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RESEARCH ARTICLE

Microbial community structure of sea spray aerosols at three California beaches

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One sentence summary: Sea spray aerosols at California beaches contain bacteria important in elemental cycling as well as potential pathogens; they may promote transport of bacteria from sand to sea.
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ABSTRACT

We characterized the microbial communities in sea spray aerosols (SSA), water and sand of three beaches in central California (Cowell Beach, Baker Beach and Lovers Point) by sequencing the V4 region of the 16S rRNA gene. Average concentrations of 16S rRNA genes in SSA ranged from 2.4×10^4 to 1.4×10^5 gene copies per m^3 of air. A total of 9781 distinct OTUs were identified in SSA and of these, 1042 OTUs were found in SSA of all beaches. SSA microbial communities included marine taxa, as well as some associated with the terrestrial environment. SSA taxa included organisms that play important roles in biogeochemical cycling of elements such as *Planctomyces* and *Synechococcus*, as well as those representing potential pathogens and fecal indicator bacteria including *Staphylococcus epidermidis* and *Enterococcus spp*. There were a large number of shared OTUs among SSA and water, and there was relatively high similarity between SSA and water communities. Results are consistent with a conceptual model where SSA is generated by breaking waves and bubble bursting in marine waters and that enables the transport of microorganisms from the sea to sand or other environments.

Keywords: sea spray aerosols; bioaerosols; microbiome; beach sand; marine; coastal

INTRODUCTION

Sandy beaches encompass 75% of the world's unfrozen shorelines (Brown and Mclachlan 2002). They provide a number of important ecosystem services (Defeo *et al.* 2009) each of which are modulated by microorganisms. Microbes mediate nutrient cycling in the water column (Mou *et al.* 2008; Sunagawa *et al.* 2015) and within the permeable sands (Santoro, Boehm and Francis 2006; Santoro *et al.* 2008; Huettel, Berg and Kostka 2014) and facilitate the exchange of molecules between different trophic levels (Defeo *et al.* 2009). Microorganisms found at beaches may also include pathogenic contaminants, which adversely affect the health of beach goers (Boehm *et al.* 2002; Wade *et al.* 2003; Colford *et al.* 2012). For example, coastal waters and beach sands adjacent to urban centers can be contaminated with fecal indicator bacteria and pathogens (Halliday and Gast 2011; Viau *et al.* 2011; Yamahara *et al.* 2012). Therefore, it is important to

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understand how various sources and processes affect microbial presence and abundance at the beach.

The beach is a dynamic environment. Tides and waves bring coastal seawater in contact with beach sands and a portion of the seawater may percolate into the beach aquifer where it enters the saltwater circulation cell (Xin *et al.* 2015) or the subterranean estuary (Moore 1999). Tides and waves also erode shorelines, causing sand to be transported alongshore in littoral cells or transported offshore (Inman, Tait and Nordstrom 1971; Defeo *et al.* 2009). The interactions between sand and sea can transfer microbes between these compartments (Gast *et al.* 2011; Russell, Yamahara and Boehm 2012; Boehm, Yamahara and Sassoubre 2014).

Airborne bacteria are present in coastal areas (Shaffer and Lighthart 1997), and sea spray aerosols (SSA) generated by breaking waves have been shown to contain microorganisms (Aller *et al.* 2005). The global production flux of dry SSA is estimated to be 10^{12} g m^{-2} yr^{-1} (de Leeuw *et al.* 2000). Depending on wind speed (Vignati *et al.* 1999; de Leeuw *et al.* 2000), SSA concentrations downwind from surf can be up to 2 orders of magnitude higher than upwind concentrations (de Leeuw *et al.* 2000). Thus, deposition of SSA represents a mechanism for transferring microbes from the seawater to beach sands (Baylor *et al.* 1977; Dueker *et al.* 2011, 2017).

Physical and chemical properties of SSA are generally well understood (Quinn *et al.* 2015). SSA can range in size from 0.01 μm to >25 μm (de Leeuw *et al.* 2011). While the largest aerosols will deposit by gravitational settling close to the land–sea interface, some of the smaller aerosols can be transported long distances over land (Quinn *et al.* 2015), more than 25 km according to a model that assumed an aerosol diameter of 10 μm and a wind speed of 2 m s^{-1} (de Leeuw *et al.* 2000). Numerous studies have shown that bacterial survival in droplets increases with increasing droplet size (Lighthart and Shaffer 1997; Shaffer and Lighthart 1997; Montero, Dueker and O'Mullan 2016), perhaps because larger droplets are better able to withstand environmental stressors (e.g. temperature and relative humidity) than are smaller droplets (Lighthart and Shaffer 1997). The chemical composition of SSA is distinct from that of seawater; SSA tend to be enriched in organic matter (Quinn *et al.* 2015) since they originate mainly from the sea surface microlayer, which is enriched in organics, including microorganisms, relative to bulk surface seawater (Aller *et al.* 2005).

Previous studies have examined the beach microbiome with an emphasis on characterizing the microbial communities in coastal waters (Giovannoni and Stingl 2005; Fuhrman *et al.* 2006; Fuhrman 2009; Moran 2015; Sunagawa *et al.* 2015) and both surficial unsaturated sands and saturated sands within the beach aquifer (Cui *et al.* 2013; Boehm, Yamahara and Sassoubre 2014; Halliday *et al.* 2014; Staley and Sadowsky 2016). Far fewer studies have investigated the microbial composition of SSA, and most of them have been conducted in the open ocean (Leck and Bigg 2005; DeLeon-Rodriguez *et al.* 2013; Seifried, Wichels and Gerdts 2015; Sharoni *et al.* 2015) or using microcosms (Cho and Hwang 2011; Fahlgren *et al.* 2015). These studies have primarily employed microscopy or culture methods to quantify microbes (Shaffer and Lighthart 1997; Leck and Bigg 2005; Cho and Hwang 2011) and Sanger sequencing of 16S rRNA from bacterial isolates or clone libraries to assess the microbial community composition (Cho and Hwang 2011; Urbano *et al.* 2011; Fahlgren *et al.* 2015; Dueker *et al.* 2017). To our knowledge, there have not been any studies comparing the microbial community structure between sand, water and SSA at multiple beaches.

The goal of this study was to characterize the microbial community in SSA collected at three sandy beaches and compare the SSA microbial community to that of water and sand. We used next generation sequencing to characterize microbial communities. We collected SSA samples contemporaneously with seawater and sand to allow cross-matrix comparisons. We tested hypotheses that (i) SSA microbial communities are similar among the three beaches, and (ii) SSA microbial communities are similar to those collected in adjacent seawater and sands. We examined the composition of the SSA, sand and water microbial communities and highlight taxa that are important in biogeochemical cycling and potential pathogens.

METHODS

Field sampling

We collected sand, water and SSA samples at Cowell Beach (hereafter 'CB', 36.962°N, 122.024°W), Baker Beach ('BB', 37.790°N, 122.486°W) and Lovers Point ('LP', 36.625°N, 121.917°W), located in central California, USA (Fig. 1). We visited CB, BB and LP on 15 August 2016, 23 August 2016 and 30 August 2016, respectively. We chose these beaches to represent different physical environments typical of California beaches: BB and CB have a surf zone with waves breaking directly on the shore, while LP is quiescent.

We gathered samples of sand in 50 mL Falcon tubes in triplicate from an alongshore transect directly above the high tide line (as evidenced from excessive wrack deposited on the shoreline). We collected the top 3–4 cm of sand using sterile scoops and placed it in sterile containers. The sand we collected was not in contact with wave run-up at the time of collection. We collected sand samples in the morning at 10:00 and in the afternoon at 15:00 local time.

We gathered samples of water in triplicate in 10% HCl acid-washed 1 L plastic bottles from ankle depth on incoming waves. We collected water samples in the morning at 10:00 and in the afternoon at 15:00.

We collected aerosol samples on PTFE filters (3 μm pore size, 25 mm diameter, manufacturer: SKC, Eighty Four, PA) using an autoclaved stainless steel filter holder attached to a vacuum pump (Leland Legacy, location) set at a volumetric flow rate of 10 L min^{-1} for 4 hr. We collected the morning sample starting at 8:15 and ending at 12:15 and the afternoon sample starting at 12:20 and ending at 16:20 local time. At the end of each sampling period, we removed the filter from the filter holder using sterile forceps and placed it in a sterile petri dish. Then, we decontaminated the filter holder using 70% ethanol. Additionally, we created morning and afternoon field blanks by wiping the filter holder with 70% ethanol, installing a filter in the holder and removing it 1 min later without starting the pump. We stored all samples in a cooler with dry ice until sample processing.

We obtained wind data for each location and day from Weather Underground at the following stations: KCASANFR909 (frequency of ~6 hr^{-1}), KMRY (1 hr^{-1}) and KCASANTA313 (~6 hr^{-1}) were for BB, LP, and CB, respectively. We calculated the onshore and alongshore components of the winds considering the strike of the shoreline and plotted hourly moving averages. We noted wave conditions during sampling.

Analysis for source of air masses

We used NOAA's HYSPLIT atmospheric dispersion model (Stein *et al.* 2015; Rolph, Stein and Stunder 2017) to confirm that

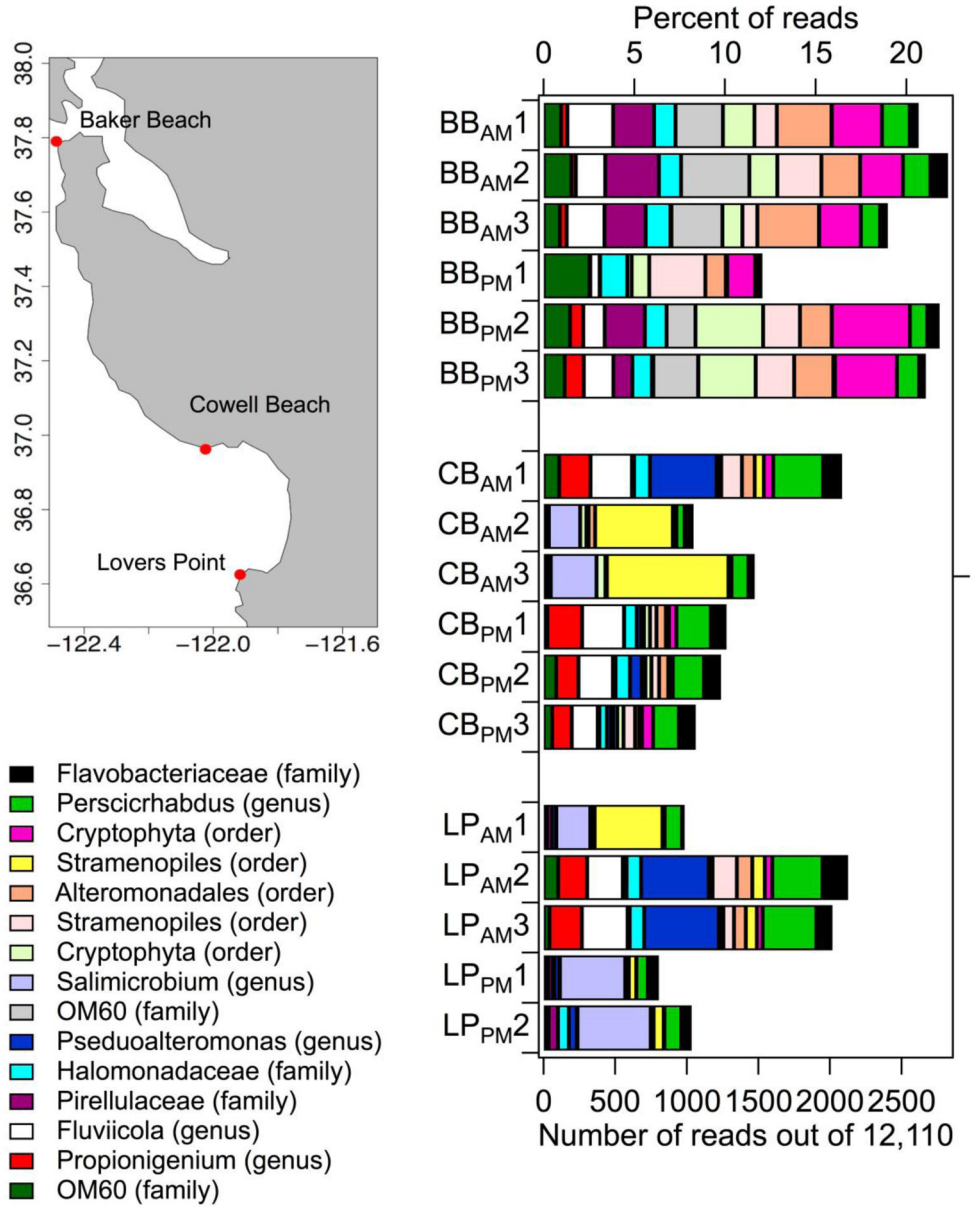

Figure 1. Map: Locations of the sampled beaches. Bar plot on right: Number of reads assigned to the 15 most abundant 'cosmopolitan taxa' in SSA. SSA samples were rarefied to 12 110 reads and 'cosmopolitan taxa' were defined as OTU that were found in SSA at all three beaches. The legend lists the taxonomy assigned to each OTU by the software in QIIME; in some cases, the taxonomic assignment to two distinct OTUs was identical (e.g. *Stramenopiles*). The name of each sample is given by its location (BB, CB or LP) followed by AM or PM and then a number referring to the replicate (1, 2 or 3).

air mass sources originated from the ocean on the days and times when we sampled. We ran 48-hr back trajectory models at 1000, 500 and 200 m above ground level, using meteorological data from the READY website (http://ready.arl.noaa.gov/HYSPLIT_traj.php).

Sample processing

We processed all samples within 8 hr of collection. We mixed 30 g (wet weight) of sand with 150 mL of sterile DNA-free water (Thermo Fisher Scientific, Waltham, MA), agitated the mixture for 3 min on a shaker table at 150 rpm. We allowed the sand to settle for 1 min, and then 100 mL of the supernatant was vacuum filtered through 0.45 μm pore size, 47 mm diameter mixed cellulose ester filters (Millipore, HAWP 04700, Hayward, CA). Water samples were vigorously shaken, and then 500 mL were vacuum filtered through the same type of filters. Each day, we created filtration blanks by filtering 250 mL of sterile DNA-free water (Thermo Fisher Scientific, Waltham, MA) using the same techniques (three filtration blanks total). We stored the filters from sand and water samples, along with the filters from aerosol sampling, at −80°C until DNA was extracted.

DNA extraction

We extracted DNA from the filters using the MoBio PowerWater extraction kit (Carlsbad, CA) according to the manufacturer's instructions with two exceptions: we extended the incubation time with PW1 at 65°C to 10 min and extended the vortexing time to 10 min to increase the yield of the DNA extracts. We

created one extraction control using only extraction reagents with no environmental sample or filter to control for contamination during the extraction process each day (three extraction controls in total). We diluted DNA extracted from water samples by a factor of 10 to reduce PCR inhibition due to substances co-extracted with the DNA from the environmental samples.

We used *Escherichia coli* K-12 (ATCC 10798) as a positive control. We grew the culture on tryptic soy agar and suspended a single colony in sterile, DNA-free water (Thermo Fisher Scientific, Waltham, MA). We centrifuged the suspension at 10 000 × g for 5 min, and then resuspended the pellet in sterile DNA-free water, heated it to 100°C for 10 min, cooled it at −20°C for 10 min, centrifuged it again at 14 000 × g for 5 min, and then used the supernatant as template in PCR reactions.

Amplicon generation, sequencing and bioinformatics analysis

We used the previously described 16S Illumina Amplicon Protocol with the 515f and 805rB primers (GTGYCAGCMGCCGCGGTAA and GGACTACNVGGGTWTCTAAT, respectively) that target the V4 region of the 16S rRNA gene (Caporaso *et al.* 2011, 2012; Apprill *et al.* 2015; Walters *et al.* 2015; Parada, Needham and Fuhrman 2016). Golay barcodes were embedded in each forward primer (Table S1, Supporting Information).

We used previously describe PCR protocols to generate 16S rRNA gene amplicons from all samples, including the negative and positive controls: 25 μl PCR reactions consisted of 2x Qiagen HotStarPlus mastermix, 10 μM of forward primer, 10 μM of reverse primer and 1 μl of template DNA. We ran PCR reactions in triplicate using the following thermocycler conditions: 94°C for 3 min, and then 35 cycles of 94°C for 45 seconds, 50°C for 60 s, 72°C for 90 s, followed by 72°C for 10 min, and a hold at 4°C (Caporaso *et al.* 2011, 2012; Apprill *et al.* 2015; Walters *et al.* 2015; Parada, Needham and Fuhrman 2016). We included a no template control reaction in every PCR run. After PCR, we visualized product size using electrophoresis on 1.5% agarose gels containing ethidium bromide. All environmental samples and the positive controls showed a band of appropriate size (~390 bp). Since we only had one SSA sample from the morning and the afternoon at each beach, we used technical replicates (triplicates) for those samples when adding barcodes during PCR, as opposed to the biological replicates collected for the sand and water samples. We did not observe any bands in the lanes of the gel containing the negative controls (including field blanks, filtration blanks, extraction controls and no template controls).

We used Qubit 2.0 (dsDNA HS assay kit; ThermoFisher) to quantify the nucleic acid concentration in PCR products. Based on those concentrations, we pooled samples and the positive control in equimolar proportions (10 μM) for sequencing. Additionally, we pooled field blanks, filtration blanks, extraction controls, no template controls and positive controls, from different PCR runs for sequencing. 10 μl of the each of the negative control pools (morning and afternoon field blanks, filtration blank, extraction control and no template control) were added to the total pool before cleaning. The total pool was cleaned using MoBio UltraClean PCR Clean-up Kit before sequencing.

We sequenced our samples using Illumina MiSeq at the Stanford Functional Genomics Center (Stanford, CA). We used 250-bp paired-end reads (2 × 250) and spiked the total pool with 20% phiX before sequencing. We sent index primers (AATGATACGGCGACCACCGAGATCTACACGCT), read 1 primers (TATGGTAATTGTGTGYCAGCMGCCGCGGTAA), and read 2 primers (AGTCAGCCAGCCGGACTACNVGGGTWTCTAAT) with the total pool (Caporaso *et al.* 2011, 2012; Apprill *et al.* 2015; Walters *et al.* 2015; Parada, Needham and Fuhrman 2016). We deposited the 16S amplicon sequences to the sequence read archive (accession number: SRP116878).

We used QIIME 1.9 (Caporaso *et al.* 2010) to merge the paired end reads, quality control, remove primers, cluster reads into OTU and rarefy OTU tables. During quality filtering, we only retained reads with Phred scores ≥ Q20. The UCLUST algorithm (Edgar 2010) chose open reference OTUs with 97% similarity and assigned taxonomy by aligning to the reference GreenGenes database (13.8). We used UCHIME (Edgar *et al.* 2011) in VSEARCH (Rognes *et al.* 2016) to remove reference-based chimeras. We excluded OTUs from our analysis that were singletons or whose sequences failed to align using PyNAST. We also excluded from our analysis any OTUs that were identified in the negative controls at relative abundances exceeding those found in the environmental samples (we removed 10 OTUs in total). There were large differences in mean library sizes of our samples and a large number of libraries (>50). Therefore, we rarefied each sand, water and SSA samples to 12 110 reads and used the rarefied OTU tables in all subsequent analyses. 53 of the 54 sequenced environmental samples had more than 12 110 reads. The single environmental sample with less than 12 110 reads was an LP SSA sample (2230 reads), and it was removed from subsequent analyses. The rarefied reads were assigned to 27 887 unique OTUs. Good's coverage for each sample ranged from 0.84 to 0.98 after rarefaction.

16S rRNA gene quantification

We quantified total bacterial concentrations in environmental samples using a 16S rRNA qPCR assay (Suzuki *et al.* 2001). We ran the reactions on an Applied Biosystems StepOnePlus thermocycler (Foster City, CA) in triplicate using the following recipe: 1x Platinum Quantitative PCR Supermix-UDG with ROX, 0.2 μM forward primer (BACT1369F: CGGTGAATACGTTCYCGG), 0.2 μM reverse primer (PROK1492R: GGWTACCTTGTTACGACTT), 0.1 μM probe (TM1389F:CTTGTACACACCGCCCGTC) and 2 μl of template. Thermocycler conditions were: 50°C for 2 min, 94°C for 10 min followed by 40 cycles of 94°C for 15 s and 56°C for 30 s. We constructed standard curves using gDNA extracts from *Staphylococcus aureus* (ATCC 25904) at concentrations ranging from 3.2×10^1 to 3.2×10^6 gene copies per reaction. We did not dilute SSA templates. We diluted sand and water templates 100-fold, to reduce PCR inhibition from environmental contaminants. We included triplicates of each standard and a single no template control on each qPCR plate. The average R^2 of the linear regression between $\log_{10}$-transformed standard concentrations (x-axis) and Cq values (y-axis) was 0.98, and the average efficiency of the reaction was 83% (slope of line −3.82).

Statistical analyses

We used the *vegan* package in RStudio (version 3.4.0) and PRIMER-E v6 software (Plymouth, PRIMER-E) to perform all statistical analyses (Clarke 1993; Clarke and Gorley 2006). We square root transformed the OTU tables before calculating the Bray–Curtis distances. We used the *metaMDS* and *anosim* functions for the nMDS and analysis of similarity calculations (all with 999 free permutations), respectively (Ramette 2007). We confirmed these results and obtained pairwise ANOSIM results using PRIMER v6. We identified overrepresented OTUs in SSA

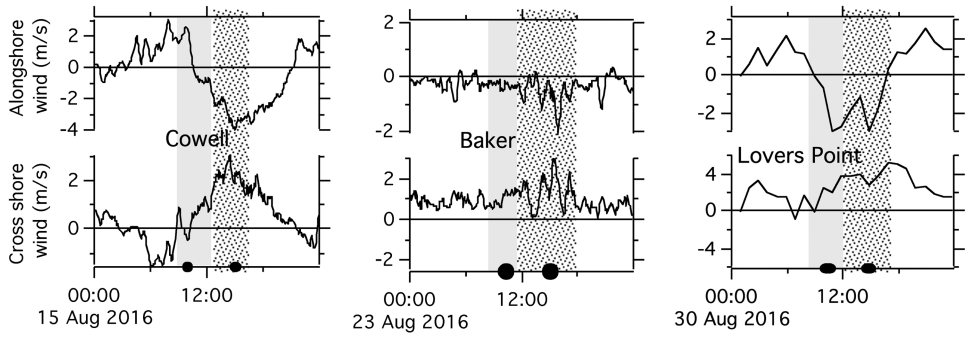

Figure 2. Winds at the beaches on the day of sampling. Positive is upcoast (poleward) and onshore for alongshore and cross shore winds, respectively. The gray background indicates when the morning SSA sample was collected and the dotted background indicates the period of time when the afternoon SSA was collected. The black dots on the x-axis indicate when the morning and afternoon water and sand samples were collected.

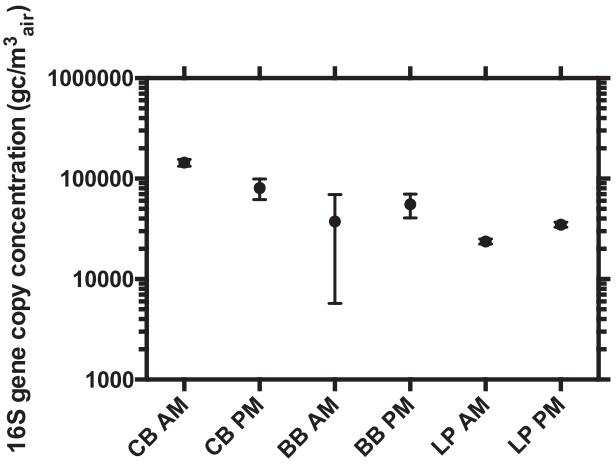

Figure 3. Concentration of 16S rRNA gene copies (gc) in SSA from Cowell Beach (CB), Baker Beach (BB) and Lovers Point (LP) collected in the morning (AM) and the afternoon (PM). Symbol indicates the mean of technical qPCR triplicates. Error bars represent 95% confidence intervals.

relative to sand and water using linear discriminants analysis (LEfSe) (Segata *et al.* 2011). We used Kruskal–Wallis to test the hypothesis that samples from each beach had equal concentrations of bacterial 16S rRNA gene copies and equal diversity measures.

RESULTS

Wind speed and direction, and oceanic conditions

Winds had an onshore component at all three beaches throughout the sampling periods (Fig. 2). According to analysis of 48-hr back trajectories, air masses originated from the ocean on all days of sampling at all heights (Fig. S1, Supporting Information). In the morning, the beaches were foggy with a thick marine layer that diminished by noon, while afternoons were clear. LP was sheltered from waves and quiescent during sampling. Breaker height was 0.5–1 m at BB and CB.

Total bacteria concentrations

The concentration of 16S rRNA genes in the SSA samples ranged from 2.4×10^4 to 1.4×10^5 gene copies per m^3 of air (Fig. 3). There was no significant difference between the concentration measured in the morning and afternoon at the beaches (Mann–Whitney test, $P = 0.43$). There were differences in concentrations among beaches (Kruskal–Wallis test, n = 18, $P < 0.0001$); CB had the highest concentration of 16S rRNA genes, followed by BB and LP had the lowest concentration. Concentrations of 16S rRNA genes in water ranged from 3.4×10^7 to 1.7×10^9 copies per liter of water (Fig. S2A, Supporting Information). The concentrations were not different between beaches (Kruskal–Wallis, n = 53, $P = 0.68$); there was also no difference between morning and afternoon samples (Mann–Whitney test, $P = 0.07$). Concentrations in sand ranged from 2.3×10^5 to 2.6×10^7 copies per g wet weight and these varied significantly among beaches (Kruskal–Wallis, n = 54, $P < 0.0001$) (Fig. S2B, Supporting Information), but not between morning and afternoon samples (Mann–Whitney test, $P = 0.14$). The no template control reactions had low levels of amplification with Ct values (average = 36.2) higher than those of environmental samples, indicating very low levels of background amplification. The background amplification is likely from the mastermix that contains Taq enzyme generated using *E. coli* cells.

Bacteria community structure

In total, we sequenced 54 environmental samples, 5 negative controls (including pooled morning and afternoon field blanks, filtration blank, extraction blank and no template control) and one positive control. In aggregate, we obtained 38 000 096 sequences, of which 30 661 824 passed initial quality filtering. 8 931 954 reads remained after processing in QIIME (median per sample: 181 549; range per sample: 2230–393 113). The positive control had a total of 233 407 sequencing reads of which 99.7% of the reads were taxonomically classified into the expected family for *E. coli* K12. Species level resolution of the positive control data was not possible, probably due to the high percentage of similarity between members of the *Enterobacteriaceae* family in the V4 region (Janda and Abbott 2007). Manual BLASTing of the representative sequences of the most abundant OTUs assigned to the positive control confirmed this.

SSA community diversity was similar among the beaches. The Shannon diversity indices were ~7 and did not vary significantly between beaches (Fig. 4, Kruskal–Wallis test, $P = 0.34$). The diversity indices of the beach water and sand were similar to those of SSA (Fig. S3, Supporting Information).

Of the 9781 unique SSA OTUs, 1042 OTUs (11%) were present at all three beaches. The OTUs found in SSA from all three beaches are hereafter referred to as 'cosmopolitan' taxa. 1838 OTUs (19% of the 9781 SSA OTUs) were present at least at two beaches, and 6901 OTUs (70%) were present at just one beach. The most abundant cosmopolitan OTUs included those assigned

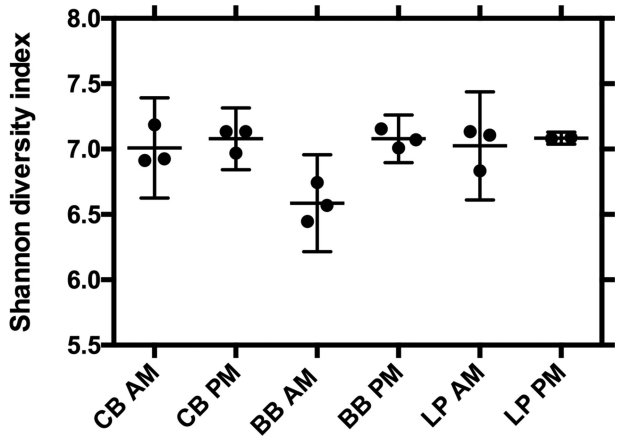

Figure 4. Alpha diversity (Shannon index) for SSA from Cowell Beach (CB), Baker Beach (BB) and Lovers Point (LP) in the morning (AM) and the afternoon (PM). Shannon indices were calculated from rarefied OTU tables in the *vegan* package in R. Technical replicates (triplicates) were averaged (horizontal line); the error bars represent 95% CIs.

to *Persicirhabdus* (genus), *Fluviicola* (genus), *Cryptophyta* (order), *Stramenopiles* (order) and OM60 (family) (Fig. 1). 231 of the cosmopolitan OTUs were annotated to the genus level and these included, for example, *Synecococcus*, *Vibrio*, *Fluviicola*, *Persicirhabdus* and *Enterococcus* (all cosmopolitan OTUs are shown in Table S2, Supporting Information).

The Bray–Curtis distance among SSA technical replicates was 0.58 ± 0.08 (n = 16, mean + 95% CI). Bray–Curtis distance between SSA collected at different beaches was higher 0.75 ± 0.02 (n = 120, mean + 95% CI) (Table S3, Supporting Information). Samples collected from the same beach tended to cluster together in an nMDS plot, although overlap between beaches was still evident (Fig. 5). The global R from an ANOSIM where SSA samples are grouped by beach was 0.48 ($P = 0.001$). Pairwise R values comparing SSA communities between beaches indicate that the community structure at BB was distinct from that of LP and CB (R = 0.71 and R = 0.72, respectively,$P = 0.002$), whereas the SSA communities from CB and LP were not different (R = −0.03, $P = 0.46$).

ANOSIMs investigating the community structure in water and sand showed dissimilarity between beaches. Pairwise comparisons between seawater samples at CB, BB, and LP revealed high levels of dissimilarity (pairwise R between 0.58 and 0.86, $P = 0.002$). Similarly, pairwise comparisons between sands at each beach showed dissimilarities between beaches (pairwise R between 0.98 and 1, $P = 0.002$).

We compared the community in samples from distinct matrices collected in the morning and afternoon at each beach and found high levels of similarity (R close to 0, and $P > 0.05$). This suggests no to minor differences in microbial community composition in the tested matrices in the morning and afternoon.

We used ANOSIM to investigate the whether the SSA communities at a beach were similar to water and sand communities at that beach by grouping samples by matrix (SSA, water or sand). Global R values were positive and statistically significant (CB global R = 0.72, $P = 0.001$; BB global R = 0.83, $P = 0.001$; LP global R = 0.88, $P = 0.001$) indicating dissimilarity between water, sand and SSA communities. Subsequent pairwise analyses produced positive and statistically significant R values (Table 1). R values were consistently lowest between water and SSA for each beach, and when beach locations were combined to form a

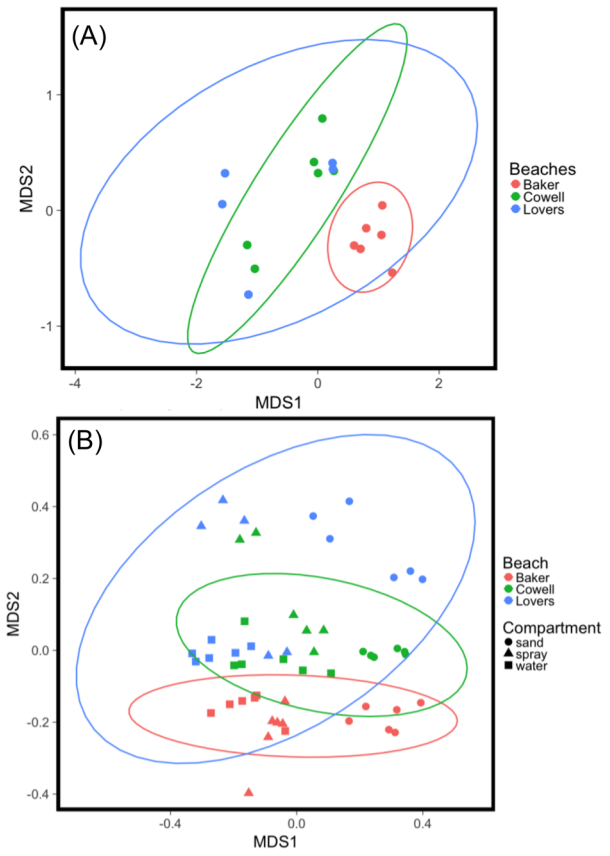

Figure 5. Panel A. nMDS plot showing SSA samples. Symbols are color-coded by beach. We used Bray–Curtis distances to generate the diagram. Ellipses represent 95% confidence intervals around the mean centroid of each beach. Stress = 0.06. Panel B. nMDS plot showing all samples from all beaches. Samples are color-coded by beach and shapes represent compartment. Bray–Curtis distances are used and ellipses represent 95% confidence intervals around the mean centroid of each beach (CB, BB and LP). Stress = 0.15.

Table 1. Pairwise ANOSIM R values (and associated p values) at each beach, and all beaches combined. CB, BB and LP are Cowell Beach, Baker Beach and Lovers Point, respectively.

	Water-sand pairwise R	SSA-sand pairwise R	SSA-water pairwise R
CB	0.93 ($P = 0.002$)	0.66 ($P = 0.002$)	0.50 ($P = 0.002$)
BB	1.0 ($P = 0.002$)	1.0 ($P = 0.002$)	0.38 ($P = 0.037$)
LP	1.0 ($P = 0.002$)	0.99 ($P = 0.002$)	0.70 ($P = 0.002$)
All beaches	0.80 ($P = 0.001$)	0.64 ($P = 0.001$)	0.36 ($P = 0.001$)

single data set suggesting that SSA and water communities were most similar of the pairings.

SSA OTUs were compared to OTUs detected in sand and in water across all beaches. 1166 OTUs (11.9% of the total 9781 OTUs found in SSA) were shared between SSA and water, while 1292 OTUs (13.2%) were shared between SSA and sand. 4438 OTUs (45.4%) found in SSA were not present in either sand or water samples and 2885 OTUs (29.5%) were found in all three compartments (Fig. S4, Supporting Information). Table S4 (Supporting Information) provides the OTUs exclusively found in SSA and their annotations; 68 of these (~2%) are also cosmopolitan (found at all three beaches). Among the OTUs exclusively found in SSA are those associated with marine (e.g. *Planctomyces*) (Ward,

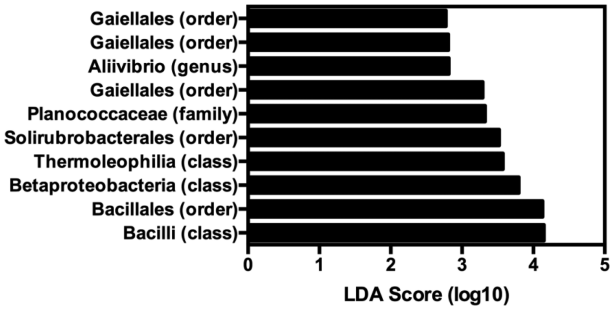

Figure 6. LEfSe analysis of abundances revealed 10 OTU that were over-represented in SSA as compared to water and sand. OTU are annotated with the lowest level of taxonomic assignment. LDA (linear discriminants analysis) Score is the output of LEfSe.

Staley and Schmidt 2015), soil (e.g. *Bacillus*) (Slepecky and Hemphill 1992), air/marine (e.g. *Aerococcus*) (Collins and Falsen 2015) and vegetative (e.g. *Streptomyces*) (Kämpfer 2015) environments, as well as ice nucleation particles (e.g. *Psychrobacter*) (Bowers *et al.* 2009). Several identified species were also associated with increased radiation tolerance and thermotolerance (e.g. *Streptomyces radiopugnans*) (Bhave *et al.* 2013).

We used LEfSe to identify OTUs that were differentially abundant in SSA compared to the sand and water. We found that 10 OTUs were over-represented in SSA using this approach (Fig. 6).

DISCUSSION

A goal of this study was to characterize the microbial communities in SSA collected from three beaches along the central Californian coast. Additionally, we aimed to test whether the SSA bacterial communities were similar to those in sand and water, and other SSA samples collected at different beaches. Two of the three beaches in this study have surf zones with waves breaking directly on the shore (BB and CB), while one is quiescent (LP), and thus the beaches represent a range of beach environments. All beaches had winds directed onshore throughout the sampling period, and sampled air masses originated from the ocean.

We measured the number of 16S rRNA gene copies in SSA to estimate the total concentration of bacteria. Assuming 3.9 copies of 16S rRNA per bacterial cell (Einen, Thorseth and Øvreås 2008), the concentrations we measured are between 6.1×10^3 and 3.7×10^4 cells m^{-3}. Because qPCR can detect potentially inactive or dead organisms, the concentration may be an overestimate. However, our estimates of SSA bacterial concentrations are consistent with those reported in other studies (Cho and Hwang 2011; Li *et al.* 2011; Fahlgren *et al.* 2015). Although the concentrations are low relative to sand and water on a per volume basis, they are similar on a per mass basis, as air is ~1000 times less dense than sand and water. The presence of bacteria in SSA suggests that SSA deposition to sand, to the water surface or to inland terrestrial environments may serve as a vector for the transport of microorganisms between environments.

SSA exhibited extensive microbial diversity. Over 1000 SSA OTUs were identified at all sampling locations. These cosmopolitan taxa were generally annotated as organisms associated with the marine environment including those important in nitrogen (e.g. *Nitrosopumilus* and *Planctomyces*), carbon (e.g. *Synechococcus*) and sulfur (e.g. *Thermodesulfovibrionaceae*) (Thauer, Stackebrandt and Hamilton 2007) cycling, as well as those that are salt (e.g. *Halomonadaceae*) (Vreeland 1992) and stress tolerant (e.g. *Octadecabacter antarcticus*) (Gosink, Herwig and Staley 1997).

The prevalence of marine taxa, along with the large number of shared OTUs among SSA and water and the relatively high similarity between SSA and water communities, is consistent with a conceptual model where SSA is generated by breaking waves and bubble bursting in marine waters (McKay *et al.* 1994; de Leeuw *et al.* 2000; Dueker *et al.* 2011; van Eijk *et al.* 2011), and SSA retains marine organisms present in the water. Previous studies have characterized the bacterial diversity in SSA using Sanger sequencing and pyrosequencing, although those studies were mostly done over the open ocean or in a laboratory microcosm setting. Two of the studies found that marine taxa dominated SSA (Fahlgren *et al.* 2010, 2015; Cho and Hwang 2011), while others (Urbano *et al.* 2011; Seifried, Wichels and Gerdts 2015; Xia *et al.* 2015; Montero, Dueker and O'Mullan 2016; Dueker *et al.* 2017) found a mix of marine and terrestrial taxa. Urbano *et al.* (2011) collected bioaerosols at a marine pier in southern California and suggested that beaches and/or coastal erosion are a more important source of the microorganisms to SSA than is the ocean. It is possible that sands at our study beaches also contribute bacteria to SSA, particularly since sand and water share OTUs.

Despite the numerous shared OTUs between SSA and water, there were still differences between their microbial communities. A majority of SSA OTUs was not present in water, and ANOSIM suggests the SSA community as a whole is somewhat distinct from that of water. There are several plausible explanations for this. First, SSA is created from the sea surface microlayer (Aller *et al.* 2005) that can harbor distinct microbial communities from the bulk water (Franklin *et al.* 2005; Cunliffe and Murrell 2009). As we sampled bulk water, we may have missed organisms preferentially associated with the microlayer. Second, some or all of the SSA may have been generated at a different location or time from that at which we sampled water and thus reflect the microbial community present in its source. The microbial community in water varied spatially across our sampling domain, although we did not detect a statistical difference between SSA, seawater or sand communities in the morning and afternoon. Third, there could be die-off or potentially growth of microbes in SSA between the time of generation and sampling that could affect the microbial community present (Aller *et al.* 2005). Finally, some of the microbes present in the aerosol samples may have not originated from the ocean and instead come from terrestrial sources; some of the taxa exclusively found in SSA are associated with non-marine environments.

Some taxa were found in higher proportions in the SSA than water and sand. These taxa include those associated with aquatic environments (e.g. *Aliivibrio*) (Ast, Urbanczyk and Dunlap 2009) and those associated with the formation of endospores (e.g. *Bacillales*) (De Vos 2015). These organisms may be over-represented in SSA because they have high survival capacity in SSA or they are preferentially associated with the sea surface microlayer where SSA is generated (Franklin *et al.* 2005; Cunliffe and Murrell 2009).

Among the annotations assigned to SSA taxa, there were at least two taxa annotated to species level that are potential pathogens according to a previously published database of potential clinical bacterial pathogens (Chiu *et al.* 2014): *Staphylococcus epidermidis* and *Rothia mucilaginosa*. Both are associated with infections in immunocompromised patients (Stackebrandt 2006; Otto 2009). The former is often found on human skin (Otto 2009) and has been shown to survive aerosolization (Thompson, Bennett and Walker 2011). Genera containing potential pathogens (*Acinetobacter, Actinomyces, Aggregatibacter, Bacillus, Burkholderia, Campylobacter, Clostridium, Corynebacterium,*

Enterococcus, Francisella, Haemophilus, Legionella, Mycobacterium, Mycoplasma, Neisseria, Nocardia, Pseudomonas, Rickettsia, Streptococcus, Treponema, and *Vibrio*) were also identified in SSA. *Enterococcus* is commonly used to assess coastal water quality where its presence is linked to increased risk of illness in swimmers (Wade *et al.* 2003). Although *E. coli*, another fecal indicator organism used to assess water quality, was not identified in SSA, OTUs assigned to its family, *Enterobacteriaceae*, were. The high percentage of similarity between members of the *Enterobacteriaceae* family may have impeded our detection of *E. coli* in these samples (Janda and Abbott 2007). All three of the beaches have been plagued by poor water quality in the past and can be impacted by raw sewage (Yamahara *et al.* 2007; Russell *et al.* 2013, https://healthebay.org). The presence of potential pathogens and fecal indicator bacteria in SSA may be a result of these organisms being present in the water. In fact, many of the potential pathogen annotations were also assigned to water and sand OTUs (data not shown). We estimated the concentrations of potential pathogens in SSA by multiplying the relative abundance of each potential pathogen, averaged across SSA samples, by the average concentration of 16S rRNA genes in the air samples. Using this estimation approach, potential pathogen genera concentrations ranged from 1 to 600 16S rRNA gene copies per m^3 air (Table S5, Supporting Information). Further research could assess whether the presence of potential pathogens in SSA represents a health risk via inhalation exposure.

O'Mullan, Dueker and Juhl (2017) put forth a conceptual model where SSA, generated in the nearshore and transported onshore, transports microorganisms from the ocean to subaerial beach sands or onto land further inland. Our data support this conceptual model. SSA and sand share OTUs, which may be explained by SSA being a source of those OTUs to sand. Some OTUs were over-represented in the SSA relative to sand, supporting the idea that SSA may be a source of these OTUs to sand. Previous work with MS2 bacteriophage showed SSA generated in a surf zone deposited on subaerial beach sands effectively transporting the bacteriophage from sea to sand (Baylor *et al.* 1977). Sands may also be seeded with microbes from ocean water, or seed microbes to ocean water, when they are periodically submerged during high tides or high wave events (Boehm, Yamahara and Sassoubre 2014). Additional work is needed to better understand the deposition of SSA microbes to beach sands.

The beaches were foggy in the mornings that we sampled. Previous work suggests that fog may affect microbial communities in SSA and SSA transport. SSA bacterial concentrations can be enriched during foggy conditions (Fuzzi, Mandrioli and Perfetto 1997; Dueker *et al.* 2011) and Dueker *et al.* (2012) found SSA and ocean surface microbial communities were more similar during foggy than non-foggy conditions. An increase in mean SSA particle size during foggy conditions could have implications for transport distance (Dueker *et al.* 2012). We did not find that the morning samples, collected during foggy conditions, were different than those collected in the afternoon after the marine layer had dissipated.

One limitation of this study is that we have assumed that the aerosol samples collected at the beach were dominated by sea spray. The rationale for this assumption is that the sampling location for aerosols was conducted just above the high tide line, very close to the ocean, and the cross-shore wind was directed onshore. Additionally, 48-hr back trajectories showed that air masses originated from the ocean. Through measurement of total aerosol concentrations and of aerosol salt content, several studies have shown that aerosols near the surf zone are dominated by sea spray (McKay *et al.* 1994; Zakey, Giorgi and Bi 2008; van Eijk *et al.* 2011). In fact, van Eijk *et al.* (2011) found that the surf zone added 0.7–1 order of magnitude to the background aerosol concentration. de Leeuw *et al.* (2000) found that aerosol concentrations downwind of the surf zone were up to 2 orders of magnitude higher than those measured upwind and suggested that surf-derived aerosols can significantly alter aerosol concentrations as far as 25 km away. SSA are typically less than 10 μm and have three peak size modes at 0.02–0.05, 0.1–0.2, and 2–3 μm (Clarke, Owens and Zhou 2006; Fuentes *et al.* 2010; Quinn *et al.* 2015). Thus, they can be transported long distances in the atmosphere, and we are confident that aerosol samples collected in this study did contain sea spray.

SUPPLEMENTARY DATA

Supplementary data are available at FEMSEC online.

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