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Diversity and biogeography of bacterial assemblages in surface sediments across the San Pedro Basin, Southern California Borderlands

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Summary

Sediment bacteria play important roles in the biogeochemistry of ocean sediments; however, factors influencing assemblage composition have not been extensively studied. We examined extractable sediment bacterial abundance, the composition of bacterial assemblages using a high-throughput molecular fingerprinting approach, and several sediment biogeochemical parameters (organic matter content and alkaline phosphatase activity), along a 35 km transect from Point Fermin, Southern California, to Santa Catalina Island, across the approximately 900-m-deep San Pedro Basin. Automated rRNA intergenic spacer analysis (ARISA) demonstrated that in two spatially isolated shallow (approximately < 60 m, on opposite sides of the channel) sediment environments, assemblages were more similar to each other than to deeper communities. Distinct communities existed in deeper and shallower sediments, and stations within the deep basin over 2 km apart contained remarkably similar assemblage fingerprints. The relative contribution to total amplified DNA fluorescence of operational taxonomic units (OTUs) was significantly correlated to that of other OTUs in few comparisons (2.7% of total), i.e. few bacterial types were found together or apart consistently. The relative proportions within assemblages of only a few OTU were significantly correlated to measured physicochemical parameters (organic matter content and wet/dry weight ratio of sediments) or enzyme (alkaline phosphatase) activities. A low percentage of shared OTU between shallow and deep sediments, and the presence of similar, but spatially isolated assemblages suggests that bacterial OTU may be widely dispersed over scales of a few kilometres, but that environmental conditions select for particular assemblages.

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Introduction

Bacteria within coastal and shelf sediments play an important role in global biogeochemical cycles, as they are the ultimate sink of most terrestrially derived compounds and a high proportion of marine particle flux. Despite a growing understanding of the global biogeochemical importance of these sediment habitats (Berelson *et al*., 1990; Blackburn and Blackburn, 1993; Vanduyl *et al*., 1993; Codispoti *et al*., 2001), little is known of the bacterial communities inhabiting them (Cifuentes *et al*., 2000; Todorov *et al*., 2000; Madrid *et al*., 2001; Kim *et al*., 2004), nor the factors influencing their distribution (Bowman *et al*., 2005; Polymenakou *et al*., 2005). Marine microbial diversity in the ocean has been the subject of intense recent study (Giovannoni *et al*., 1990; Fuhrman and Ouverney, 1998; Giovannoni and Rappe, 2000; Venter *et al*., 2004). Only in the past two decades have molecular tools for addressing microbial ecology become available, which circumvent culture biases associated with an estimated 95% of bacterial taxa (Giovannoni *et al*., 1990; Fuhrman *et al*., 1992). Pelagic studies have revealed a high diversity of marine prokaryotes which are capable of utilizing unexpected metabolic pathways (Beja *et al*., 2000; Fuhrman, 2003). Several other studies, using semi-quantitative approaches to observe assemblage composition, have allowed greater understanding of dispersal of different taxonomic units within marine and estuarine environments (Hollibaugh *et al*., 2000; Moeseneder *et al*., 2001; Troussellier *et al*., 2002; Stepanauskas *et al*., 2003; Hewson and Fuhrman, 2004). These studies have demonstrated that some bacterial taxonomic units within water column assemblages are capable of existing in a wide range of environmental conditions and habitats, confirming earlier studies of ubiquitous dispersal of some bacterial clades (Giovannoni *et al*., 1990; Morris *et al*., 2002; 2004). Within sediments, most studies to date examining bacterial community diversity have focused upon a single

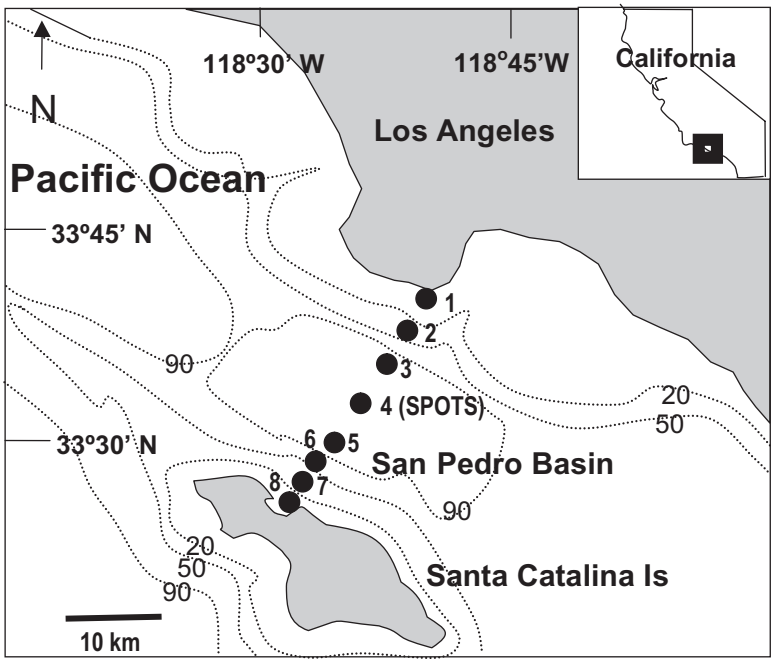

Fig. 1. Map showing location of transect and sampling sites in the San Pedro Basin (California Borderlands). Bathymetry is indicated by dashed lines and given in tens of metres. Station 4 is the San Pedro Ocean Time Series station (SPOTS).

functional group of bacteria (Scala and Kerkhof, 2000; Burns *et al*., 2002; Bowman *et al*., 2005), a single location or environment type (Cifuentes *et al*., 2000; Todorov *et al*., 2000; Madrid *et al*., 2001) or effects of biological (Hewson *et al*., 2003; Luna *et al*., 2004) and chemical factors upon assemblage composition (Hewson *et al*., 2003; Bowman *et al*., 2005).

Microbial composition in the oceans is believed to be influenced by combinations of resource availability (Rowe *et al*., 1991; Torsvik *et al*., 2002), temperature (Pomeroy and Deibel, 1986), pressure (Kato *et al*., 1998), and selective loss factors such as grazing (Sherr and Sherr, 1994; Simek *et al*., 2001) and viral lysis (Suttle *et al*., 1990; Bratbak *et al*., 1992; Fuhrman, 1992; Fuhrman and Suttle, 1993; Thingstad and Lignell, 1997; Hewson *et al*., 2003; Schwalbach *et al*., 2004; Winter *et al*., 2004). Planktonic communities are believed to be well mixed as there are few barriers to microbial dispersal (Falkowski and de Vargas, 2004). Along with arguments of ubiquity of free-living microeukaryotes (Finlay, 2002), this has been argued to confirm Beijerinck and Winogradsky's statement that 'everything is everywhere, the environment selects' (Beijerinck, 1913). While sediments may seem unlikely to support ubiquitous dispersal of microorganisms relative to the water column, some bacterial taxa can be isolated from a very wide range of terrestrial soil habitats (Beijerinck, 1913). Thus, environmental factors are believed to select from a ubiquitously dispersed bacterial assemblage, and thus similar environments are hypothesized to give rise to similar assemblages. While there is little empirical evidence to support the idea of ubiquitous dispersal, there have been several studies suggesting habitat specificity of different types of bacteria (Hewson and Fuhrman, 2004; 2006a; Hewson *et al*., 2006).

Previous study of estuaries, which typically contain strong gradients in physical and chemical conditions, indicated habitat specificity of some bacterial phylotypes, but ubiquitous distribution of others (Troussellier *et al*., 2002; Stepanauskas *et al*., 2003; Hewson and Fuhrman, 2004). Over vertical gradients in sediments, the assemblage composition of denitrifying bacteria (Scala and Kerkhof, 2000) and the entire bacterial assemblage (Hewson and Fuhrman, 2006b) has been observed to change dramatically. Furthermore, the water column addition of PO_4^{3-} to carbonate sediment-water mesocosms significantly stimulated the richness [total number of operational taxonomic units (OTU)] and diversity (rank distribution of OTU relative proportions) of sediment bacterial assemblages, indicating nutrient limitation of bacteria (Hewson *et al*., 2003). A recent study in deep sediments found that the diversity and richness of meso- and bathypelagic sediment bacterial assemblages were positively correlated to the number of active bacterial cells (Luna *et al*., 2004), suggesting that resource availability plays a key role in their structure.

The aim of this study was to determine the distribution of sediment bacterial assemblages along a 35 km transect from the North American mainland to Santa Catalina Island, which passes through the 900-m-deep San Pedro Basin (Fig. 1). The study was initiated in an attempt to understand: (i) the distribution of bacterial diversity in sediments along kilometre scales within deep-sea sediments, (ii) whether habitat specificity of sediment bacterial assemblages occurs and (iii) whether this habitat speci-

ficity is linked to ecosystem functions. We used a molecular fingerprinting technique, automated rRNA intergenic spacer analysis (ARISA) (Fisher and Triplett, 1999) to examine sediment bacterial assemblages at eight sites ranging in water depth from 27 to 912 m, from coarse to fine muds, and underlying different water column trophic conditions ranging from highly productive and anthropogenically disturbed to comparatively low productivity oceanic conditions. Our results demonstrate that diversity indices of sediment bacterial assemblages do not vary strongly across the transect, that phosphatase activity does not correlate with any component of the assemblages, and that the composition of assemblages is different between shallow and deep water sediments.

Results and discussion

Extractable bacterial abundance, fingerprint richness and diversity

Extractable bacterial abundance ranged from 0.7×10^8 to 1.6×10^8 cells per cubic centimetre of sediment and negatively correlated with total water depth ($r = -0.89$, $P < 0.05$), sediment organic matter content ($r = -0.88$, $P < 0.05$) and water content ($r = -0.91$, $P < 0.01$) (Fig. 2). ARISA fingerprints of sediment bacterial communities contained 105–129 OTUs per fingerprint, and fingerprint diversity (Simpson Index) ranged from 28.4 to 46.9. However, both richness and diversity of fingerprints did not change significantly with increasing depth nor distance along transect (Fig. 2). ARISA fingerprints were less rich than fingerprints of bacterial communities in carbonate sediments of Florida Bay (Hewson *et al*., 2003), but notably higher than the water column of Moreton Bay (Hewson and Fuhrman, 2004). This observation is supported by earlier study of deltaic muds off French Guiana and Papua New Guinea that indicated higher richness in sediments compared with water column assemblages (Madrid *et al*., 2001). The higher richness of phylotypes recorded in other studies in sediments may be due to niche availability, as there may be greater complexity of resources, physical parameters and chemical parameters than in overlying waters.

The lack of change in richness across the San Pedro Basin was unexpected as the different environments (deep and shallow water) presumably have different resource availability, productivity and temperature controls on growth. Primary and secondary productivity have been linked in terrestrial environments to enhanced richness and diversity in several environments which has further been attributed to greater ecological niche availability (Waide, 1999). Other studies of bacteria have demonstrated increased morphological diversity with intermediate disturbance (Kassen *et al*., 2000), which is consistent with hypotheses of coral diversity (Glynn, 1976; Connell, 1978) and artificial disturbance – incubation studies of phytoplankton (Sommer, 1995). As the productivity of most sediments (except those in proximity to seeps or hydrothermal vents) is ultimately linked to the productivity of overlying waters (Berelson *et al*., 1997; Nodder *et al*., 2003), the shallower, sandy sediments in this study are believed to be more productive than deeper muds. However, ARISA fingerprint richness or diversity did not vary strongly along the transect from the mainland to Santa Catalina Island. Shallower sediments are more heavily affected by tidal currents, grazing and resuspension than deeper sediments in the San Pedro Basin. However, we did not find any difference in richness or diversity between these two environments. Therefore, either productivity and disturbance are too weak to create significant changes in overall diversity, or there may be competing effects that generally compensate for each other to yield no net change in richness or diversity.

The relative proportion that each OTU contributed to total fingerprint DNA fluorescence correlated significantly ($P < 0.004$) with that of other OTUs in a total of 136 comparisons (of 5512 possible correlations, or 2.7%). This suggests that the dynamics of individual OTU within assemblage fingerprints are independent and there may be few mutualistic or directly antagonistic bacteria within these environments. At the same time, this also suggests that the large majority of bacteria do not have multiple operon copies with different ARISA lengths as these would correlate to each other, and such correlated OTUs comprised a small proportion of total amplified DNA fluorescence (all each < 1%).

Bacterial assemblage composition

Duplicate ARISAs from the same sediment DNA extract shared a Whittaker Index of 0.90 ± 0.01 and Sorensen Index of 0.93 ± 0.06. ARISA fingerprints of deep basin and slope samples were remarkably similar, despite large distances between adjacent stations. Stations 2, 3, 4, 5 and 6 shared a Whittaker Index of 0.66 ± 0.01 and Sorensen Index of 0.76 ± 0.01 but Station 7 clustered differently when using the Whittaker Index and Sorensen Index. The results of ARISA fingerprint analysis suggest that assemblages are homogeneously dispersed between sediment stations but not between surface and deep sediments, and that the environmental conditions give rise to distinct assemblages within particular habitats, a finding similar to a recent report of sediment bacteria in the Ionian Sea (Polymenakou *et al*., 2005). ARISA fingerprints formed two distinct groups (Fig. 3). Study of bacterioplankton over similar distances in mesotrophic estuaries demonstrated more heterogeneity in assemblage composition than in these sediments (Troussellier *et al*., 2002;

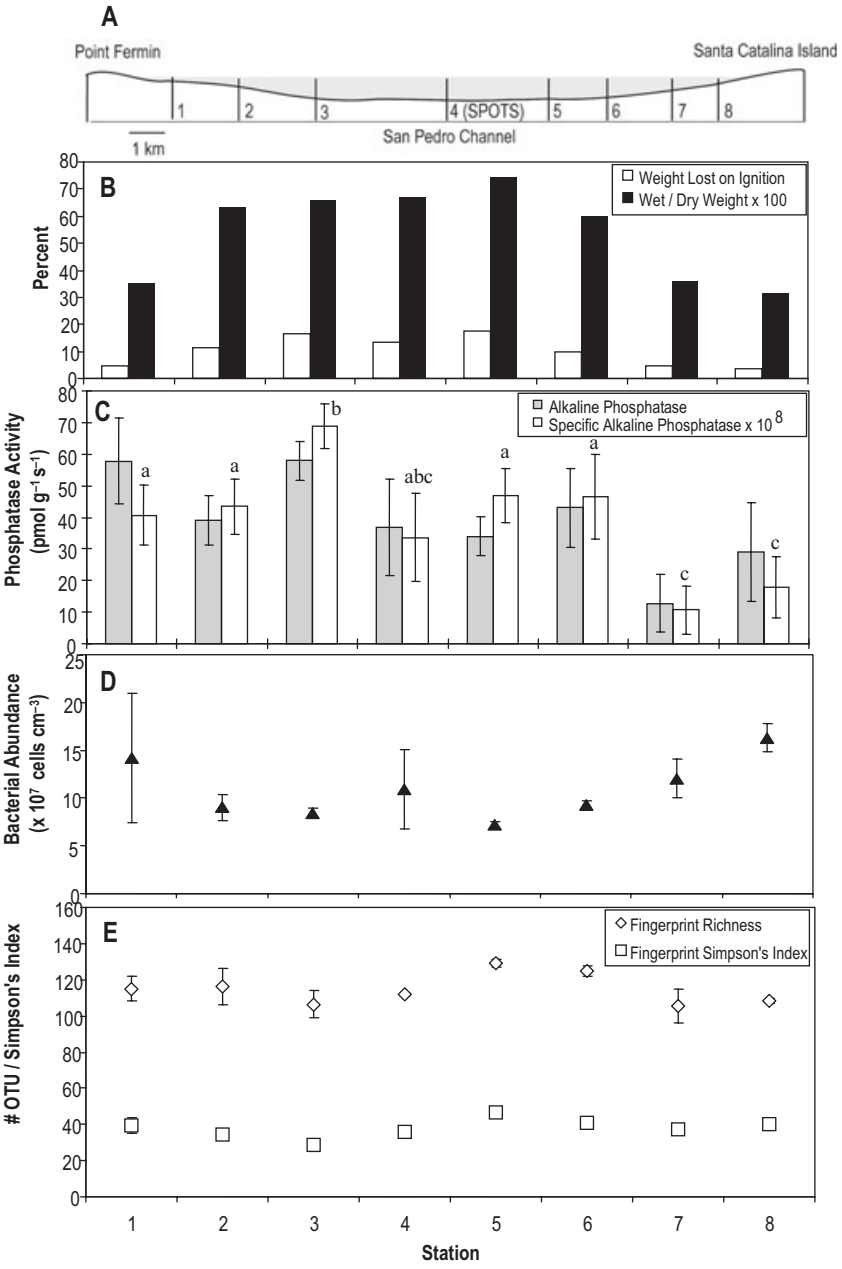

Fig. 2. A. Cross-section of the San Pedro Basin (to scale).
B. Sediment characteristics based upon organic matter content and water content (i.e. wet/dry weight ratio × 100) (Dean, 1974).
C. Activity of alkaline phosphatase and specific activity of alkaline phosphatase (i.e. normalized per bacterium). Significant ($P < 0.05$, Student's *t*-test) differences between specific phosphatase activities are denoted by different letters.
D. Extractable bacterial abundance as determined by SYBR Green I staining (Noble and Fuhrman, 1998) and epifluorescence microscopy of extracted sediments (pore water + surface-associated prokaryotes) (Hewson and Fuhrman, 2003).
E. ARISA total fingerprint richness and Simpson Index.
Error bars = SE ($n = 3$).

Hewson and Fuhrman, 2004). Stations 1 and 8 shared a Whittaker Index of 0.47 and Sorensen Index of 0.68. The similarity between the two shallow, sandy sediment stations (1 and 8) suggests that local selective factors may cause similar assemblages to arise. Furthermore, interaction with overlying waters is much higher in shallower sediments, and as such, factors such as genetic exchange between pelagic and benthic populations may give rise to similar assemblages. As assemblages within deep basin sediments are not resuspended by physical motion or currents, mixing of communities is unlikely to cause homogeneity within muds. Rather, the sediments of the San Pedro Basin experience relatively constant physical and chemical conditions and are isolated from shallower depths of the California Borderland by a shallow (approximately 750 m deep) sill (Berelson, 1991). These constant physical and chemical conditions relative to shallower sediments probably place similar selective pressures upon assemblage development. In contrast, the shallow sediment stations were less similar to each other than between basin stations, which could be due to differences in productivity between the two locations. High variability between nutrient-amended assemblages depending on inorganic nutrient type added (PO_4^{3-}, NH_4^+) in shallow sediments has been observed in sediment-water mesocosms (Hewson *et al.*, 2003) and demonstrate

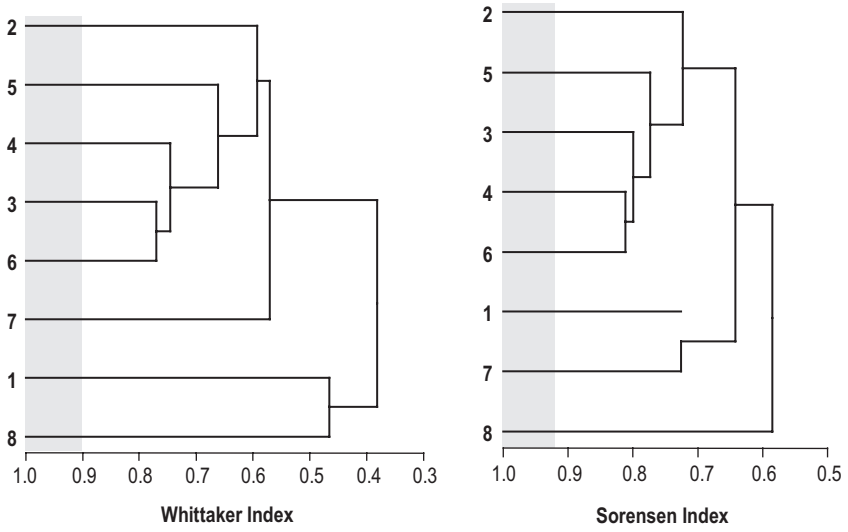

Fig. 3. Dendrograms of whole bacterial assemblage similarity along transects, based upon Whittaker and Sorensen Indices of similarity (Legendre and Legendre, 1998). Clustering was conducted by unweighted pair-group mean average (UPGMA) using the XLStat program (Sokal and Rohlf, 1995). The grey shaded region indicates the average similarity between replicate fingerprints in this study.

that within surface sediments, resource type may shape bacterial communities.

Relationship between assemblage composition and biogeochemical parameters

Microorganisms able to mobilize organic matter macromolecules using free (exoenzymes) or cell-surface attached (ectoenzymes) are able to colonize and thrive in variety of nutrient-defined habitats. The availability of nutrients in an ecosystem is a function of both the amount of nutrient present and the stoichiometic ratio of nutrients present in an environment. These enzymes may be present at constitutive levels and/or induced depending on the relative rate of nutrient supplied to the ecosystem as well as the balance between different types of nutrients.

Bulk sediment phosphatase activity correlated significantly with distance to the mainland ($r = 0.74$, $P < 0.05$) where phosphatase activity decreased from the mainland to Santa Catalina Island. These activities may correspond to higher nutrient inputs associated with the coastal processes (Fig. 2). Alkaline phosphatase specific activity was calculated by dividing the enzyme activity by microbial abundance. The specific activity of stations closest to Catalina Island (Stations 7 and 8) was significantly (Student's *t*-test; $P < 0.05$) lower than specific activity at all other stations with the exception of Station 4. These stations had lower bacterial abundance and specific alkaline phosphatase activity. A significant increase (Student's *t*-test; $P < 0.05$) in specific activity at Station 3 relative to stations closer to the mainland (Stations 1 and 2) was also evident (Fig. 2). Specific enzyme activity for Stations 5 and 6 is similar to Stations 1 and 2 (Fig. 2).

In this study we found that ectoenzymes were related positively with distance from the mainland with decreasing activity between offshore stations and in proximity to the mainland. High alkaline phosphatase activity has been associated with nutrient deficiency as well as with nutrient imbalance in microbial communities (Sala *et al*., 2001; Hoppe, 2003). Ectoenzyme activities appear to be partially constitutive, as well as induced, and are biomass dependent (Sala *et al*., 2001; Taylor *et al*., 2003) but independent from overall bacterial production (Lavigne *et al*., 1997). Taylor and colleagues (2003) found aquatic environments studied to have unique ectohydrolytic profile associated with each area analysed. Ectoenzyme activity may be a systematic response to environmental nutrient flux, particularly in sediments (Vetter and Deming, 1994; Polemba, 1995; Lavigne *et al*., 1997; Talbot and Bianchi, 1997; Fabiano and Danovaro, 1998). Kirchman and colleagues (2004a) found covariance of ectoenzyme activity with the beta protobacteria showing the highest correlation with phosphatase activity in a lotic ecosystem. This may suggest acclimation of bacterial communities to different nutrient regimes. Fandino and colleagues (2001) found attached bacteria had a higher cell-specific hydrolytic enzyme activity than free living bacteria. Rapid enzyme inductions and changes in bacterial activity as measured by Fandino and colleagues (2001) and Kirchman and colleagues (2004a) may reflect change in community structure which possibly is related to changes in nutrient stoichiometry.

The relative contribution to fingerprint DNA fluorescence of 4 OTUs significantly and negatively and 2 OTUs significantly and positively correlated to total water depth at the sampling locations (Table 1). The relative proportions within fingerprints of 2 OTUs significantly and positively correlated to bacterial abundance. The amplified DNA fluorescence of 2 OTUs significantly and negatively and 2 OTUs significantly and positively correlated to organic matter content. DNA fluorescence proportion of a

Table 1. Correlation analysis of biogeochemical parameters and the relative fluorescence intensity of OTU.

	Water depth	Alkaline phosphatase activity	Organic matter content	Moisture content	Extractable bacterial abundance	OTU					
						557 bp	763 bp	888 bp	943 bp	963 bp	967 bp	978 bp
Water depth	1	Ns	0.95	0.97	−0.89	Ns	0.94	0.93	−0.90	−0.89	−0.91	−0.89
Alkaline phosphatase activity		1	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns
Organic matter content			1	0.96	−0.88	−0.87	0.90	0.85	−0.89	Ns	Ns	Ns
Moisture content				1	−0.91	−0.90	0.95	0.82	−0.92	−0.87	−0.89	Ns
Extractable bacterial abundance					1	Ns	Ns	Ns	Ns	Ns	0.94	0.89

Ns, not significant, $P > 0.007$.

further 4 OTUs correlated significantly and negatively but 1 OTU significantly and positively correlated with the moisture content of sediments. The relative contribution of no OTU significantly correlated with alkaline phosphatase activity. The lack of correlation between alkaline phosphatase activity and OTU relative DNA fluorescence in this study contrasts with previous study comparing enzyme activities with the composition of lotic bacterioplankton (Kirchman *et al.*, 2004a). This correlation analysis demonstrates habitat specificity of only a small percentage of bacterial OTUs (153 total OTUs) as the relative proportions of most OTU within assemblage fingerprints is not directly tied to the measured environmental parameters. The results also suggest that alkaline phosphatase activity is not specifically related to any single OTU group, as been observed in previous studies of stream bacterioplankton (Kirchman *et al.*, 2004b). Further study of the interaction of bacterial physiological response to nutrient changes and community structure in natural systems using finer-scale quantitative molecular analyses over a broader range of locations may offer insight into the relationship between ectoenzyme activity, stoichiometric imbalance and community structure.

Conclusions

The similarity of two spatially isolated shallow-water communities (i.e. Point Fermin and Santa Catalina Island, across the channel from each other) and similarity between stations along the floor of the San Pedro Basin suggest that sediment bacteria may be widely dispersed. Dispersion could be by movement on suspended particles or in faecal pellets or animals, or through the sediment layer itself. While these assemblages may be dispersed over large distances, similarity between communities in similar environmental settings suggests that factors present within habitat types may select for similar communities, at least on the scale of < 25 km. Finally, this study demonstrates that while the abundance and diversity of microbial assemblages may not change over wide geographic scales and across environmental gradients, the composition of assemblages may change dramatically, highlighting the need for taxonomic unit-level studies in biogeochemistry.

Experimental procedures

Sampling location

Samples for fingerprinting analysis were collected onboard the R/V *Point Sur* in May 2004 as part of the San Pedro Ocean Time Series (SPOTS) Microbial Observatory. Samples were collected using a 40 cm-by-30 cm contained Van Veen grab at eight sites across the San Pedro Basin from Point Fermin to Two Harbors, Santa Catalina Island within the

California Borderland region (Fig. 1). Two locations (Stations 1 and 8) were in shallow (60 and 27 m respectively) waters within the euphotic zone. Station 2 was on the slope of the continental shelf (170 m total water depth) and Stations 6 and 7 were on the Catalina Rise in 400 m and 700 m respectively. The remaining three stations (3, 4 and 5) were within the San Pedro Basin in 890–911 m. The transect from Two Harbors to Point Fermin is characterized by a north-south gradient in productivity, caused by wastewater discharges close to Point Fermin and possibly by coastal upwelling which are equivalent to natural terrestrial inputs due to prevailing semiarid conditions in the Los Angeles basin. Once retrieved onboard, sediments (top 1 cm) were subsampled within 2 min for DNA and viral/bacterial abundance analysis. Within each grab, only relatively undisturbed, oxic sediments were sampled (as distinguished by colour and presence of worm tubes). Single samples from each grab were collected using sterile 5 ml syringes with the needle end removed, and placed immediately into sterile 15 ml centrifuge tubes, which were placed on dry ice until processing. Furthermore, samples for microorganism enumeration were collected using cut-off syringes to a total depth of 1 cm then discharged immediately into 15 ml centrifuge tubes containing 10 ml of 0.02 μm filtered elution buffer (10 mM sodium pyrophosphate, 5 mM EDTA, 3% formaldehyde) (Hewson and Fuhrman, 2003), and kept at 4°C prior to analysis (within 6 h of collection).

Determination of extractable bacterial abundance

Extractable bacterial abundance was determined as described previously (Hewson and Fuhrman, 2003). Briefly, samples in elution buffer were shaken for 10 min on a shaker table at 400 r.p.m. After shaking, 1 ml of this homogenate was removed and centrifuged at 3000 *g* for 5 min to remove suspended sediments. Duplicate subsamples were then prepared for SYBR Green I staining and epifluorescence microscopy (Noble and Fuhrman, 1998), using 50 μl of centrifuged subsamples further diluted in 2 ml of elution buffer to prevent low-volume meniscus artefacts on the Anodisc surface. This method resulted in removal of approximately 60% of sediment bacteria in a previous study (Hewson and Fuhrman, 2003). More than 200 cells of bacteria were counted in 20 ocular fields on an Olympus BH-60 microscope at 1000× magnification.

Sediment biogeochemical parameters

A subsample of sediment from each site was either frozen or kept cold (4°C) until analysis. Frozen samples as well as cold stored samples were used for alkaline phosphatase analysis. Independent tests on sediments from 400 m depths and from peat-rich sites indicate that alkaline phosphatase activity in these samples are not affected by freezing when compared with those that were not frozen. Enzyme activity was assayed using modification of established methods (Hoppe, 2003). The fluorogenic substrate methylumbelliferone phosphate (MUF-P) was used to measure phosphatase activity. Enzyme activity was measured by adding a single concentration of substrate (2 μM). The final concentrations result in linear rates over the time tested. Samples were measured on a Turner TD-700 fluormeter, using filters for excitation (380 nm) and emission (440 nm). Molar rates were calculated from the linear range of the activity curve against pure MUF as a fluorophore standard. Fluorescence quenching was tested by running fluorophore-spiked sediment blanks, and any corrections necessary are within the final calculation. Assays were initiated by suspending 1 g of sediment in 29 ml of cold, 0.05 M Trizma buffer pH 8.5 (Sigma). Substrate was added to the final concentration mentioned above, and then an immediate zero time sample was extracted, and frozen in liquid nitrogen. Subsequent time-course samples were taken and frozen. Samples were then centrifuged and defrosted and added to 10.3 pH, 0.5 M carbonate/bicarbonate buffer in a 1:1 ratio. This enabled running the assay in an alkaline range while reading samples at the optimum pH of the fluorophore.

An estimate of the organic matter content was determined by loss on ignition (LOI). Briefly, small subsamples of sediments (< 0.5 g) were placed in aluminium dishes and weighed, before being placed in an oven at 60°C overnight to dry. After drying the samples were re-weighed, then combusted for 8 h at 550°C, after which the combusted samples were weighed. Sediment water content was calculated by subtracting the dried weight of samples from the wet weight of samples and expressed as a percentage of the wet weight. The percentage LOI was calculated by subtracting the combusted weight of samples from their dry weight and expressing LOI as a percentage of dried weight (Stephens *et al.*, 1992).

Extraction of sediment assemblage DNA

DNA from 0.5 ml of subsamples of each station was extracted using Bio101 (QBIOGENE) kits according to manufacturer's protocols. The final DNA elution volume was 50 μl, and contained 17–19 ng DNA μl^{-1}.

Automated rRNA intergenic spacer analysis

Automated rRNA intergenic spacer analysis was conducted on 2.5 ng of extracted DNA as measured by Pico Green (Molecular Probes) fluorescence (Fisher and Triplett, 1999), as described previously (Hewson and Fuhrman, 2004). Briefly, the ITS region (plus about 285 bases of 16S and 23S rRNA) of DNA extracts was amplified using the polymerase chain reaction (PCR). Polymerase chain reaction was carried out in 50 μl of reactions containing 1× PCR buffer, 2.5 mM MgCl, 1.25 mM PCR Nucleotide Mix (Promega), 200 nM each of universal primer 16s–1392F (5′-G(C/T)AC ACACCGCCCGT-3′) and eubacterial primer 23s–125R labelled with a 5′-TET phosphoramidite (5′-GGGTT(C/G/T)CCCCATTC(A/G)G-3′) (Borneman and Triplett, 1997), BSA (40 ng μl^{-1}, final concentration) and 2.5 U Taq polymerase (Promega). These primers targeted specifically bacteria, hence archaea are not included in our analysis. Thermocycling was preceded by a 3 min heating step at 94°C, followed by 30 cycles of denature at 94°C for 30 s, anneal at 55°C for 30 s, extend at 72°C for 45 s, with a final extension step of 5 min at 72°C. Polymerase chain reaction amplification products were purified in Zymo Research Clean and Concentrator-5 Kits, then diluted to 5 ng μl^{-1} as mea-

sured by Pico Green fluorescence. ARISA products were run in duplicate on an ABI 377XL automated slab gel sequencer using a custom-made Bioventures 1500 bp size standard, and analysed densitometrically using the ABI Genescan program.

Polymerase chain reaction-based fingerprinting approaches, like all methods in ecology, are not perfect and have been criticized because of unequal template-to-product ratios (Suzuki and Giovannoni, 1996; Polz and Cavanaugh, 1998; Suzuki *et al*., 1998), unequal extractability of cells (Polz *et al*., 1999) and differences in operon copy number (Crosby and Criddle, 2003); however, ARISA is reproducible (Hewson and Fuhrman, 2004) and allows detection of clear differences between assemblages. Furthermore, slow-growing bacteria (characteristic of deeper sediment bacteria; Turley and Dixon, 2002) have been shown to contain few operon copies per cell (Klappenbach *et al*., 2000), and therefore are unlikely to influence our results.

Statistical analysis of assemblage fingerprints

Outputs from Genescan were transferred to Microsoft Excel for subsequent analysis. Peaks less than five times the baseline fluorescence intensity were discarded because they were considered 'noise'. The area under each peak was expressed as a percentage of the total integrated area under the electropherogram. Simpson Indices (D) were calculated manually using descriptions given in Legendre and Legendre (1998) according to the following equation:

$$D = \sum_{i=1} (P_i)^2$$

where P_i is the fraction of each peak of total integrated area.

Whole communities (i.e. all OTU each comprising > 0.09% of total amplified DNA) were compared by calculating the Sorensen Index of similarity using the following equation:

$$\text{Sorensen Index} = 2W/(a_1 + a_2)$$

where W is the number of shared ITS peaks between population 1 and 2, and a_1 and a_2 are the total number of ITS lengths in population 1 and 2 respectively. This index scales from 1 (completely identical) to 0 (completely different). Whole assemblages (i.e. all OTU each comprising > 0.09% of total amplified DNA) were analysed by calculating Whittaker Index of association (S_w) using the following equation (Whittaker, 1952):

$$S_w = 1 - \sum_{i=1,2}^{n} |(b_{i1} - b_{i2})|$$

where b_1 and b_2 are the percentage contributions to amplified DNA of the *i*th OTU in samples 1 and 2 respectively. S_w scales from 0 (completely different) to 1 (identical).

To account for variability in size associated with standards, ARISA fingerprints were binned ±1 bp from 400 to 700 bp, ±2 bp from 700 to 1000 bp and ±5 bp > 1000 bp using a shifting bin window approach described in Hewson and Fuhrman (2006b). Cluster analysis was conducted using the XLStat (AddinSoft SARL) program using either the Sorensen or Whittaker Index and clustering via unweighted group-pair mean average method (UPGMA).

Correlation analysis

Pairwise comparisons between the relative contribution to total DNA of each binned OTU within ARISA fingerprints and measured parameters comprised calculation of Pearson's correlation coefficient using the XLStat program. The significance level for comparisons was determined by dividing 0.05 by the number of correlates, which was used to avoid Type II error (Sokal and Rohlf, 1995). Only OTU for which there were three or more non-zero values were used in the multiple correlation analyses.

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