of P and available organic C (and possibly also N). The observation of carbohydrase activities confirms the presence of microorganisms with a high potential in decomposing refractory material (Zaccone et al., 2002; Caruso, 2010). Lipase activities in the water column, as a C provider, rely on lipid availability which depends on biotic (e.g. shift in phytoplankton population, degradation of zooplanktonic material, etc.) and abiotic (e.g. summer photodegradation) factors (Zaccone et al., 2002; Williams and Jochem, 2006; Bourguet et al., 2009; Frka et al., 2011).

Gacia et al. (2002) estimated that 42% of the buried organic carbon of a *Posidonia* bed is derived from magnoliophytes and their epibiontes. A significant fraction is refractory when just a minor percentage (18% on average) is found in the biopolymeric fraction (lipids, carbohydrates and proteins) (Danovaro, 1996). Notable differences between metabolic fingerprints of pore-water bacterial communities (Fig. 4A) are attributed to the heterogeneity of the sedimentary environment, associated to the metabolic particularities of organisms living in it. Prokaryotes of the suboxic zone (denitrifying bacteria, metallic oxide reducing bacteria) might use a large variety of organic substrates in their energetic metabolism. With burying in sediment, the usable carbon substrate spectrum is reduced; sulfate-reducing bacteria are practically incapable to oxidize amino acids or sugars. These monomeric compounds are nevertheless usable by fermentative bacteria and converted in a large spectrum of fermentation products, including mainly volatile fatty acids used in the energetic metabolism of sulfate-reducing bacteria as electron donors (Capone and Kiene, 1988; Jørgensen and

Table 4
Mean enzymatic activity levels and mean energetic metabolism sustained by heterotrophic bacterial communities (nmole of hydrolysed substrates by hour and ml) or by bacteria (nmole of hydrolysed substrates by hour and cell) from pore-water samples and from the water column in November, March and May.

Sample	Season	Densities (cell ml^{-1})	Mean enzymatic activity		Mean energetic metabolism	
			nmol h^{-1} ml^{-1}	nmole h^{-1} $cell^{-1}$	nmol h^{-1} ml^{-1}	nmole h^{-1} $cell^{-1}$
Free water	Nov.	2.25×10^5	1.80	0.80×10^{-5}	1.80	0.80×10^{-5}
	March	6.40×10^5	2.04	0.32×10^{-5}	2.55	0.40×10^{-5}
	May	5.33×10^5	4.38	0.82×10^{-5}	3.50	0.66×10^{-5}
Pore-water	Nov.	7.88×10^6	3.50	0.44×10^{-6}	4.81	0.61×10^{-6}
	March	4.24×10^6	4.67	1.10×10^{-6}	4.67	1.10×10^{-6}
	May	5.57×10^6	8.36	1.50×10^{-6}	6.42	1.15×10^{-6}

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Kasten, 2006). This metabolism of carboxylic acids by benthic bacteria (Table 2) is linked to the important lipolytic activities (esterase lipase and lipase) measurable in pore-water for the 3 sampling periods (Table 3).

Zaconne et al. (2002) observed an increasing trend in the warm period (July–October) for phosphatase, lipase and peptidase exoenzymatic activities in the Adriatic Sea water column. Lopez et al. (1995) highlighted this seasonality in the sediment of 5 *Posidonia* beds along the Catalane coast (Spain): exoglucosidase and exoprotease activities, measured with fluorogenic substrates, were 2–3 times higher in June than in November and March. The late spring increase of lipase, protease and phosphatase activities (Table 3) results from the larger quantity of available OM, the direct consequence of spring augmentation of seagrass meadow primary production (Lopez et al., 1995; Zaccone et al., 2002; Marbà et al., 2006; Gobert et al., 2006a). In November and May, bacterial enzymatic activity and energetic metabolism (Table 4) of free water were similar (0.80 10^{-5} $nmoles_{substrates}$ h^{-1} $cell^{-1}$ and 0.82 and 0.66 10^{-5} $nmoles_{substrates}$ h^{-1} $cell^{-1}$, respectively). Thermal conditions are favourable in both cases (around 20 °C; Fig. 2A). The high bacterial metabolism recorded in November is supported by the autumnal seagrass leaf senescence and decay and the higher phototrophic plankton growth (Chl.*a* concentrations were 3–4 times higher in November than in May; Fig. 2B; Champenois and Borges, 2012; Lepoint et al., 2002). Seagrass meadows channel a large part of their primary production, higher from late winter to summer (Gobert, 2002), through the benthic detrital food chain (Holmer et al., 2004; Marbà et al., 2006). The higher enzymatic activity and energetic metabolism of pore-water bacteria are henceforth measured in March (1.10 10^{-6} $nmoles_{substrates}$ h^{-1} $cell^{-1}$) and May (1.50 and 1.15 10^{-6} $nmoles_{substrates}$ h^{-1} $cell^{-1}$, respectively; Table 4).

4.3. Biological activity of pore-water

BART™ tests successively detected the dominant presence and activity of indigenous SRB. This is consistent with previous studies investigating sulfate reduction rates with radioactive tracers ($^{35}SO_4$) in iron-depleted carbonate sediments colonized by *Posidonia* (Holmer et al., 2003, 2004; Holmer and Frederiksen, 2007), as for the Calvi Bay (Moulin et al., 1985; Luy et al., 2012). The elevated quantity of OM trapped and produced by seagrasses favour the rapid installation of anoxic conditions in sediments, with the exception of the oxygenated peri-root microzone (Gobert, 2002). SRB, previously considered as strictly anaerobic, present however a group of differentiated reactions to oxygen (Brune et al., 2000). Their importance results from the narrow association between diazotroph SRB and the oxic rhizosphere of seagrasses, as supported by several studies using specific inhibitors of sulfate reduction (Welsh, 2000). There are few reports on iron reduction activities in seagrass sediments, but visual observations of rhizosphere sediments from a range of seagrass species indicate that oxidized iron concentrations are probably low (Marbà et al., 2006). The reduced bioaccessibility of ferric iron, particularly in iron-depleted carbonate sediments (Holmer et al., 2005), makes this mineralization process of lower importance (Haese, 2006). In the rhizosphere, coupled reactions of nitrification-denitrification rely on the relative influence of oxygen liberation by marine magnoliophyte roots and on the competition between these roots and nitrifying and denitrifying bacteria for ammonium, nitrates and nitrites (Welsh et al., 2000). The absence of detectable denitrification activity might be due to nitrification inhibition resulting from sulphide exposition (Joye and Hollibaugh, 1995), to a lack of requested oxygen for nitrification (Caffrey et al., 1993), to the important detrital OM charge coming from the seagrass bed (Hemminga et al., 1991), and to the probable competition for these limited resources (Fig. 3B) in nitrogen (Welsh et al., 2000).

5. Conclusions

The estimation of bacterial densities by luminescence in *Posidonia* meadows and the study of their metabolism with microtitration techniques gave results similar to the ones obtained with commonly used methods in microbial ecology. The underestimation of benthic bacterial densities was a consequence of the adopted sampling strategy (densities in pore-water samples, without detaching bacteria from sediment grains by sonication). Bacterial numbers in the water column were similar to other seagrass meadows, and the observed variations could be linked to environmental forcings. We revealed the importance of phosphatase, protease and lipase activities, compared to carbohydrases, and the efficient utilisation of the resulting monomeric products of these enzymatic activities. We also observed a seasonal pattern of bacterial metabolism, linked to the temperature regimes and to the seagrass meadow productivity. Main benthic mineralization processes, *i.e.* sulfate and iron reduction, were also demonstrated. However, quantitatively less important processes such as carbohydrase or denitrification activities were little or not observed. Although these techniques do not replace commonly used methods for bacterial counting and metabolism studying, this semi-quantitative approach allows us to obtain rapid estimations of bacterial ecological key processes. Their low cost, rapid and easy use, and the low level of expertise and sophistication they require, means that these techniques can be employed by numerous workers engaged in environmental surveys.

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Appendix A. Supplementary material

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Site	Punta Bianca		Calvi city		Stareso-1						Stareso-2						Stareso-3				Site	Carbon substrates
Sample	FW	PW	FW	PW	FW	FW	FW	PW	PW	PW	FW	FW	FW	PW	PW	PW	FW	FW	PW	PW	Sample	
Season	Nov.	Nov.	Nov.	Nov.	Nov.	March	May	Nov.	March	May	Nov.	March	May	Nov.	March	May	March	May	March	May	Season	
A1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	A1	Water
A2	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	1	A2	α-cyclodextrin
A3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	A3	dextrin
A4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	A4	glycogen
A5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	A5	tween 40
A6	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	A6	tween 80
A7	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	A7	N-acetyl-D-galactosamine
A8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	A8	N-acetyl-D-glucosamine
A9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	A9	adonitol
A10	0	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	A10	L-arabinose
A11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	A11	D-arabitol
A12	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	1	A12	D-cellobiose
B1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	B1	l-erythritol
B2	0	1	0	1	0	1	1	0	1	1	0	1	1	1	0	0	0	0	1	1	B2	D-fructose
B3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	B3	L-fucose
B4	1	1	1	1	1	1	1	1	1	1	1	0	0	1	0	0	1	1	1	1	B4	D-galactose
B5	0	1	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	B5	gentiobiose
B6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	B6	α-D-glucose
B7	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	B7	m-inositol
B8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	B8	α-D-lactose
B9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	B9	lactulose
B10	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	B10	maltose
B11	0	1	0	1	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	1	B11	D-mannitol
B12	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	B12	D-mannose
C1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	C1	D-melibiose
C2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	C2	β-methyl-D-glucoside
C3	0	0	0	0	0	1	1	0	0	0	0	0	0	0	1	1	0	0	0	0	C3	D-psicose
C4	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	C4	D-raffinose
C5	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	C5	L-rhamnose
C6	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	C6	D-sorbitol
C7	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	C7	sucrose
C8	0	1	0	1	0	0	0	1	0	0	0	1	0	1	0	0	0	0	1	1	C8	D-trehalose
C9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	C9	turanose
C10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	C10	xylitol
C11	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	C11	pyruvic acid methyl ester
C12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	C12	succinic acid mono-methyl ester
D1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	D1	acetic acid
D2	0	1	0	1	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	1	D2	cis-aconitic acid
D3	0	1	0	1	0	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	D3	citric acid
D4	0	1	0	1	0	0	0	1	0	0	1	0	0	1	1	1	0	0	1	1	D4	formic acid
D5	0	1	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	D5	D-galactonic acid lactone
D6	0	0	0	1	0	0	0	1	0	0	1	0	0	1	1	1	0	0	1	1	D6	D-galacturonic acid
D7	0	1	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	D7	D-gluconic acid
D8	0	1	0	1	0	0	0	1	0	0	1	0	0	1	1	1	0	0	1	1	D8	D-glucosaminic acid
D9	0	1	0	1	0	0	0	1	1	1	1	0	0	1	1	1	0	0	1	1	D9	D-glucuronic acid
D10	0	1	0	1	0	1	1	1	1	1	1	0	0	1	0	0	1	1	1	0	D10	α-hydroxybutyric acid
D11	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0	D11	β-hydroxybutyric acid
D12	0	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	D12	γ-hydroxybutyric acid
E1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	E1	p-hydroxy-phenylacetic acid
E2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	E2	itaconic acid
E3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	E3	α-ketobutyric acid
E4	0	1	0	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	1	E4	α-ketoglutaric acid
E5	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	E5	α-ketovaleric acid
E6	1	1	1	1	0	0	0	1	1	1	0	0	0	1	1	1	0	0	1	1	E6	D,L-lactic acid
E7	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	E7	malonic acid
E8	1	1	0	1	1	1	1	1	1	1	0	0	0	1	1	1	0	0	1	1	E8	propionic acid
E9	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	E9	quinic acid
E10	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	E10	D-saccharic acid
E11	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	E11	sebacic acid
E12	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	E12	succinic acid
F1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	F1	bromosuccinic acid
F2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	F2	succinamic acid
F3	0	0	1	0	0	1	1	0	0	0	0	1	1	0	1	1	0	0	0	0	F3	glucuronamide
F4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	F4	L-alaninamide
F5	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	F5	D-alanine
F6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	F6	L-alanine
F7	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	F7	L-alanyl-glycine
F8	0	1	0	1	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	1	F8	L-asparagine
F9	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	F9	L-aspartic acid
F10	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	F10	L-glutamic acid
F11	0	1	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	F11	glycyl-L-aspartic acid
F12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	F12	glycyl-L-glutamic acid
G1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	G1	L-histidine
G2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	G2	hydroxy-L-proline
G3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	G3	L-leucine
G4	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	G4	L-ornithine
G5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	G5	L-phenylalanine
G6	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	G6	L-proline
G7	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	G7	L-pyroglutamic acid
G8	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	G8	D-serine
G9	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	G9	L-serine
G10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	G10	L-threonine
G11	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	G11	D,L-carnitine
G12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	G12	γ-aminobutyric acid
H1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	H1	urocanic acid
H2	0	1	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	H2	inosine
H3	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	H3	uridine
H4	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	H4	thymidine
H5	0	0	0	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	H5	phenylethylamine
H6	1	0	1	1	0	1	1	0	1	1	0	0	0	1	1	1	0	0	1	1	H6	putrescine
H7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	H7	2-aminoethanol
H8	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	H8	2,3-butanediol
H9	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	H9	glycerol
H10	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	H10	D,L,α-glycerol phosphate
H11	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	H11	α-D-glucose-1-phosphate
H12	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	H12	D-glucose-6-phosphate
Season	Nov.	Nov.	Nov.	Nov.	Nov.	March	May	Nov.	March	May	Nov.	March	May	Nov.	March	May	March	May	March	May	Season	
Sample	FW	PW	FW	PW	FW	FW	FW	PW	PW	PW	FW	FW	FW	PW	PW	PW	FW	FW	PW	PW	Sample	Carbon substrates
Site	Punta Bianca		Calvi city		Stareso-1						Stareso-2						Stareso-3				Site	

Appendix A: Detailed table of carbon substrates used, given as an on-off signal (1 yellow vs 0 white cells), by heterotrophic bacterial communities from pore-water samples (PW) and from the water column (FW) in the Punta Bianca, the Calvi city, Stareso-1*P. oceanica* bed 12m depth, Stareso-2 bare sand patch and Stareso-3 *P. oceanica* bed 30m depth in November, March and May.

Site	Punta Bianca		Calci city		Stareso-1						Stareso-2						Stareso-3			
Sample	FW	PW	FW	PW	FW	FW	FW	PW	PW	PW	FW	FW	FW	PW	PW	PW	FW	FW	PW	PW
Season	Nov.	Nov.	Nov.	Nov.	Nov.	March	May	Nov.	March	May	Nov.	March	May	Nov.	March	May	March	May	March	May
Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Alkaline phosphatase	0,19 ± 0,34	2,33	-	2,33	-	-	2,57 ± 2,66	1,17	1,17	9,33	-	-	2,10 ± 1,73	0,58	2,33	2,33	0,10 ± 0,24	1,07 ± 1,07	-	-
Esterase (C4)	-	-	-	-	-	-	-	-	-	0,58	-	-	-	-	0,58	-	-	-	-	-
Esterase lipase (C8)	0,19 ± 0,34	2,33	-	2,33	-	0,35 ± 0,52	2,10 ± 0,52	1,17	2,33	4,67	-	-	1,75 ± 0,82	0,58	2,33	4,67	0,29 ± 0,32	0,88 ± 0,80	1,17	2,33
Lipase (C14)	-	-	-	-	-	-	0,70 ± 0,26	-	-	0,58	-	-	0,93 ± 0,32	-	0,58	2,33	0,10 ± 0,24	0,39 ± 0,30	-	1,17
Leucine arylamidase	-	-	-	-	-	0,35 ± 0,32	0,82 ± 0,88	1,17	-	1,17	-	0,12 ± 0,26	1,28 ± 0,64	0,58	1,17	2,33	0,29 ± 0,49	0,68 ± 0,24	0,58	1,17
Valine arylamidase	0,58 ± 0,00	1,17	0,78 ± 0,34	1,17	1,17 ± 0,00	1,17 ± 0,00	0,93 ± 0,32	1,17	-	0,58	1,56 ± 0,67	1,17 ± 0,00	1,05 ± 0,26	1,17	1,17	1,17	0,88 ± 0,49	1,17 ± 0,00	1,17	1,17
Cystine arylamidase	0,19 ± 0,34	-	-	0,58	-	0,35 ± 0,32	0,12 ± 0,26	-	-	0,58	0,78 ± 0,34	-	0,35 ± 0,32	-	0,58	0,58	0,29 ± 0,32	-	0,58	-
Trypsin	-	-	-	-	0,39 ± 0,67	-	-	-	-	-	0,39 ± 0,67	0,12 ± 0,26	-	0,58	0,58	-	0,10 ± 0,24	-	-	-
α-chymotrypsin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0,58	-
Acid phosphatase	0,19 ± 0,34	1,17	-	1,17	-	-	-	1,17	0,58	2,33	-	0,35 ± 0,52	-	0,58	0,58	-	0,19 ± 0,48	0,10 ± 0,24	1,17	0,58
Naphtol-AS-BI-phosphohydrolase	0,39 ± 0,34	1,17	0,58 ± 0,00	0,58	1,17 ± 0,00	2,10 ± 1,52	1,56 ± 0,00	2,33	1,17	1,56	4,67 ± 0,00	1,40 ± 1,87	1,56 ± 0,00	4,67	2,33	1,56	3,50 ± 1,95	1,56 ± 0,00	4,67	1,56
α-galactosidase	-	-	-	-	-	-	-	-	-	-	0,19 ± 0,34	0,12 ± 0,26	-	-	-	-	-	-	-	-
β-galactosidase	-	-	-	-	-	-	-	-	-	-	-	0,12 ± 0,26	-	-	-	-	0,10 ± 0,24	-	-	-
β-glucuronidase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
α-glucosidase	-	-	-	-	-	-	-	-	-	-	-	0,12 ± 0,26	-	-	-	-	-	-	-	-
β-glucosidase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
N-acetyl-β-glucosamidase	-	-	0,39 ± 0,67	-	0,58 ± 1,01	-	-	-	-	-	-	-	-	-	0,58	-	-	-	-	-
α-manosidase	-	-	-	-	-	-	-	-	-	-	-	0,12 ± 0,26	-	-	-	-	-	-	-	-
α-fucosidase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Appendix B: Detailed table of enzymatic activities (nmoles of substrates hydrolysed by hour and ml) sustained by heterotrophic bacterial communities from pore-water samples (PW) and from the water column (FW) in the Punta Bianca, the Calvi city, Stareso-1 *P. oceanica* bed 12m depth, Stareso-2 bare sand patch and Stareso-3 *P. oceanica* bed 30m depth in November, March and May.