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## ORIGINAL ARTICLE

# Bacterial diversity and community composition from seasurface to subseafloor

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**We investigated compositional relationships between bacterial communities in the water column and those in deep-sea sediment at three environmentally distinct Pacific sites (two in the Equatorial Pacific and one in the North Pacific Gyre). Through pyrosequencing of the v4–v6 hypervariable regions of the 16S ribosomal RNA gene, we characterized 450 104 pyrotags representing 29 814 operational taxonomic units (OTUs, 97% similarity). Hierarchical clustering and non-metric multi-dimensional scaling partition the samples into four broad groups, regardless of geographic location: a photic-zone community, a subphotic community, a shallow sedimentary community and a subseafloor sedimentary community (⩾ 1.5 meters below seafloor). Abundance-weighted community compositions of water-column samples exhibit a similar trend with depth at all sites, with successive epipelagic, mesopelagic, bathypelagic and abyssopelagic communities. Taxonomic richness is generally highest in the water-column $O_2$ minimum zone and lowest in the subseafloor sediment. OTUs represented by abundant tags in the subseafloor sediment are often present but represented by few tags in the water column, and represented by moderately abundant tags in the shallow sediment. In contrast, OTUs represented by abundant tags in the water are generally absent from the subseafloor sediment. These results are consistent with (i) dispersal of marine sedimentary bacteria via the ocean, and (ii) selection of the subseafloor sedimentary community from within the community present in shallow sediment.**
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## Introduction

Previous studies indicate that microbial community composition varies from one marine environment to another (Delong *et al.*, 2006; Zinger *et al.*, 2011; Hamdan *et al.*, 2013), but can be relatively consistent in similar marine environments separated by long distances (Inagaki *et al.*, 2006; Agogué *et al.*, 2011; Walsh *et al.*, 2015). For example, adjacent marine water masses with different environmental properties have distinct microbial communities (Agogué *et al.*, 2011; Hamdan *et al.*, 2013). Similarly, subseafloor sedimentary environments with different properties separated by a few tens of kilometers also have distinct communities (Inagaki *et al.*, 2006; Hewson *et al.*, 2007; Schauer *et al.*, 2010). Despite these differences over relatively short geographic distances, microbial community composition in individual

deep-seawater masses can be relatively constant for thousands of kilometers (Agogué *et al.*, 2011). And broadly, similar microbial communities inhabit similar subseafloor sedimentary environments separated by thousands of kilometers (Inagaki *et al.*, 2006). These observations are consistent with the old adage, 'Everything is everywhere but the environment selects' (Baas-Becking, 1934), in which microorganisms are considered to be ubiquitously dispersed because of their small size, large numbers and low extinction rates (Martiny *et al.*, 2006). Despite these intriguing biogeographic patterns, few previous studies have examined (i) the vertical distribution of bacterial taxa throughout the entire open-ocean water column, or (ii) the relationship of microbial communities in marine sediment to those in the overlying water.

Distributions of abundant bacterial taxa in surface marine sediment (to 1.1 meters below seafloor (mbsf)) suggest that the seafloor is colonized via the overlying water column (Hamdan *et al.*, 2013). However, the relationship of microbial communities in deep subseafloor sediment (⩾ 1.5 mbsf) to those in

surface marine sediment and the overlying ocean has not been previously examined.

Vertically sampling the water column and sediment in distinct oceanographic regions provides a means to evaluate the influence of local oceanographic properties such as nutrient availability, sedimentation rate and distance from land (Fuhrman *et al.*, 2006; Kallmeyer *et al.*, 2012) on microbial diversity throughout both water column and sediment. High productivity in surface water can lead to the formation of oxygen-deficient zones in deeper water, where unique communities may form in response to the deoxygenation (Beman and Carolan, 2013). In high-productivity regimes, such as the eastern equatorial Pacific upwelling region (EQP-1) and the moderately productive central equatorial Pacific upwelling region (EQP-8), a relatively high flux of organic debris to the seafloor sustains an active anaerobic subseafloor sedimentary community (D'Hondt *et al.*, 2004). In contrast, low-productivity regions, such as the North Pacific gyre (EQP-11), are characterized by a continuously oxic water column, extremely low rates of sedimentation and organic matter deposition, and a relatively low-activity aerobic subseafloor community (D'Hondt *et al.*, 2009; Røy *et al.*, 2012; D'Hondt *et al.*, 2015).

(i) To document vertical distributions of bacterial diversity and community composition, (ii) to investigate how these communities vary from one oceanographic region to another, and (iii) to test the extent to which bacterial communities of deep subseafloor sediment may originate in the water column, we used 454 pyrosequencing technology and the v4–v6 hypervariable region of the bacterial 16S ribosomal RNA (rRNA) gene to examine bacterial community composition (presence–absence and relative abundance) in the water column, near-seafloor sediment (0–10 centimeters below seafloor (cmbsf)), and subseafloor sediment at three environmentally distinct Pacific sites: the very high-productivity eastern equatorial upwelling region (EQP-1), the moderately high-productivity open-ocean central

equatorial upwelling region (EQP-8) and the very low-productivity northern gyre (EQP-11) (Figure 1).

## Materials and methods

### Sampling methodology

We collected the sediment and seawater samples during a 43-day transect of the *R/V Knorr* through the eastern equatorial Pacific and the North Pacific gyre (*Knorr* Expedition 195-3). We collected the water samples using a 24-Niskin bottle CTD rosette (Sea-Bird SBE-911 CTD system plus package). Once the CTD/Niskin system was on deck, we clamped the Niskin bottles and applied compressed air to the vent plugs to enhance filtration. We filtered 5 l from each bottle onto 0.2 μM Supor-200 47 mm filters at a rate of approximately 200 ml min$^{-1}$, and then stored the filters at −80 °C. We targeted a combination of standard depths and oceanographic horizons (seasurface, $O_2$ minimum, chlorophyll *a* maximum, thermocline and deep water) and analyzed 7–14 sampling depths at each station from 3 to 5500 m water depth depending on location. We measured water depths using the center depth of the SeaBeam 2112 multibeam. We estimated the average sedimentation rate for each site by dividing the crustal age (Müller *et al.*, 2008) by the sediment thickness in the NDGC global thickness grid (Divins, 2003). We used mean annual average chlorophyll *a* data (Behrenfeld and Falkowski, 1997; Gregg *et al.*, 2005), collected from September 1994–December 2004, as a proxy for seasurface productivity.

At each station, we collected sediment using a multi-corer (0–30 cmbsf], gravity corer (up to 4 mbsf) and long-piston coring device (up to 35 mbsf). We subsampled the sediment using sterile 60 cc cut-off syringes and immediately froze the subsamples at −80 °C for later DNA extraction. For each site in this study, we analyzed one sediment sample taken with the multi-corer at the sediment-water interface
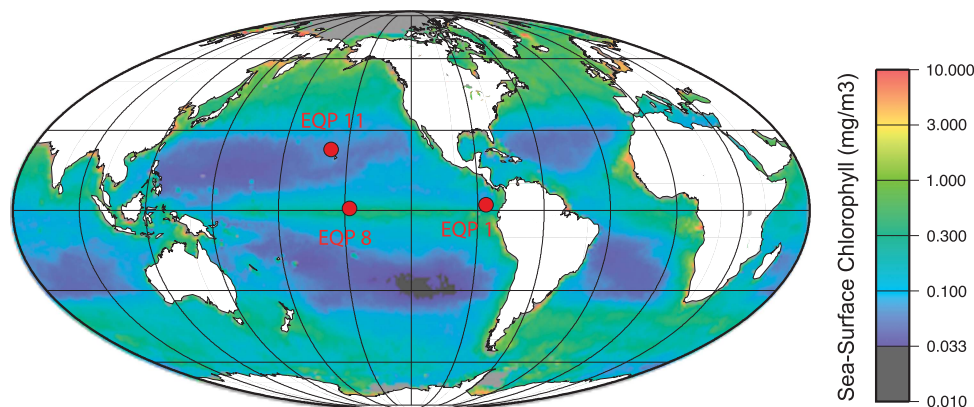


**Figure 1** Locations of sampling stations. Superimposed chlorophyll *a* content is annual average data (mean from September 1994–December 2004) from the SeaWiFS satellite uploaded into GeoMapApp (Behrenfeld and Falkowski, 1997; Gregg *et al.*, 2005).

(0–10 cmbsf) and two to five sediment samples taken at greater depths (between 0.25 and 34 mbsf). In total, this study includes 39 tag-pyrosequencing samples: 28 from the water column, 3 from the sediment-water interface and 8 from subseafloor sediment.

*Site descriptions*

All three sites are located in the deep open ocean, with fully pelagic sediment.

EQP-1 (1° 48.21' N, 86° 11.29' W) was the eastern-most site in our equatorial Pacific transect. This site is located in a relatively flat region of 1.6-Ma basement (Müller *et al.*, 2008), with water depth of 2856 m (Figure 1). It is in the eastern equatorial upwelling regime. Consequently, it contains a highly productive ecosystem and is characterized by a high mean sedimentation rate (~75 m Ma$^{-1}$). Its sediment is predominantly foraminifera-rich nannofossil ooze. Total sediment thickness is estimated to be 120 m (Divins, 2003). We began sampling with the CTD/Niskin cast on 14 January 2009 (bottles on deck 19:33 GMT). The site has a pronounced O$_2$ minimum zone. The chlorophyll maximum was strongly developed at our time of sampling (at 34 m) and located above the depth of the pycnocline. The O$_2$ minimum zone was within the thermocline and contained O$_2$ concentrations as low as 6 µmol kg$^{-1}$ (Figure 2). In the sediment, dissolved oxygen was limited to the upper 7 mm (Røy *et al.*, 2012).

EQP-8 (0° 0.36' N, 147° 47.50' W) was the western-most site of our equatorial transect (Figure 1). It is located on a flat abyssal plain, with basement age of 80 Ma (Müller *et al.*, 2008) and water depth of 4336 m. Total sediment thickness is estimated to be 380 m (Divins, 2003). This site is in the Central Pacific equatorial upwelling region. Consequently, it

has high surface productivity for an open-ocean location. Its sediment is calcareous nannofossil ooze. The mean sedimentation rate at this site (4.8 m Ma$^{-1}$) is substantially lower than at EQP-1. We conducted water-column sampling here on 5 February 2009 (CTD/Niskin system on deck 4:54 GMT). At this site, the chlorophyll maximum was slightly deeper in the water column than at EQP-1 at the time of sampling (41 m), but in contrast to EQP-1, was above the depth of the pycnocline. The O$_2$ minimum at this site was within the thermocline, with a concentration of 29 µmol kg$^{-1}$ (Figure 2). In the sediment, dissolved oxygen penetrated ~10 cm below seafloor (Røy *et al.*, 2012).

EQP-11 (30° 21.32' N, 157° 52.23' W) is in the North Pacific gyre. It is located on a very slight topographic high with basement age of 88.7 Ma (Müller *et al.*, 2008). With water depth of 5813 m, this is our deepest water site. Total sediment thickness is estimated to be 100 m (Divins, 2003). We began water-column sampling on the 19th of February (CTD on deck 06:12 GMT). The deep chlorophyll maximum (DCM) was in relatively deep water (127 mbsl) and not strongly developed. The O$_2$ minimum at this site (18 µmol kg$^{-1}$) was also deep in the water column (900 mbsl). As at the other sites, this O$_2$ minimum was located within the thermo-cline. Mean sedimentation rate is extremely low at EQP-11 (~1.1 m Ma$^{-1}$). The sediment is pelagic clay and dissolved oxygen penetrates past the greatest depth cored (28.06 mbsf; Røy *et al.*, 2012).

*DNA extraction, pyrosequencing and statistical analyses*

We applied a high-resolution v4–v6 tag-pyro-sequencing approach of the bacterial 16S rRNA gene to a total of 39 water and sediment samples taken
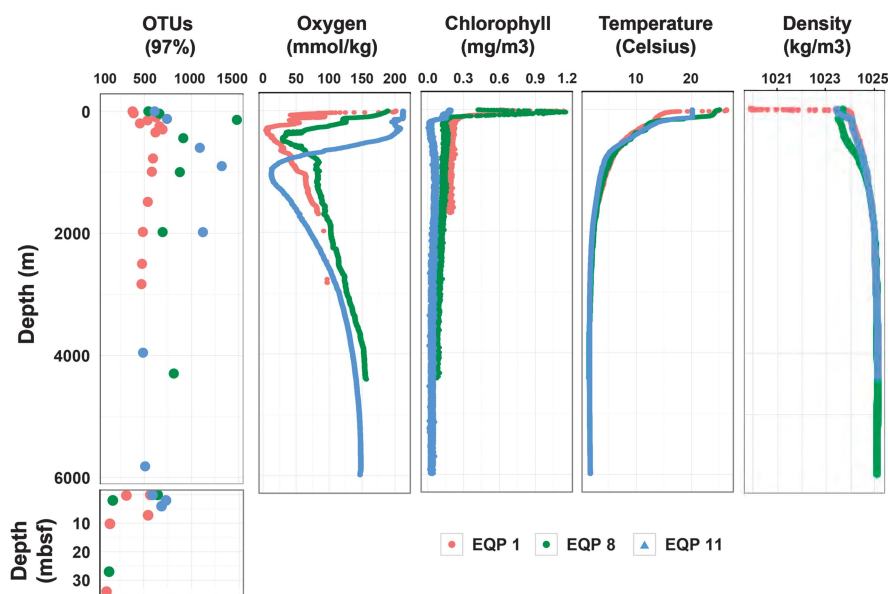


**Figure 2** Scatterplot of sequencing and CTD data including (from left to right): OTUs (97%), oxygen, chlorophyll *a*, temperature and density.

**Table 1** Sampling information and diversity estimates for water and sediment collected from our three sites

| Sample ID | Sample type | Water or sediment depth (m) | Oxygen (mmol kg⁻¹) | Chlorophyll (mg m⁻³) | AOU (µmol kg⁻¹) | OTUs (97%) | Chao-1 (97%) | PD | Evenness (97%) |
|---|---|---|---|---|---|---|---|---|---|
| EQP-1 (3 m) | Seawater | 3 | 201 | 0.61 | 7 | 376 | 593 | 51 | 0.69 |
| EQP-1 (34 m) | Seawater | 34 | 102 | 1.08 | 136 | 387 | 778 | 47 | 0.76 |
| EQP-1 (101 m) | Seawater | 101 | 93 | 0.35 | 159 | 625 | 1254 | 64 | 0.82 |
| EQP-1 (150 m) | Seawater | 150 | 45 | 0.24 | 211 | 535 | 884 | 60 | 0.83 |
| EQP-1 (200 m) | Seawater | 200 | 55 | 0.24 | 203 | 454 | 679 | 61 | 0.81 |
| EQP-1 (249 m) | Seawater | 249 | 21 | 0.20 | 241 | 660 | 1393 | 67 | 0.81 |
| EQP-1 (299 m) | Seawater | 299 | 6 | 0.22 | 264 | 693 | 1748 | 77 | 0.80 |
| EQP-1 (348 m) | Seawater | 348 | 6 | 0.22 | 268 | 618 | 1308 | 67 | 0.78 |
| EQP-1 (774 m) | Seawater | 774 | 43 | 0.20 | 260 | 592 | 1092 | 66 | 0.79 |
| EQP-1 (991 m) | Seawater | 991 | 52 | 0.19 | 258 | 580 | 1078 | 61 | 0.78 |
| EQP-1 (1485 m) | Seawater | 1485 | 73 | 0.19 | 249 | 537 | 1010 | 65 | 0.78 |
| EQP-1 (1978 m) | Seawater | 1978 | 92 | NA | 237 | 485 | 736 | 62 | 0.80 |
| EQP-1 (2500 m) | Seawater | 2500 | 96 | NA | 236 | 476 | 857 | 61 | 0.78 |
| EQP-1 (2832 m) | Seawater | 2832 | 97 | NA | 237 | 470 | 685 | 61 | 0.81 |
| EQP-1-SEDA | Sediment | 0.05 | 0 | NA | NA | 569 | 677 | 68 | 0.89 |
| EQP-1-SEDB | Sediment | 0.25 | 0 | NA | NA | 319 | 392 | 44 | 0.82 |
| EQP-1-SEDC | Sediment | 7.2 | 0 | NA | NA | 546 | 736 | 72 | 0.85 |
| EQP-1-SEDD | Sediment | 10.2 | 0 | NA | NA | 147 | 201 | 14 | 0.72 |
| EQP-1-SEDE | Sediment | 34 | 0 | NA | NA | 111 | 168 | 14 | 0.63 |
| EQP-8 (2 m) | Seawater | 2 | 188 | 0.43 | 18 | 543 | 1786 | 61 | 0.73 |
| EQP-8 (41 m) | Seawater | 41 | 191 | 1.07 | 28 | 660 | 3017 | 72 | 0.74 |
| EQP-8 (142 m) | Seawater | 142 | 124 | 0.18 | 105 | 1479 | 7467 | 143 | 0.89 |
| EQP-8 (442 m) | Seawater | 442 | 30 | 0.16 | 249 | 912 | 3370 | 100 | 0.82 |
| EQP-8 (998 m) | Seawater | 998 | 84 | 0.14 | 227 | 876 | 3188 | 91 | 0.81 |
| EQP-8 (1980 m) | Seawater | 1980 | 102 | 0.05 | 227 | 694 | 2209 | 83 | 0.79 |
| EQP-8 (4296 m) | Seawater | 4296 | 154 | 0.07 | 182 | 812 | 2858 | 99 | 0.78 |
| EQP-8-SEDA | Sediment | 0.05 | 34 | NA | NA | 646 | 927 | 80 | 0.84 |
| EQP-8-SEDB | Sediment | 2 | 0 | NA | NA | 177 | 229 | 28 | 0.58 |
| EQP-8-SEDC | Sediment | 27 | 0 | NA | NA | 138 | 173 | 19 | 0.57 |
| EQP-11 (3 m) | Seawater | 3 | 212 | 0.18 | 13 | 609 | 1627 | 69 | 0.71 |
| EQP-11 (127 m) | Seawater | 127 | 212 | 0.14 | 13 | 740 | 2378 | 82 | 0.74 |
| EQP-11 (600 m) | Seawater | 600 | 125 | 0.05 | 174 | 1087 | 3532 | 118 | 0.84 |
| EQP-11 (900 m) | Seawater | 900 | 19 | 0.07 | 297 | 1319 | 5539 | 138 | 0.87 |
| EQP-11 (1981 m) | Seawater | 1981 | 70 | 0.05 | 261 | 1120 | 4470 | 122 | 0.85 |
| EQP-11 (3955 m) | Seawater | 3955 | 136 | 0.03 | 201 | 487 | 1132 | 67 | 0.74 |
| EQP-11 (5816 m) | Seawater | 5816 | 148 | 0.03 | 186 | 509 | 1242 | 72 | 0.77 |
| EQP-11-SEDA | Sediment | 0.05 | 101 | NA | NA | 592 | 1216 | 74 | 0.80 |
| EQP-11-SEDB | Sediment | 2 | 73 | NA | NA | 733 | 2978 | 71 | 0.59 |
| EQP-11-SEDC | Sediment | 4 | 60 | NA | NA | 687 | 2924 | 68 | 0.57 |

Abbreviations: AOU, apparent oxygen utilization; NA, not applicable; OTU, operational taxonomic unit; PD, phylogenetic diversity.
Diversity estimates are based on 16S rRNA 454 pyrosequencing data with each sample randomly subsampled to the same number of reads as the sample with the initial lowest number of reads (2880).

vertically from seasurface to subseafloor at our three sites (Figure 1, Table 1). We extracted DNA from filtered water samples using the Power Water DNA Isolation Kit (MoBio, Carlsbad, CA, USA) and from sediment samples using the Power Soil DNA Isolation Kit (MoBio). We modified the extraction protocol for the deep EQP-11 sediment samples (2 and 4 m) to overcome effects of low biomass and DNA-binding clay (Cai *et al.*, 2006; Kallmeyer *et al.*, 2012). These modifications were (i) pooling of 10x the sediment (10 vs 1 g) and (ii) use of extended PCR cycle numbers (40 × vs 30 × ). In addition, we used a phenol–chloroform DNA extraction for water samples from EQP-1. We used fusion primers 518 f and 1064r, targeting the v4–v6 hypervariable regions of the 16S rRNA gene, to construct amplicon libraries using the following PCR conditions: 94 °C for 2 min, 30 cycles of (94 °C for 30 s, 55 °C for 20 s, 72 °C for 1 min). We pooled three separate PCR reactions per sample to eliminate the potential for early cycle PCR-induced error. We ran negative controls during all PCR reactions. All sequenced reactions produced bands, whereas controls did not. We sequenced pooled amplicons using a FLX 454 pyrosequencer (Roche, Branford, CT, USA).

We discarded low-quality reads, defined as (i) reads with undefined residues and (ii) reads that did not contain the PCR primers at the beginning of each read (Huse *et al.*, 2007). We submitted our sequence data to the GenBank database under study accession no. SRP058974. We taxonomically assigned reads, based on ⩾80% sequence similarity, using the GAST system for sequence identification (Sogin *et al.*, 2006).

To ensure inter-sample comparability for our taxonomic richness estimates and statistical analyses, we utilized the QIIME software (Boulder, CO, USA) to randomly reduce the number of reads in each sample to the lowest number of reads in any individual sample (2880). We also used the QIIME program to calculate taxonomic richness (total operational taxonomic units (OTUs), Chao-1, ACE and phylogenetic diversity (PD); Caporaso *et al.*, 2010).

We used the Primer-E software package (Clarke and Gorley, 2006) for (i) calculation of Bray–Curtis similarity indices and (ii) all ordination and statistical methods including non-metric multidimensional scaling, hierarchical group-average clustering and Spearman rank correlation tests. We used this output to construct figures using the ggplot2 package (Wickham, 2009) in R computer language (R Core Team, 2014).

To test the hypothesis that OTUs in subseafloor sediment are preferentially from the rare biosphere in the water column, rather than a random sample of water-column OTUs, we developed a Matlab simulation to randomly resample the water-column OTU frequency data with replacement. We repeated this sampling 5000 times to create a distribution of the number of OTUs randomly drawn from the rare (rank abundance >100) water-column OTUs. We then used this distribution to estimate the probability of randomly picking 'n' rare OTUs from the water-column abundance distribution.

## Results

### Similarity among bacterial communities

Hierarchical 'group-average' clustering and non-metric multidimensional scaling analyses separate our samples into four distinct abundance-weighted community compositions, based on Bray–Curtis similarity (Figure 3a). Regardless of site, water-column samples partition into (i) photic-zone communities, which include samples from at or above the DCM, and (ii) aphotic communities (below the DCM). The GAST taxonomy demonstrated that Cyanobacteria, Flavobacteria and Alphaproteobacteria dominate the photic-zone samples, whereas the aphotic-zone samples are heavily dominated by Alphaproteobacteria, Gammaproteobacteria, Deltaproteobacteria and Deferribacteres (Figure 4).
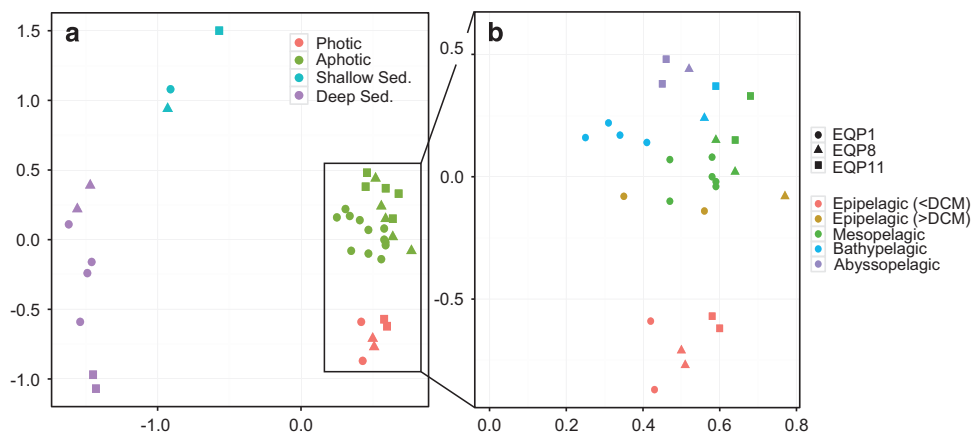


**Figure 3** (**a**) Non-metric multidimensional scaling (nMDS) plot of the water column and sediment community compositions. Axes do not represent any measured parameter, but define a 2-D space that allows the best spatial representation of sample similarity, based on Bray–Curtis similarity indices. (**b**) Enlargement of the nMDS plot for the water-column communities with a superimposed color gradient representing depth from seasurface (red) to the abyssopelagic water of EQP-8 and EQP-11 (blue).
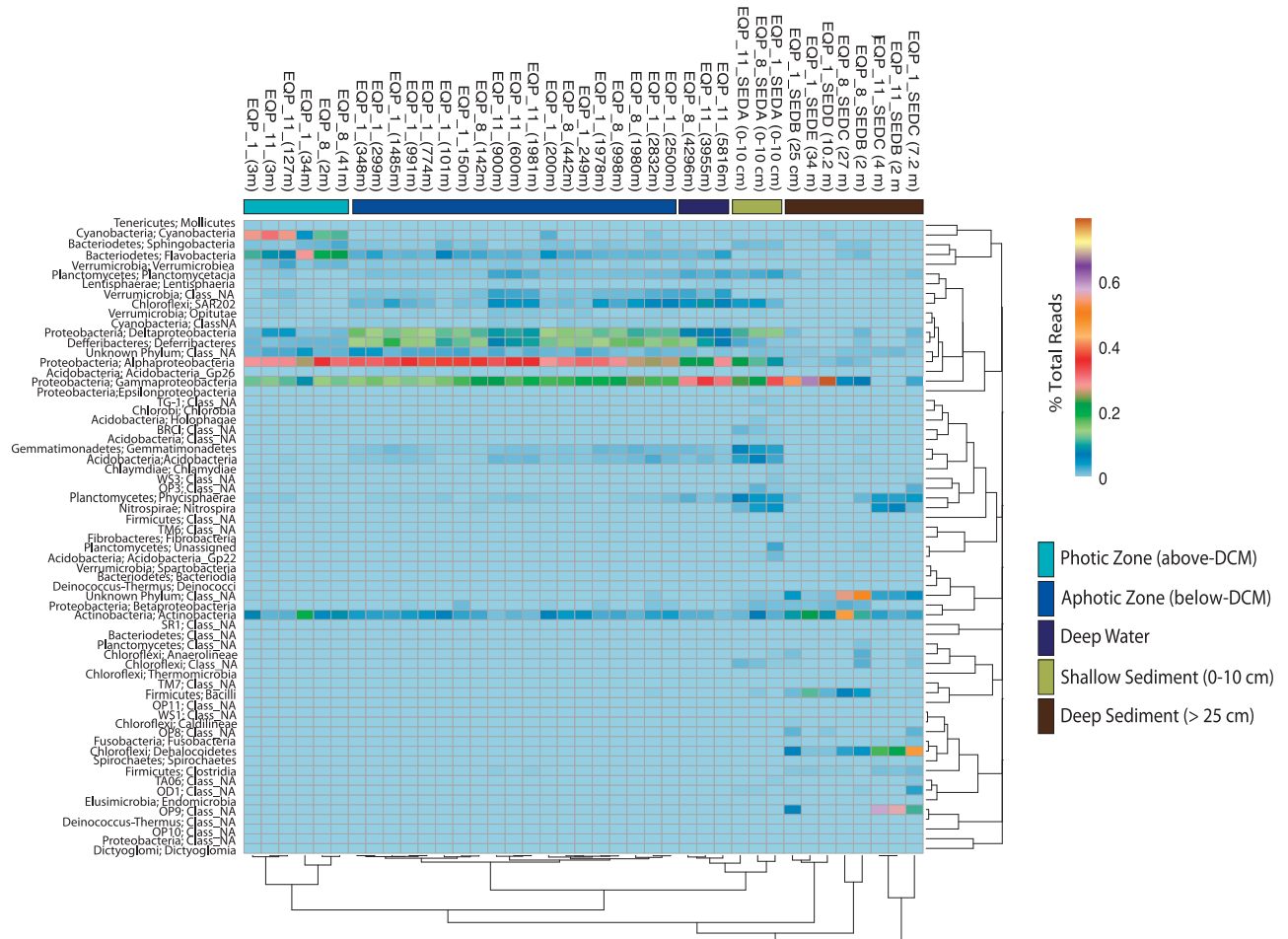
**Figure 4** Heatmap clustering visualization of water column and sediment bacterial communities (x axis) and class-level GAST taxonomy (y axis). The heatmap is color coded by the percentage contributed by a given taxon to any given sample. Hierarchal clustering is based on the Morista–Horn similarity index.

Regardless of site, aphotic-zone community composition exhibits a consistent relationship to water depth, with a clear compositional gradation from epipelagic communities below the DCM to abyssopelagic communities at depths between 3500 and 6000 mbsl (Figure 3b). The photic-zone samples cluster by site, whereas the aphotic-zone samples vary less from site to site. Water-column samples decline in similarity with increasing water depth regardless of site location (Figure 3b).

We also observed two distinct groups in the sediment: a near seafloor (0–10 cmbsf) community and a subseafloor (0.25–34 mbsf) community. The common members of the shallow sedimentary community overlap with those of the deep-water community. The shallow sedimentary community is dominated by Alphaproteobacteria, Gammaproteobacteria and Deltaproteobacteria, as well as *Nitrospira* and Planctomycetes (Figure 4). The deep sedimentary community primarily comprises members of the obsidian pool (OP) candidate phylum, including OP9, OP8 and OP3, as well as Chloroflexi (Dehalococcoidetes and Anaerolineae),

Actinobacteria and another phylum of unknown lineage (Figure 4).

*The rank abundance and distribution of shared OTUs*
To examine the extent to which OTUs are shared between the previously described communities (photic, aphotic, shallow sediment and deep sediment) (Figure 3a), we generated rank abundance histograms of the 100 most abundant OTUs (97% similarity) in each community. Figures 5a and c highlight the OTUs shared between the water column (photic and aphotic combined) and shallow sediment communities, between shallow and deep (≥1.5 mbsf) sediment communities, and between the deep sedimentary and water-column communities. Although our clustering results binned an EQP-1 sample from 0.25 mbsf with the deep sedimentary community (Figure 4), we excluded this sample from this shared-OTU analysis because it was taken close to the seafloor and we wish to ensure that only OTUs truly selected for subseafloor conditions are compared.
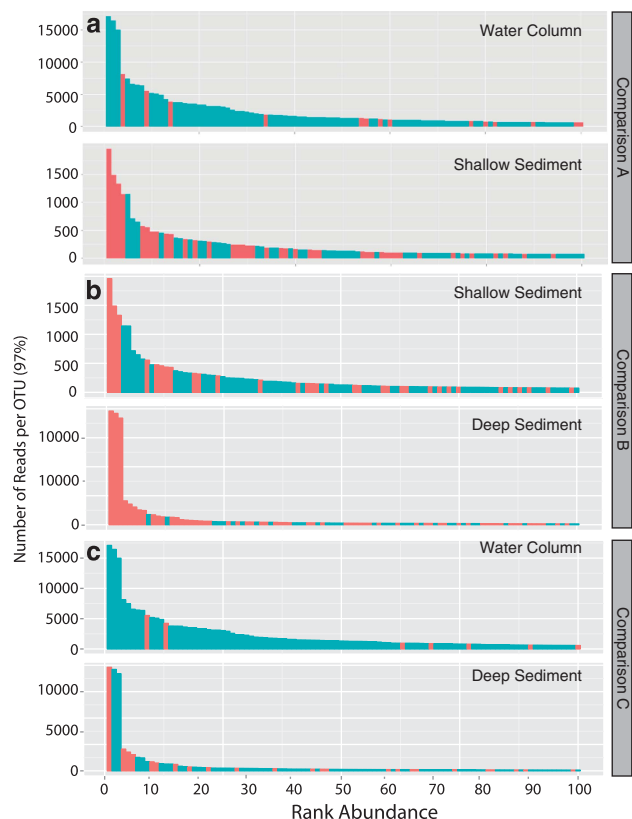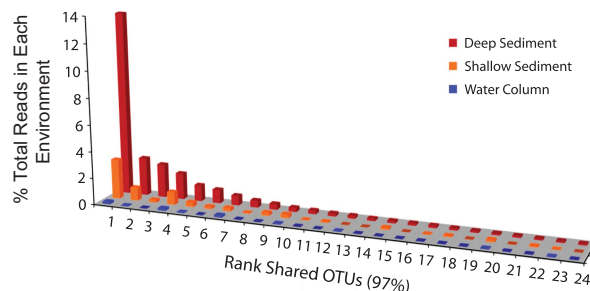
**Figure 5** Rank abundance histograms of community overlap between (**a**) water column and shallow sediment (0–10 cmbsf), (**b**) shallow sediment and deep sediment (≥1.5 mbsl) and (**c**) water column and deep sediment (≥1.5 mbsl). OTUs highlighted in pink are shared between compared environments, whereas OTUs highlighted in blue are unique to each environment.



**Figure 6** Rank abundance histograms for the top OTUs shared (x axis) between the oceanic and sedimentary environments. The y axis indicates the percentage of the total number of reads per OTU within our subseafloor sediment samples (≥1.5 mbsf, red), shallow sediment samples (orange) and water-column samples (blue).

**Table 2** Shared OTUs in the 100 most abundant OTUs found in the pooled data from each environment (water column, shallow sediment and deep sediment)

| Top 100 OTUs | Number of OTUs/% reads that overlap with: | | |
|---|---|---|---|
| *Base data set* | *Water column* | *Shallow sediment* | *Deep sediment* |
| Water column | – / – | 14/11.9% | 7/5.8% |
| Shallow sediment | 41/56.3% | – / – | 33/40.6% |
| Deep sediment | 24/37.0% | 62/87.8% | – / – |

Abbreviation: OTU, operational taxonomic unit.
For the environment shown in each row, each pairwise comparison shows the number of shared OTUs and the percent of total reads in the top 100 OTUs comprised by those shared OTUs.

Many of the abundant OTUs in our shallow and deep sedimentary communities are present in the water at trace levels (<0.025% of total reads) (Table 2, Figures 5a and c). To quantify the statistical significance of so many shared OTUs being rare in the water column, we randomly resampled with replacement the water-column OTU frequency data until 24 unique OTUs were selected. These 24 randomly selected OTUs represent the 24 OTUs in the 100 most abundant deep-sediment OTUs that are shared with the water column (pink bars in lower panel of Figure 5c). Of these 24 shared OTUs, 22 are rare in the water column. Given the water-column abundance distribution and 5000 repetitions of our random resampling procedure, the probability of randomly selecting 22 of 24 OTUs from the pool of OTUs rarer than the top 100 in the water column is vanishingly small ($P < 0.0001$).

These shared OTUs belong to Dehalococcoidetes, OP9, OP8 and other taxa. Furthermore, the OTUs that are abundant in the deep (subseafloor) sediment are also often abundant in the shallow sediment (Figure 5b). In contrast, most of the OTUs abundant in the water column are not present in our subseafloor sediment tags. The most abundant water-column OTUs that appear in our deep sedimentary tags include members of the Actinobacteria, Planctomycetes and Firmicutes (Figure 4). In short, the individual OTUs that are abundant in the deep sediment are also often abundant in the shallow sediment and often present but very rare (<0.025% of the tag population) in the overlying water column (Figure 6). The exceedingly small probability of getting so many rare water-column OTUs (22 of 24) by randomly sampling the water column ($P < 0.0001$) indicates that water-column OTUs surviving in subseafloor sediment are preferentially from the rare community in the water column.

*Taxonomic richness, evenness and PD*
Vertical trends in bacterial diversity (# of observed OTUs, Chao-1, ACE and PD) and evenness (H') are similar at all three sites, with all measurements of richness and evenness peaking within the mesopelagic zone (Table 1). Overall, diversity is highest in the water column and lowest in the deep subseafloor sediment (Figure 2a). Within the water column, the vertical profile of bacterial diversity mirrors the $O_2$ profile and matches the profile of apparent oxygen utilization at each site (Figures 2a and b, Table 1). Diversity in the water column is generally lowest in the surface ocean and highest at the $O_2$ minima (Figures 2a and b). In the sediment, richness is

highest near the seafloor (Figure 2a). Bacterial diversity in the water column varies from site to site; for example, richness is highest in the oxygen minimum zone of North Pacific gyre site EQP-11 and much lower in the oxygen minimum zone of eastern equatorial upwelling Site EQP-1 (Figure 2a).

## Discussion

*Vertical trends in bacterial diversity*
Water-column bacterial communities at all sites exhibit a clear depth profile, with samples declining in similarity from the seasurface to the abyssopelagic waters, in accordance with previously described trends (for example, DeLong *et al.*, 2006; Brown *et al.*, 2009; Treusch *et al.*, 2009). Although changes in community composition with depth have previously been well documented, studies of taxonomic richness with depth have yielded mixed results. Some studies have reported declines in taxonomic richness and/or PD with increasing ocean depth (Brown *et al.*, 2009; Agogué *et al.*, 2011; Bryant *et al.*, 2012), whereas others have observed higher richness deeper in the water column (for example, Pommier *et al.*, 2010, Kembel *et al.*, 2011; Ghiglione *et al.*, 2012). By sampling the water column more comprehensively than previous studies, we find that bacterial richness (taxonomic richness, PD and Chao-1) and evenness in the water column is lowest at the surface and highest within the $O_2$ minima of the mesopelagic zone at each of our sites. These results are consistent from site to site regardless of the diversity metric applied. Consequently, they provide clear evidence of vertical changes in bacterial richness across a range of environments. Some previous studies also reported elevated bacterial richness within the mesopelagic zone (Kembel *et al.*, 2011; Jing *et al.*, 2013; Ladau *et al.*, 2013), but did not explicitly associate this result with the $O_2$ minimum zone. The relationship we observe between oxygen and taxonomic richness suggests that respiration may have a significant role in shaping bacterial richness in the open ocean.

Only a few studies have examined bacterial diversity in both the water column and marine sediment (Feng *et al.*, 2009; Zinger *et al.*, 2011; Hamdan *et al.*, 2013). None of these studies included sediment deeper than 1.1 mbsf. Most previous studies of seafloor bacterial communities agree that those communities are highly diverse (Ravenschlag *et al.*, 1999; Madrid *et al.*, 2001; Luna *et al.*, 2004; Hewson *et al.*, 2007; Schauer *et al.*, 2010). Some studies have suggested that seafloor communities are taxonomically richer than communities in the water column (Lozupone and Knight, 2007; Feng *et al.*, 2009; Zinger *et al.*, 2011). In our study, taxonomic richness was generally higher in the water and lower in the sediment. This difference from previous studies may be partly due to the absence of subseafloor (>1 mbsf) sedimentary communities in

those studies (>1 mbsf communities at our sites tend to be consistently less diverse than seafloor communities). It may also be due to the different oceanographic context of our studies relative to some previous studies (for example, Feng *et al.*, 2009). It is possible that the open-ocean seafloor sediment is less taxonomically rich than seafloor sediment of continental margins and estuaries. This difference may also partly result from different sampling approaches. Our study and Feng *et al.* (2009) sample both seawater communities and sediment communities at each location, while Luzopone and Knight (2007) and Zinger *et al.* (2011) compared seawater communities from diverse locations with sediment communities from very different locations.

Within the sediment at all of our sites, taxonomic richness is highest at the seafloor and declines with increasing sediment depth (Figure 2). There is extensive taxonomic overlap between the communities of the deep sediment and those of the shallow sediment (Figure 5). These results suggest that communities in deep marine sediment are derived from a subset of the community that lived in the sediment when it was near the seafloor.

*Taxonomic overlap between the ocean and sediment*
Zinger *et al.* (2011) examined the bacterial beta-diversity of seafloor and seawater ecosystems and demonstrated that pelagic communities and epibenthic communities differed greatly at all taxonomic levels. Our study supports this observation and extends it to subseafloor sediment, as the abundance-weighted bacterial community compositions of the sediment are clearly distinct from those in the overlying water column (Figure 3).

To examine the extent of taxonomic overlap between the ocean communities and the sediment communities, we separately pooled the DNA sequences from the water samples of all three sites and the DNA sequences from the sediment samples of all three sites. We pooled the data for these comparisons, rather than doing the comparisons site by site, because sinking particles may be advected large distances, and in different directions at different water depths, before settling to the seafloor. Consequently, the material that settles to the seafloor rarely originates in the immediately overlying water. Although our three sites are separated by thousands of kilometers, spanning over 70 degrees of longitude and 30 degrees of latitude, we think it is justifiable and necessary to pool the samples because (i) intense sequencing at a single site has demonstrated that the marine bacterial rare biosphere is highly cosmopolitan (Gibbons *et al.*, 2013) and (ii) pelagic communities from the same oceanographic horizon (the DCM) are highly similar for thousands of kilometers (Walsh *et al.*, 2015).

When we consider the presence–absence of individual taxa, we observe overlap between environments. The most prominent example of this overlap

is the presence in seawater and shallow sediment of many OTUs that are abundant in the deep-sediment samples (Figures 5 and 6). The overlap is very lopsided considering relative abundance; OTUs represented by abundant sequences in the sediment tag populations are often present but represented by rare sequences in the seawater tag population, whereas OTUs with abundant tags in the seawater population are rarely present in the sediment populations. This result is consistent with microbes being carried through the ocean and deposited in the sediment. The absence of known phototrophs (for example, *Prochlorococcus*, *Synechococcus*) in our subseafloor tag populations indicates that taxonomic overlap between the seawater and subseafloor sedimentary communities does not simply result from preservation of detritus. Instead, the subseafloor conditions appear to select for preferential survival of taxa that were represented by relatively few sequences in the seawater populations. The relatively low abundance of these sequences in the seawater populations is not surprising, as subseafloor sedimentary conditions are very different from water-column conditions; for example, anoxic conditions and low energy availability are much more ubiquitous in subseafloor sediment than in the overlying ocean (Jørgensen and D'Hondt, 2006). It is possible that microbes associated with the sedimentary environment are transported long distances through the ocean as components of the rare biosphere, from which they may or may not re-colonize the seafloor. Although resuspension and downslope sediment transport may be important sedimentary depositional processes in some marine environments, they are not significant processes at our sites; all three sites are located on flat abyssal regions or topographic highs and the cores from all three sites show no evidence of turbidites, indicating little to no redeposition of sediment. Consequently, advective transport through the water column provides the simplest explanation of microbial input to this sediment, consistent with discovery of thermophiles in marine sediment far from hydrothermal vents (Lee *et al.*, 2005; Hubert *et al.*, 2009), and with Hamdan *et al.*'s (2013) identification of hydrography as a forcing factor for OTU composition in near-seafloor marine sediment.

Microbial transport through the water column may explain the incidence of large-scale biogeographic patterns in both near-seafloor sediment (Hamdan *et al.*, 2013) and subseafloor sediment. A previous study (Inagaki *et al.*, 2006) observed that similar taxa (90% similarity, for example, Dehalococcoidetes, OP9 and Desulfobacteriales) are present in environmentally similar subseafloor sedimentary environments separated by large geographic distances. Our results show this similarity at a much finer taxonomic resolution (97% similarity) and indicate that these clades may dominate in a broad range of subseafloor sedimentary environments. Transport through the water column is far easier than through

a sedimentary matrix where both advection and biologically accessible electron donors are limited.

## Conclusions

Our study provides the first vertical profiles of microbial diversity from seasurface to subseafloor sediment (down to 34 mbsf). Bacterial richness is generally higher in the water column than in the sediment. It is highest in the $O_2$-minimum zone of the water column and lowest in the subseafloor sediment. Despite the differences in abundance-weighted community compositions in the water column, the shallow sediment and the subseafloor sediment, the most abundant taxa in the subseafloor sedimentary communities are also often abundant in the shallow sedimentary communities and present but rare in the water column. This result suggests that deep subseafloor communities (i) constitute a subset of the diverse taxa that were present in the sediment at the time of burial and (ii) are ultimately seeded via the water column.

## Conflict of Interest

The authors declare no conflict of interest.

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