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Biodiversity of prokaryotic communities in sediments of different sub-basins of the Venice lagoon

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Abstract

Microbial community structure and diversity in the wide and shallow Venice lagoon were assessed, prior to construction of mobile dams, at nine stations representative of four different sub-basins previously selected on the basis of international guidelines for sediment quality. The sediments were mainly anoxic and were colonized by microbial communities the species richness of which was quantitatively correlated with total elemental sulfur and acid-volatile sulfide. Automated ribosomal intergenic spacer analysis clustered the stations into three groups. One station for each group was hence analyzed in detail for bacterial and archaeal diversity by screening of 16S rRNA gene clone libraries. The dominance of *Gammaproteobacteria* clones (84% with a high proportion of *Vibrionaceae*, indicator of urban pollution) determined significant divergence of the station adjacent to industrial and metropolitan areas. *Bacteroidetes* were widespread, especially where prairies of aquatic plants are located. The other two analyzed stations were dominated by bacterial taxa implicated in the sulfur cycle: the anoxygenic photosynthetic *Chromatiales*, sulfate- and sulfur-reducing *Desulfobacterales* and *Desulfuromonadales*, and members of the *Alpha-* and *Epsilonproteobacteria*.
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1. Introduction

Natural lagoons are crucial environments for coast preservation in many geographic areas. They are transition environments with shallow waters, rapid temperature changes on the water surface and high sedimentation rates. These ecosystems are highly productive [30] and need careful monitoring because their very fragile status could rapidly change in response to natural or human-driven events, especially where they are close to metropolitan areas. Coastal lagoons are subjected, at intermediate latitudes, to high evaporation and rainfalls that, together with tidal currents, the low exchange rate of oxygenated water with the open sea and high sedimentation, can determine anoxia in the sediments [11]. Anoxia is sometimes added to heavy pollution, as in the case of the Venice lagoon, due to the heavily industrial site of Marghera [6].

A number of studies carried out in recent years in the Venice lagoon have shown the presence of a wide range of contaminants such as heavy metals, hydrocarbons, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, dioxins and hexachlorobenzene, and many risk assessment screenings have been performed to evaluate sediment quality [4]. Recently, an integrated evaluation of sediments with a view toward new management policies, which ideally would provide a holistic approach, was reported. In that study, management of sediment contamination was considered to be only one of the factors necessary for achieving ecosystem health and 'satisfactory ecological status' [4]. Thus there

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exists a need for an integrated evaluation so as to completely elucidate the ecological quality of Venice lagoon sediments. Current work should help to define and locate areas posing a threat to the environment, and hence, to develop more sustainable management of sediments. This would improve planning in sediment dredging for navigational purposes, along with other interventions such as the building of three mobile dams currently in progress at the three entrances to Venice lagoon.

Within this integrated evaluation process concerning sediment quality, the study of microbial community structure and biodiversity has thus far been ignored. However, the microbial health hazard has been evaluated [1] and continuous monitoring is being carried out by the national health authority. Nevertheless, investigations on microbial diversity, which plays an important role in pollutant degradation and natural attenuation of contamination, are inconclusive, although bacterial strains responsible for degradation of linear and aromatic polycyclic hydrocarbons have been isolated [5,16].

This work aims to fill in the gap in well-defined subregions of the Venice lagoon before starting the building of mobile dams. We report here the characterization of bacterial and archaeal communities in sediments having similar hazard quotients, namely, the ratio between the pollutant concentration and its value, established for each contaminant by sediment quality guidelines [4].

2. Materials and methods

2.1. Study area

The coastal lagoon of Venice is a shallow body of water 550 km^2 wide and 1.3 m deep, bordered by a large industrial and agricultural watershed roughly four times wider (2038 km^2). The lagoon communicates with open sea through 3 inlets, Lido, Malamocco and Chioggia, and water exchange is calculated as $400 \times 10^6\ m^3\ d^{-1}$, whereas freshwater input from the watershed is $30 \times 10^6\ m^3\ d^{-1}$. The lagoon is divided into four geographical sub-basins: north (VL-N), central-north (VL-CN), central (VL-C) and south (VL-S) (Fig. 1). This division was previously defined on the basis of relative hazard quotients calculated using international sediment quality guidelines for each contaminant during a 25-year-long survey on a data set of 278 samples [4,26].

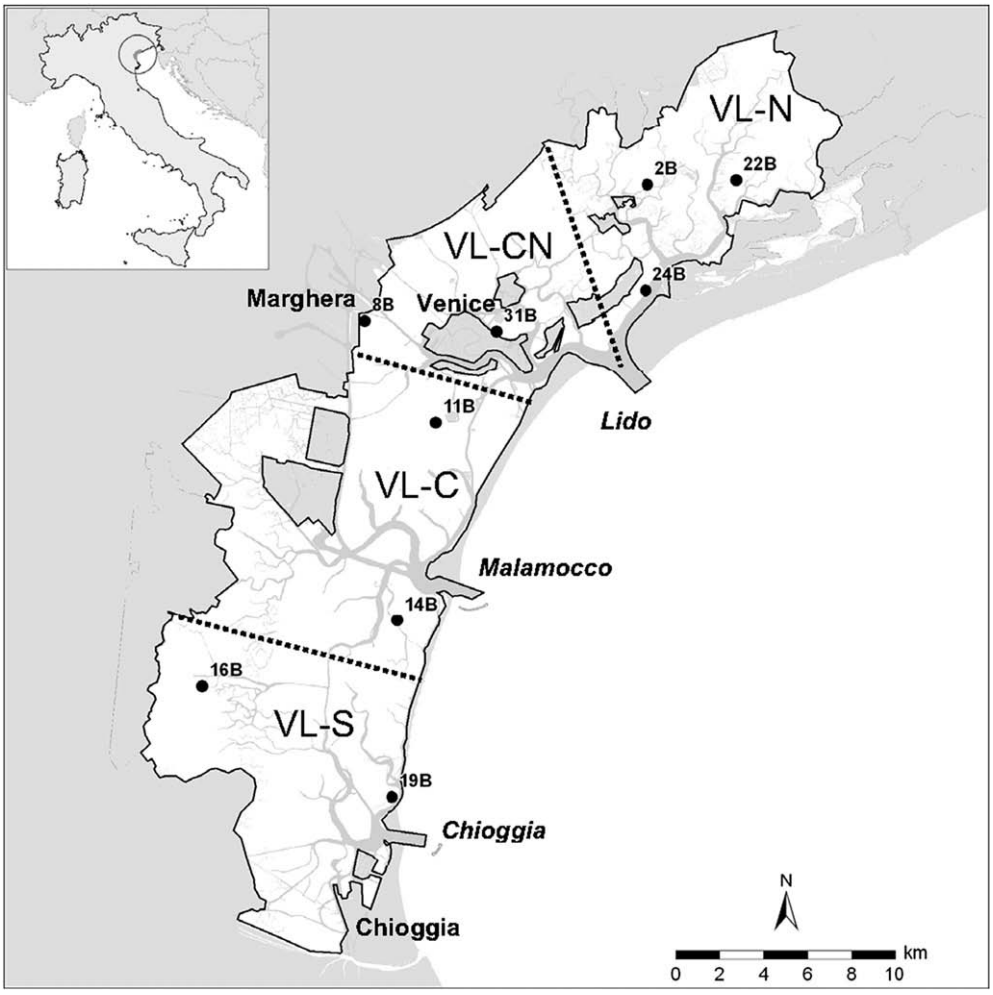

Fig. 1. Map of the Venice lagoon with reported sample stations. Dashed lines border the 4 sub-basins based on risk analyses following international sediment quality guidelines [4]. VL-N: north basin; VL-CN: central-north basin; VL-C: central basin; VL-S: south basin.

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Artificial mobile dams (Mose system) are under construction at the three lagoon entrances aimed at blocking exceptionally high tides to protect the city from floods.

2.2. Sediment sampling

Sediments were collected in nine stations of the Venice lagoon located in the four sub-basins. Sampling sites are reported in Fig. 1 and briefly described in Table 1. In May 2005, five sediment cores were collected for each of the nine stations using a 5 cm diameter handheld corer following an integrated sampling design [31]. At each station, one core was immediately frozen for subsequent geochemical analysis, and a second was used for measuring in situ pH, redox potential and temperature. The first cm of three cores in each station was collected with a sterile scalpel, pooled, homogenized, aliquoted and stored at −20 °C for subsequent microbiological analyses. Sampling could not be repeated, since dredging sediment operations in the lagoon were initiated following this first sampling trial.

2.3. Sediment physico-chemical analysis

The samples were 1 mm sieved to eliminate mollusk shell debris and other coarse materials. Sediment grain size was measured by wet sieving and grain sizes were classified on the basis of Shepard's ternary diagram [20]. Total organic carbon was measured with a CHN-S elemental analyzer (mod. EA1110, Carlo Erba Instruments, Milan, Italy) by total combustion at 1000 °C of organic carbon to CO_2 that was quantified by gas chromatography with a TCD detector as previously reported [28]. Elemental sulfur (S^0) and acid-volatile sulfide (AVS) concentrations were determined following the procedures described by Chen et al. [9] and Allen et al. [2], respectively.

2.4. Total cell counts

Sediments were fixed immediately after sampling by the addition of 2% glutaraldehyde and stored at 4 °C in the dark. Before analysis, sediment slurries were prepared by thoroughly shaking the sediments 1000-fold diluted in 0.1 M particle-free tetrasodium pyrophosphate dispersing solution. A 1–3 ml aliquot of the suspension was incubated 15 min with 4,6-diamidino-2-phenylindole (DAPI, final concentration 1 μg mL^{-1}) and filtered through a black polycarbonate membrane (0.2 μm pore size, 25 mm diameter, Isopore, Millipore, Milan, Italy). Cell counts were determined by counting stained cells on 20 randomly selected fields using a Zeiss Axioplan epifluorescence microscope with excitation/emission filters of 365/420 nm.

2.5. DNA extraction from sediments

Total DNA was extracted and purified using a FastDNA™ spin kit for soil (BIO 101 Systems Q-BIO gene; CA, USA) following the manufacturer's instructions. From each sample, total DNA was extracted from six separate aliquots of 0.5 g, pooled three by three in order to obtain two cumulative extracts each from a total of 1.5 g of sediment.

2.6. Bacterial community fingerprinting by ARISA

Amplified ribosomal intergenic spacer analysis (ARISA) fingerprinting was performed twice on extracted DNA as described elsewhere [8]. Intergenic transcribed spacers (ITSs) were amplified with the ITSF primer labelled at its 5′ end with phosphoramidite dye (6-FAM) [8]. Denatured ARISA fragments were loaded on an ABI Prism 310 capillary electrophoresis system and run in triplicate in denaturing conditions. Output peak matrix was subjected to principal component analysis (PCA). Pairwise distances were calculated with the SimQual function of the NTSYSpc 2.01 computer program (Applied Biostatistics Inc., USA) by employing Jaccard's coefficient for two-state data. The significance of the resulting PCA was checked by ANOVA and Tukey's test, considering a *P*-value threshold of 0.05.

Table 1
Details of sampling stations and physico-chemical characteristics of the sediments.

Station ID	Station name	Location[a]	Sediment type	*T* (°C)	Eh[b] (mV)	pH	Clay (%)	TOC[c] (%)	AVS[d] (mg kg^{-1})	*S*[e] (mg kg^{-1})
2B	Palude della Rosa	VL-N	Silty-clay-loam	21.5	−115	7.99	39.6	1.22	1342	678
22B	Palude Maggiore	VL-N	Silty-clay-loam	20.9	−160	7.88	34.8	1.3	403	207
24B	S. Erasmo	VL-N	Sandy-loam	21	+80	6.24	8.6	2.69	25	19
8B	Tresse	VL-CN	Clay-loam	20.9	−114	7.78	30.9	1.82	273	143
31B	Fondamenta Nuove	VL-CN	Silty-loam	21	−70	6.9	18.3	1.67	132	104
11B	Sacca Sessola	VL-C	Silty-clay-loam	21.5	−125	6.51	29.2	1.64	191	67
14B	San Pietro in Volta	VL-C	Clay-loam	21	−160	7.5	17.6	1.6	103	86
16B	Millecampi	VL-S	Clay-loam	23	−113	7.2	35.9	4.32	51	23
19B	Ca' Roman	VL-S	Sand	21.8	+50	7.93	1	0.42	3	38

[a] Sub-basins of the lagoon according to Ref. [4]: VL-N: north; VL-CN: central-north; VL-C: central; VL-S: south (Fig. 1).
[b] Redox potential.
[c] Total organic carbon.
[d] Acid-volatile sulfide.
[e] Elemental sulfur.

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2.7. 16S rRNA gene libraries and phylogenetic analysis

16S rRNA genes were amplified from total DNA extracted from sediments using universal primers for bacteria and archaea as described by Van der Wielen et al. [29]. For archaea, nested PCR was performed using as template the first PCR product and using PArch 340F (5′-CCTACGGGGYG-CASCAG-3′) and PArch 934R (5′-CCTACGGGGYGCAS-CAG-3′) primers [10].

The amplicons were purified using the Qiaquick PCR purification kit (Qiagen, Milan, Italy), ligated in the pGEMT vector and cloned in JM300 competent *Escherichia coli* cells (Promega, Milan, Italy) following the instructions of the manufacturer. Positive clones were randomly picked and screened for insert presence by PCR using universal primers T7 and U19 (Promega, Milan, Italy), and about 800 bp were sequenced (Primm, Milan, Italy), with the forward primers used for 16S rRNA amplification.

Sequences were checked using the CHECK_CHIMERA program to determine the presence of hybrid sequences. Phylogenetic affiliations were preliminarily obtained using BLASTN and confirmed with the naive Bayesian rRNA Classifier provided by the Ribosomal Database Project website [3]. Nucleotide sequences were multialigned with ClustalX v.1.83 [27]. Alignments were checked manually and poorly aligned, or divergent regions were eliminated using GBlocks v.0.91b [9] with a minimum block of five and allowed gap positions equal to half. Operational taxonomic units (OTUs) were defined at 95% of similarity by the use of DNA_DIST software from the PHYLIP package. The significance of the DNA clone libraries was evaluated using ∫-LIBSHUFF [23].

Sequences were deposited in the EMBL nucleotide sequence database (GenBank/EMBL/DDBJ) under accession numbers from AM501576 to AM501918.

3. Results and discussion

3.1. Physico-chemical and biological characterization of sediments

The nine stations analyzed in this work represent the main different sub-basins present in the Venice lagoon ecosystem. The four sub-basins have similar ranges of contamination based on hazard quotients, with higher levels in VL-CN and lower ones in VL-S [4]. Temperature and pH were relatively homogeneous among samples, with values of 21.4 ± 1.2 °C and 7.33 ± 0.65, respectively (Table 1). The superficial sediments were mostly anoxic, except for stations 19B and 24B that showed hypoxic conditions (Table 1). These stations are adjacent to Chioggia and Lido entrances, respectively, and are sandy and more subjected to prevailing winds, tidal currents and ship movements that determine partial oxygenation. Station 14B is adjacent to the third lagoon entrance, but its sediments are protected here from erosion and consequent oxygenation by a wide prairie of marine phanerogams and macroalgae [20]. These results confirmed the general anoxic state of sediments from the Venice lagoon, which could be due to rapid oxygen consumption of total organic carbon in the presence of high concentrations of nitrogen species and phosphorus in pore waters [19].

Cell counts showed that all the stations were colonized by high numbers of microbial cells, varying between 6.4×10^7 and 5.6×10^9 cells g^{-1} d.w.$^{-1}$ (Table 2). Cell concentrations were positively correlated with the content of AVS ($r = 0.90$; $P < 0.05$, $n = 9$) and S^0 ($r = 0.91$; $P < 0.05$; $n = 9$), with good linear regression between them ($S^0 = 0.49 \times AVS + 13$; $r = 0.99$; $n = 9$). Given the unlimited quantity of nutrients in the lagoon, S^0 and AVS are the key elements that drive prokaryotic colonization of sediments. Sulfate present in the brackish coastal lagoon waters was previously demonstrated to be significantly reduced in anoxic sediments to sulfur or sulfide by anaerobic microbial activity [24,25].

3.2. ARISA fingerprinting

The structure of bacterial communities colonizing the sediments was analyzed by ARISA, and PCA was applied to ARISA fingerprints. The nine samples were divided into three groups, A, B and C (Table 2), by ANOVA and Tukey's post hoc test (Table SI1), with a *P*-value of 0.05. Ecological diversity indices were calculated from the normalized ARISA peak matrix (Table 2). A high Shannon–Weaver index, between 3.0 and 4.1, indicated relatively high bacterial community diversity, confirmed by high Menhinick and

Table 2
Microbial cell counts and ecological diversity indices of bacterial communities, as deduced from ARISA profiles.

Station	Cell count (cell g_{dw}[a])	ARISA[a]						
		No. of taxa	Shannon–Weaver	Menhinick	Margalef	Dominance	Equitability	Group[b]
2B	$5.67 \pm 1.83 \times 10^9$	75	3.878	0.652	7.796	0.034	0.898	B
22B	$1.48 \pm 0.66 \times 10^9$	68	3.842	0.622	7.135	0.032	0.911	C
24B	$5.84 \pm 1.67 \times 10^8$	82	4.128	0.722	8.557	0.022	0.937	B
8B	$2.71 \pm 0.54 \times 10^9$	65	3.024	0.370	6.191	0.088	0.724	A
31B	$8.35 \pm 2.78 \times 10^7$	87	4.059	0.673	8.845	0.026	0.909	B
11B	$1.37 \pm 0.27 \times 10^8$	67	3.303	0.548	6.865	0.081	0.786	A
14B	$3.5 \pm 0.82 \times 10^8$	73	4.085	0.746	7.855	0.021	0.952	C
16B	$1.48 \pm 0.39 \times 10^9$	46	3.624	0.619	5.222	0.033	0.947	C
19B	$3.08 \pm 0.79 \times 10^8$	74	3.426	0.480	7.246	0.060	0.796	A

[a] Diversity values calculated from electropherograms obtained from ARISA fingerprinting.
[b] Grouping of samples as calculated by PCA analysis of ARISA fingerprints.

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Margalef indexes. Low dominance and high equitability indicated substantial evenness in all sediments, with rather uniform species diversity distribution, but low predominance of specific groups (Table 2). ANOVA analysis of diversity indices showed that ARISA group A was significantly different from the other two groups in dominance, Shannon–Weaver and equitability (Table SI1). The three identified ARISA groups did not correlate with the four sub-basin divisions identified on the basis of their hazard quotients.

3.3. 16S rRNA gene libraries and phylogenetic analysis

One sample for each ARISA group was selected for further detailed description of bacterial and archaeal communities by screening of 16S rRNA clone libraries. Within each group, we selected samples that showed the highest number of cell counts: station 8B from sub-basin VL-NC, 2B from VL-N and 16B from VL-S.

A total of 343 clones were sequenced from six clone libraries, obtaining 298 bacterial and 45 archaeal sequences. We detected a total of 145 and 16 distinct bacterial and archaeal OTUs, respectively, which belonged to nine different known phyla. No significant affiliations were obtained for eleven 16S rRNA sequences in public databases, which suggests new taxonomic bacterial groups. Table 3 and Fig. SI1 reported the relative abundance of the different taxa found in the clone libraries, listed in function of their taxonomic rank.

Proteobacteria were predominant in all 16S rRNA sequence libraries which accounted for between 72% and 91% of clones (Fig. SI1). Sample 8B was dominated by *Gammaproteobacteria* (84%), while *Deltaproteobacteria* were less represented (3%). In contrast, samples 2B and 16B showed a relevant proportion of the delta subgroup (18% and 17% respectively) with predominance of bacteria belonging to *Desulfobacterales* (10% and 13% respectively) (Table 3 and Figs. SI1, SI2). In all samples, significant fractions of *Gammaproteobacteria* (between 12% and 27%) belonged to unknown families (Fig. SI3). The identified *Gammaproteobacteria* were differentially distributed among samples at the family and genus level (Table 3). The sediments of station 8B were colonized by *Oceanospirillales* (6%), *Thiotrichales* (4%), and *Alteromonadales* represented by the genera *Thalassomonas* (2%) and *Shewanella* (2%). This station showed a numerically significant presence (28%) of clones related to the genus *Photobacterium* within the *Vibrionales* that was absent in the other samples. Anoxygenic photosynthetic *Chromatiales* was the dominating phylum in the sediments of stations 2B and 16B (21% and 22% respectively), and was also present at a relatively high percentage in station 8B (13%). *Chromatiales* are anaerobic microorganisms specialized in sulfur-anoxygenic photosynthesis by consuming H_2S with S^0 production, typical of pristine anaerobic environments [13]. The presence of these bacteria indicated that sediments are often well exposed to solar irradiation due to tide oscillations and shallow water depth. Purple spots, attributed to the flourishing of anoxygenic purple photosynthetic bacteria, are often observable in the intertidal part of lagoon sediments.

Epsilonproteobacteria that were absent in station 8B and accounted for up to 4% in stations 2B and 16B, were mainly represented by sequences close to the *Sulfurimonas* genus, known as sulfide oxidizer [7] (Fig. SI4). *Alphaproteobacteria* were found at low percentages in all the libraries, with the exception of the family *Rhodobacteraceae* that represented 10% of clones only in the sediments of station 2B. This family comprises non-sulfur purple anoxygenic photosynthetic bacteria, typically found in lagoon ecosystems [13]. Bacteria belonging to *Bacteroidetes* phylum accounted for 20% of clones in station 2B, and 3% and 8% in stations 8B and 16B respectively (Table 3, Figs. SI1, SI4). Within this phylum, the *Flavobacteraceae* family was the most abundant, comprising genera *Robiginitalea* and *Maribacter*. These bacteria had been previously isolated from the marine algae *Ulva fenestrata*, able to degrade proteins and complex polysaccharides [17]. *U. fenestrata* colonizes sediments of Palude della Rosa (station 2B), where intense cellulase activity has been measured, up to 800 mg $O_2\,kg^{-1}\,h^{-1}$ (d.w.), four times higher than in the other lagoon areas [21].

Stations close to the urban centers of Venice, Marghera and Chioggia, (ARISA A cluster) were significantly different from the others in terms of bacterial taxonomic composition. The bacterial clone library of station 8B contained 28% of *Vibrionaceae* that were absent in the other stations. *Vibrionaceae* are typical markers of urban polluted marine areas [12], and the occurrence of *Vibrio* species in sewage effluents has been considered a potential serious threat to public health [15]. The presence of bacteria belonging to this family in the lagoon sediments, confirmed by cultivation-based surveys [1], indicates that in the urban stations, microbial communities were mainly selected on the basis of urban waste input. Stations not influenced by urban areas showed, on the contrary, significant clone numbers related to *Deltaproteobacteria*. This phylogenetic group is putatively responsible for sulfate (*Desulfobacterales*) and sulfur (*Desulfuromonadales*) reduction, which complement the activity of *Chromatiales* in the sulfur cycle [13,14]. The use of sulfide as electron donor by anaerobic photosynthetic bacteria creates a beneficial loop by sustaining the microbial growth of *Desulfuromonadales*, which consume the excess of sulfur produced during anoxygenic photosynthesis [14]. In these stations, clones related to *Sulfurimonas*, typical inhabitants of sulfidic habitats [7], were found. The presence of these bacteria that use reduced sulfur compounds as electron donor, together with the sulfur-oxidizing *Thiotrichales* retrieved in station 8B, suggest a complete sulfur cycle in lagoon sediments.

A large fraction of retrieved sequences (up to 80%) did not show high sequence identity with previously described species, giving values higher than 96% only with sequences of uncultured unidentified bacteria. Sample 8B also differed from the others in this feature, showing the highest percentage of sequences (33%) with 99–100% nucleotide identity with described species, mainly constituted by bacteria belonging to the *Photobacterium* genus.

Sequences present in the archaeal libraries belonged to *Euryarchaeota* (56–92%) and *Crenarchaeota* (8–44%, Table 3). Sample 8B was also shown to be different from the

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Table 3
Results of screening of 16S rRNA libraries from samples 2B (VL-N), 8B (VL-CN) and 16B (VL-S). The percentage of identified clones by phylum, class, order, and/or family and/or genus is reported for each taxonomical rank.

Phylum	Stations			Class	Stations			Order and/or family and/or genus	Stations		
	2B	8B	16B		2B	8B	16B		2B	8B	16B
Archaea											
Euryarchaeota	76	56	92								
Crenarchaeota	24	44	8	*Crenarchaeota* unknown	18						
				Thermoprotei	6	44	8				
Bacteria											
Unknown bacteria		2	9								
Bacteroidetes	20	3	8	Unknown *Bacteroidetes*	5	1	3				
				Flavobacteria	12	2	3	Unknown *Flavobacteriaceae*	5		1
								Flavobacteriaceae, *Maribacter*	2	1	
								Flavobacteriaceae, *Robiginitalea*	4		
								Flavobacteriaceae, *Ulvibacter*			1
				Sphingobacteria	3		5	*Sphingobacterales* unknown			1
								Sphingobacterales, *Flexibacteraceae*			2
Chloroflexi		1									
Cyanobacteria	7	1	4	*Cyanobacteria*	7	1	4	*Cyanobacteria* unknown	7	1	3
								Plochlorales, *Prochlorococcus*			1
Deferribacteres		2	1	*Deferribacteres*		2	1	*Deferribacterales*		2	1
Firmicutes	1			*Clostridia*	1			*Clostridiales*	1		
Planctomycetes			1	*Planctomycetacia*			1	*Planctomycetales*, *Planctomycetaceae*			1
Proteobacteria	72	91	77	*Proteobacteria* unknown	1	1					
				Alfaproteobacteria	12	3	4	*Alfaproteobacteria* unknown	2	1	1
								Rhodobacterales, *Rhodobacteraceae*	10	1	3
								Rhodospirillales		1	
								Rhizobiales			1
				Betaproteobacteria			1	*Burkolderiaceae*, *Schlegelella*			1
				Gammaproteobacteria	36	84	52	*Gammaproteobacteria* unknown	12	28	24
								Alteromonadaceae unknown		1	6
								Alteromonadaceae, *Thalassomonas*		2	
								Alteromonadaceae, *Shewanella*		2	
								Chromatiales unknown	21	13	5
								Chromatiales, *Ectothiorhodospiraceae*			17
								Oceanospirillales unknown	3	4	
								Oceanospirillaceae, *Thalassolitus*		2	
								Thiotrichales, *Piscirickettsiaceae*		4	
								Vibrionaceae, *Photobacterium*		28	
				Deltaproteobacteria	18	3	17	*Deltaproteobacteria* unknown	4		2
								Desulfobacteraceae unknown	9	1	7
								Desulfobacteraceae, *Desulfobacula*		1	
								Desulfobacteraceae, *Desulfofaba*			1
								Desulfobacteraceae, *Desulfonema*			1
								Desulfobulbaceae	1		4
								Desulfuromonadaceae unknown	2	1	1
								Desulfuromonadaceae, *Pelobacter*	1		1
								Bdellovibrionales	1		
				Epsilonproteobacteria	4		3	*Campylobacterales* unknown	1		2
								Helicobacteraceae, *Sulfurimonas*	3		1

others in the composition of the archaeal communities. At this station, 44% of the clones were *Crenarchaeota*, while stations 2B and 16B were dominated by *Euryarchaeota*, accounting, respectively, for 76% and 92% of the libraries. In addition to *Thermoprotei*, known to comprise anaerobes and sulfur reducers [32], the majority of archaeal sequences in all samples nevertheless belonged to unidentified classes (Table 3, Fig. SI5). Little information could hence be gained from taxonomic distribution of archaeal communities, recently estimated by FISH analysis to constitute around 10% of Venice lagoon sediment microbiota [11].

Surprisingly, no sequences related to known methanogenic archaea were found in the 16S rRNA clone libraries. Sequences distantly affiliated with *Methanomicrobia* were retrieved at all three stations by denaturing gradient gel electrophoresis (DGGE)–fingerprinting analysis of archaeal 16S rRNA gene (data not shown). We hypothesized that most methanogenic archaea might even be restricted to deeper layers of the sediments. These layers may have more suitable redox conditions, while on the surface they can be outcompeted by bacteria capable of using more energetic electron acceptors [18].

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3.4. Statistical analyses of gene libraries

Rarefaction analysis of clone libraries indicated that we explored between 43.2% and 50.2% of the theoretical total number of bacterial taxa present, and between 47.0% and 57.7% of archaeal taxa (Table 4). Although rarefaction values did not indicate complete saturation for any of the clone libraries, estimation of the coverage (Table 4) revealed high values for archaeal clone libraries (76–92%). The sequenced clones were therefore in sufficient number for describing archaeal communities. Bacterial libraries showed lower values of coverage (57–72%), indicating that bacterial diversity remained partially unexplored. However, this approach led to the description of the most numerically abundant phylogenetic groups, which putatively constitute the populations that colonize sediments and contribute to ecosystem functioning.

Bacterial taxonomic diversity proved to be very high at all three stations examined, much higher than archaeal communities, as shown by coverage values and diversity indexes (Table 4). Sediments from station 8B were the most divergent, with reduced taxonomic diversity with respect to the other stations, and were dominated by specific taxa, like the *Vibrionaceae*, present only in this sample.

In order to verify the significance of divergences observed among clone libraries, we used the ∫-LIBSHUFF approach [23]. ∫-LIBSHUFF comparison demonstrated that sediments of station 8B were significantly different in taxonomic composition of both archaeal and bacterial communities from those of stations 2B and 16B, that did not show significant differences from one another (data not shown).

Compilation of 16S rRNA sequence databases and the study of microbial biodiversity distribution and population structure are important in understanding potential biogeochemical processes that currently occur in sediments, but also in constructing a state-of-the-art picture useful for evaluating the future impact of mobile dams. A further RNA-based survey would allow access to active prokaryotic populations for better characterization of microbial components involved in functioning of this particular ecosystem.

Based on the total microbial diversity of the superficial sediments, two areas were differentiated in the Venice lagoon. VL-N and VL-S, considered relatively pristine for the standard ecological parameters of coastal lagoons [4], showed a significant presence of bacteria related to the sulfur cycle. VL-NC, located between the two most impacted areas of the lagoon, i.e. the canals of Venice and the harbor of Porto Marghera [4], showed a predominance of *Vibrionaceae*. These results indicated that the major discriminating factor in microbial biodiversity in the historical Venice lagoon might be urban waste rather than organic debris from colonizing algae and aquatic plants [22]. In the relatively pristine areas of the lagoon, the organic fraction is subjected to a cascade of catalytic events, starting from the hydrolytic activity of *Enterobacteraceae* and *Bacteroidetes* toward polymeric substances. At the end of the carbon cycle, the uptake of low molecular weight residues is essential for sustaining growth of anaerobic chemoheterotrophic bacteria linked to the sulfur cycle. Sulfur- and sulfate-reducing bacteria provide electron donors for anaerobic sulfide oxidizers and for anoxygenic photosynthetic bacteria, which fuel the nutritional loop providing primary production of organic nutrients.

Table 4
Rarefaction analysis and diversity indexes for bacterial and archaeal 16S rRNA clone libraries.

Libraries			2B	8B	16B
Bacteria	General features	n° clones	99	99	100
		n° taxa	49	42	54
		singletons	28	34	43
		coverage (%)	72	66	57
	Rarefaction	described taxa (%)	50.2	43.2	47.6
		st dev	3.2	3	3
	Diversity indices	dominance	0.036	0.136	0.052
		Shannon–Weaver	3.629	2.841	3.522
		Menhinick	4.925	4.221	5.4
		Margalef	10.45	8.92	11.51
		equitability	0.932	0.76	0.883
Archaea	General features	n° clones	17	16	12
		n° taxa	6	6	4
		singletons	4	3	1
		coverage (%)	76	81	92
	Rarefaction	described taxa (%)	54.2	57.7	47.0
		st dev	1.3	1.3	1.0
	Diversity indices	dominance	0.315	0.218	0.391
		Shannon–Weaver	1.305	1.555	1.136
		Menhinick	0.354	0.34	0.372
		Margalef	0.755	0.744	0.706
		equitability	0.811	0.966	0.706

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Appendix. Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.resmic.2009.04.005.

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