

Regional Similarities and Consistent Patterns of Local Variation in Beach Sand Bacterial Communities throughout the Northern Hemisphere

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ABSTRACT

Recent characterization of the bacterial community structure in beach sands has revealed patterns of biogeography similar to those observed in aquatic environments. Studies to date, however, have mainly focused on subtidal sediments from marine beaches. Here, we investigate the bacterial diversity, using Illumina-based sequencing of the V5-V6 region of the 16S rRNA gene, at 11 beaches representing those next to the Great Lakes, Florida, and the Pacific Ocean. The alpha diversity differed significantly among regions ($P < 0.0001$), while the within-region diversity was more similar. The beta diversity also differed by region ($P < 0.001$), where freshwater sands had significantly higher abundances of taxa within the *Actinobacteria*, *Betaproteobacteria*, and *Verrucomicrobia* than marine environments. In contrast, marine sands harbored greater abundances of *Gammaproteobacteria* and *Planctomycetes*, and those from Florida had more *Deltaproteobacteria* and *Firmicutes*. Marine beaches had significantly different phylogenetic community structures ($P \leq 0.018$), but freshwater and Florida beaches showed fewer within-region phylogenetic differences. Furthermore, regionally distinct patterns in taxonomic variation were observed in backshore sands, which had communities distinct from those in nearshore sands ($P < 0.001$). Sample depth minimally influenced the community composition. The results of this study reveal distinct bacterial community structures in sand on a broad geographic scale but moderate regional similarity and suggest that local variation is primarily related to the distance from the shoreline. This study offers a novel comparison of the bacterial communities in freshwater and marine beach sands and provides an important basis for future comparisons and analyses to elucidate factors affecting microbial ecology in this underexplored environment.

IMPORTANCE

This study presents a large-scale geographic characterization of the bacterial communities present in beach sands. While previous studies have evaluated how environmental factors influence bacterial community composition, few have evaluated bacterial communities in freshwater sands. Furthermore, the use of a consistent methodology to characterize bacterial communities here allowed a novel comparison of communities across geographic regions. We reveal that while the community composition in sands at individual beaches is distinct, beach sands within the same region harbor similar assemblages of bacteria and these assemblages differ greatly between regions. In addition, moisture, associated with distance from the shoreline, strongly influences the bacteria present in sands and more strongly influences the bacteria present than sample depth does. Thus, the data presented here offer an important basis for a broader characterization of the ecology of bacteria in sands, which may also be relevant to public health and resource management initiatives.

Beach sands are dynamic ecosystems that harbor diverse microbial communities vital to ecosystem services, including water purification, biogeochemical cycling, and the mineralization of organic compounds (1–3). In marine systems, a beach cross section can be divided into three major compartments that vary in physical and chemical properties in large part due to tidal cycles (depicted in Fig. S1 in the supplemental material): (i) the near-shore, subtidal zone, which is permanently saturated by the overlying water column; (ii) the intertidal zone, which experiences periodic tidal wetting and drying; and (iii) the backshore, supratidal region, which does not experience tidal wetting (4). Variation also likely occurs due to depth, with a vadose zone of dry sand sitting above an ephemeral intertidal saltwater cell that often overlies fresh groundwater (5). The last two saturated zones interact with a deep saltwater wedge located below the subtidal sands, resulting in complex chemical gradients and the potential exchange of nutrients and bacteria (3, 6). Similarly, in the Great Lakes, seiche dynamics (a tide-like standing wave found in enclosed bodies of water) play a role similar to that played by the

tidal cycles of marine beaches (7), and there is also frequently a continual discharge of groundwater to many lakes (8).

There are several methods by which bacterial communities in sands may be shaped by stochastic variables, as well as by transport and exchange between sand and water (4). The majority of microbial species in beach sands are thought to be autochthonous, and a cosmopolitan assemblage has been identified in a limited geo-

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TABLE 1 Summary of beach locations

Region	Location	Beach	Sand moisture content (%)	Latitude	Longitude
Great Lakes	Duluth, MN, USA	Minnesota Point	7.5 ± 8.0	46.728	−92.048
	Chicago, IL, USA	63rd Street Beach	26.1 ± 7.6	41.782	−87.573
	Burlington, Ontario, Canada	Burlington Beach	20.7 ± 4.4	43.314	−79.800
	Toronto, Ontario, Canada	Marie Curtis Park	12.0 ± 6.5	43.583	−79.543
Florida	Tampa, FL, USA	Fort DeSoto	20.2 ± 2.6	27.617	−82.737
	Miami, FL, USA	Crandon Park	14.6 ± 6.5	25.713	−80.151
Pacific Ocean	Southern California, USA	Huntington Beach	19.8 ± 5.3	33.656	−118.000
	Oahu, HI, USA	Sandy Beach	8.2 ± 5.4	21.286	−157.673
	Northern Japan	Otaru Dream Beach	28.6 ± 12.6	43.158	141.208
	Southern Japan	Fukiage-hama Beach	6.9 ± 4.5	31.521	130.325
	Jeju, South Korea	Jeju Beach	ND ^a	33.445	126.294

^a ND, not determined.

graphic area (3). However, contamination of beach sands by pollution in the water has also been suggested (9). Bacteria in beach sands may also enter the water column via overbeach transport, where tidal events or wave action release bacteria attached to sand particles or those residing in interstitial spaces and these bacteria then enter the water directly (10, 11). Alternatively, through-beach transport can also occur when bacteria in unsaturated sands are mobilized downward due to tidal or wave action, enter the groundwater, and are transported via subterranean discharge to the water column (3, 12). Consequently, beach sand microbes and those in the lake water or in sediments can be thought of as being in a state of dynamic equilibrium.

Recently, bacterial communities in beach sands have begun receiving increasing attention due to their relevance to public health (4, 13–18). While recreational waters have long been monitored for indicators of human fecal pollution and their presumed indication of risk to humans because of the presence of human pathogens (19, 20), beach sands have historically been neglected in this area. Over the last several years, both marine and freshwater sands have been recognized to be important reservoirs of fecal indicator bacteria (21–23) and environments that support naturalized microbial populations consisting of prokaryotes and eukaryotes (10, 24). Furthermore, the number of studies that have identified bacterial, fungal, and viral pathogens in beach sands is steadily increasing (4, 18, 22), and not all of these pathogens have been of fecal origin. Therefore, a more thorough understanding of the bacterial communities typical of beach sands is likely to offer novel insights into the ecology of these complex ecosystems and perhaps guide monitoring efforts to protect public health.

The evolution of next-generation sequencing has allowed a more comprehensive characterization of the microbial communities present in a variety of environments, including in marine waters (25), in riverine ecosystems (26, 27), in soils (28), and in and on humans and animals (29). Over the last 5 years, many studies have employed next-generation sequencing to study primarily subtidal sands and sediments (2, 30, 31). These studies have revealed that members of the rare biosphere, i.e., taxa of low abundance, fluctuate in abundance in relation to physicochemical parameters and sample depth (2, 30, 31). A recent study of intertidal sand samples collected from California beaches revealed the presence of abundant, cosmopolitan taxa that were active in through-beach transport (3). Furthermore, a biogeographic relationship in

the microbial community composition has been revealed, where the communities from sands with similar characteristics or an increased anthropogenic impact were more similar to each other (3). Taken together, these studies indicate that, similar to what has been noted in soils, water and water availability play a prominent role in the distributions, types, and abundances of microbes in sand environments.

To date, nearly all of the next-generation sequencing studies of sands have focused on sand from marine beaches (2, 3, 31–35), but one recent study has focused on sand from freshwater beaches (17), and due to differences in methodology, including the sequencing platform and biases associated with the sequencing region associated with primer targets (36), the ability to compare these data sets with each other has been limited. In this study, beach sands from 11 beaches throughout the United States, Japan, and South Korea, including 4 beaches from the Great Lakes, were extensively characterized by using Illumina next-generation sequencing of the 16S rRNA gene. Generation of this data set allowed assessment of the variation in communities at global, regional, and local scales. Samples were collected from nearshore, intertidal, and backshore segments of all beaches at three depths at 10-cm intervals. We hypothesized that, due to geographic separation, each beach would harbor a unique bacterial community but that patterns in local variation would be similar at all locations. The results of this study revealed novel patterns in the bacterial community composition and structure at beaches throughout the world and further emphasized that local differences in bacterial community composition and structure due to distance from the tidal zone and sample depth are likely.

MATERIALS AND METHODS

Sample collection and processing. Samples were collected from 11 beaches throughout the United States, Japan, and South Korea by local collaborators at each site (Table 1; see also Fig. S2 in the supplemental material). Due to geographic proximity, similarity in water type (i.e., fresh or marine), and statistical grouping based on the bacterial communities present, the sampling sites were grouped as Great Lakes, Florida, and Pacific Ocean. All samplings were performed between 29 September and 3 October 2014. For beaches influenced by tides, samples were collected at an outgoing, low tide. Samples were collected at depths of 0 to 10 cm, 10 to 20 cm, and 20 to 30 cm at the same spot using an ethanol-sterilized auger. Triplicate locations 2 m apart were sampled at each distance from the shoreline, and the sampling distances from the shoreline included (i) at

the shoreline, (ii) 1 m from the shoreline, and (iii) 10 m from the shoreline (see Fig. S1 in the supplemental material). Samples were stored in 50-ml conical tubes or Whirl-Pak bags (Nasco, Fort Atkinson, WI, USA) and transported back to the lab on ice. Samples were shipped on dry ice to the University of Minnesota for DNA extraction, or DNA was extracted as described below and shipped on dry ice to the University of Minnesota. Samples were stored at -20°C prior to DNA extraction. DNA extraction was done using 250 to 300 mg of sand and PowerSoil DNA isolation kits (Mo Bio Laboratories, Inc., Carlsbad, CA, USA) as described by the manufacturer.

Sequencing and bioinformatics. PCR amplification, amplicon purification, sample pooling, and sequencing were performed by the University of Minnesota Genomics Center (Minneapolis, MN, USA) as previously described (37). The V5-V6 hypervariable region of the 16S rRNA gene was amplified using the BSF784/1064R primer set (25, 38), and the amplicons were gel purified. Negative controls consisting of sterile water were included to test the PCR mixtures, and amplification products were not obtained from those controls. Purified amplicons were pooled in equal amounts, and paired ends were sequenced at a read length of 150 nucleotides (nt) on a HiSeq2500 platform (Illumina, Inc., San Diego, CA, USA).

All sequence processing was performed using mothur (version 1.34.0) software (39). Sequences were paired-end joined using fastq-join software (40) and quality trimmed using a mean quality score of 35 and a 50-nt window. Sequences containing homopolymers of >8 nt, ambiguous bases, or more than 2 nt mismatches from the primer sequences were removed. High-quality sequences were aligned against the SILVA database (version 119) (41), screened to remove sequences that did not fall within the region amplified by the primers, and subjected to a 2% preclustering step (42). UCHIME software was used to identify and remove chimeric sequences (43). Sequences were normalized by random subsampling to 25,000 sequence reads per sample for statistical comparisons (44). Operational taxonomic units (OTUs) were assigned at a 97% identity using the furthest-neighbor algorithm, and taxonomic assignments were made using the Ribosomal Database Project taxonomy (version 10) described previously (45).

Statistical analyses. Analysis of variance (ANOVA) and Spearman correlations were performed using XLSTAT (version 2015.1.01) software (Addinsoft, Belmont, MA, USA). All other diversity indices and statistics were calculated using mothur software. Shannon indices and abundance-based coverage estimates (ACEs) were calculated to assess parametric and nonparametric diversity. Comparisons between samples were performed on the basis of Bray-Curtis dissimilarity matrices (46). Beta diversity was compared using analysis of similarity (ANOSIM) (47), ordination was performed via principal coordinate analysis (PCoA), and clustering of sample groups was evaluated using analysis of molecular variance (AMOVA) (48). Determination of OTUs that significantly affected ordination was performed by the Spearman method using mothur software. Variations in the abundances of OTUs among sample groups were determined using the Kruskal-Wallis test (49), and differences in phylogenetic structure were assessed on the basis of both unweighted and weighted UniFrac distances (50). All statistics were evaluated at an α level of 0.05.

Nucleotide sequence accession numbers. Fastq files containing the raw sequencing data were deposited in the Sequence Read Archive of the National Center for Biotechnology Information under accession number SRP064396.

RESULTS

The mean sand moisture content was $15.7\% \pm 9.7\%$ among all samples, and the moisture content was significantly different among all beaches ($P < 0.001$; Table 1). Moisture content was negatively correlated with distance from the shoreline (Spearman's $r = -0.483$, $P < 0.0001$), but not sample depth ($r = 0.019$, $P = 0.776$). The ACE index was also negatively correlated with distance from the shoreline ($r = -0.130$, $P = 0.046$) and posi-

TABLE 2 Alpha diversity indices

Beach	Mean index value \pm SD ^a	
	Shannon	ACE
Minnesota Point	5.38 ± 0.41^A	$1,702 \pm 516^A$
63rd Street Beach	$5.50 \pm 0.41^{A,B,C}$	$1,479 \pm 867^A$
Burlington Beach	$5.81 \pm 0.13^{B,C}$	$2,056 \pm 658^{A,B}$
Marie Curtis Park	$5.94 \pm 0.28^{B,D}$	$2,830 \pm 598^{B,C}$
Fort DeSoto	6.43 ± 0.21^C	$7,247 \pm 2189^D$
Crandon Park	6.43 ± 0.24^C	$4,564 \pm 1235^E$
Huntington Beach	$5.94 \pm 0.24^{B,D}$	$2,853 \pm 476^{B,C}$
Sandy Beach	$6.20 \pm 0.40^{C,D}$	$3,750 \pm 1601^{C,E}$
Otaru Dream Beach	$5.88 \pm 0.31^{B,D}$	$3,021 \pm 598^{B,C}$
Fukiage-hama Beach	$5.43 \pm 1.02^{A,C}$	$1,313 \pm 667^A$
Jeju Beach	$6.12 \pm 0.11^{B,C,D}$	$6,661 \pm 1135^D$

^a Horizontal spaces divide sampling regions. The results for beaches sharing the same superscript letter (A, B, C, D, and E) did not differ significantly at an α value of 0.05.

tively correlated with the moisture content ($r = 0.302$, $P < 0.0001$).

Large-scale variation in bacterial communities. Among all samples included in the analysis ($n = 258$), a mean coverage of $96.7\% \pm 2.0\%$ was achieved, with the coverage ranging from 89.4% to 99.9%. Mean Shannon indices for individual sites, taking all depths and distances from the shoreline together, ranged from 5.38 to 6.43, and Shannon diversity differed significantly on the basis of the sampling region, where the ranking of diversity was as follows: Florida beaches $>$ Pacific Ocean beaches $>$ Great Lakes beaches ($P < 0.0001$; Table 2). This trend was also observed using the ACE index ($P < 0.0001$).

Taking all sampling sites together, samples taken 10 m from the shoreline had significantly lower Shannon diversity indices ($P \leq 0.025$) than samples taken at the shoreline and samples taken 1 m from the shoreline, for which the Shannon diversity indices did not differ significantly from each other ($P = 0.992$). This trend was maintained regionally within the Great Lakes and Pacific Ocean sands (see Tables S1 and S2 in the supplemental material) but not within Florida sands (see Table S3 in the supplemental material). In contrast, differences in ACE indices for samples collected at all distances were not significant ($P = 0.092$). Regionally, this trend was maintained in the Great Lakes and Pacific Ocean sands, but in Florida sands, ACE diversity was significantly lower for samples taken 1 m from the shoreline than samples taken either at the shoreline or 10 m from the shoreline ($P = 0.041$).

Differences in Shannon diversity at various depths were significant ($P = 0.018$), although *post hoc* tests revealed that only the difference between the samples obtained at 10-cm and 20-cm depths was significant ($P = 0.026$). Regionally, however, differences in Shannon diversity were significantly different only among Pacific Ocean sands ($P = 0.042$; see Table S2 in the supplemental material). Sample depth did not significantly affect ACE diversity among all samples ($P = 0.324$) or samples from the same region ($P \geq 0.305$).

The bacterial communities found in all samples primarily comprised the phyla *Proteobacteria*, *Bacteroidetes*, *Firmicutes*, and *Actinobacteria* (Fig. 1). The communities in samples collected from beaches in the same region (i.e., the Great Lakes, Florida, or Pacific Ocean) tended to be more taxonomically similar to each

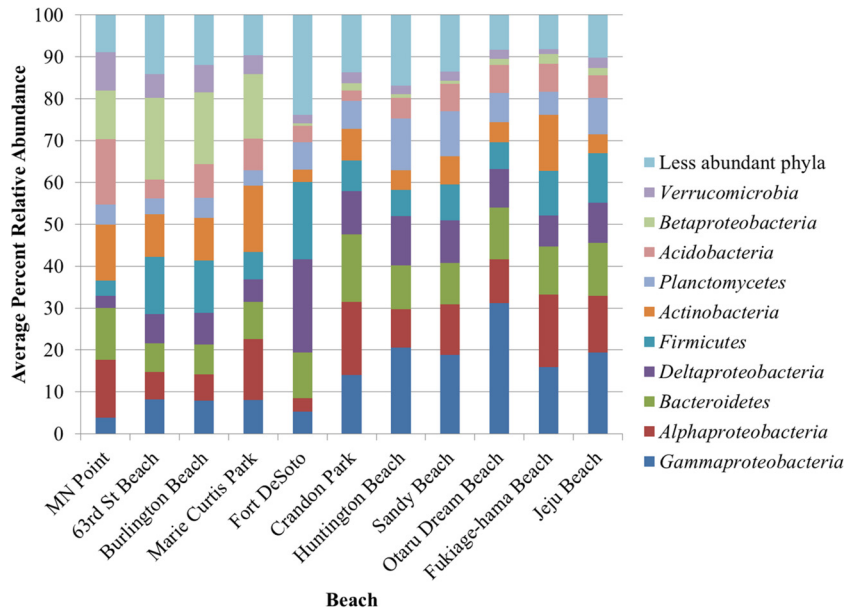


FIG 1 Distribution of phyla and classes of *Proteobacteria*, averaged among all samples, for each beach.

other than the communities in samples collected from different regions. Notably, the freshwater beaches tended to have greater relative abundances of *Actinobacteria*, *Betaproteobacteria*, and *Verrucomicrobia*, while the marine beaches had greater relative abundances of *Gammaproteobacteria*, *Deltaproteobacteria*, *Firmicutes*, and *Planctomycetes*. The observed differences in the phylum distribution were statistically significant ($P < 0.05$; Fig. 2) on the basis of Kruskal-Wallis tests of differences in the relative abundances of OTUs among regions. Furthermore, differences in the sand communities between Florida and Pacific Ocean beaches were resolved in greater detail. While Florida beach sand communities had greater abundances of *Deltaproteobacteria* (e.g., *Desul-*

fobacteraceae) and *Firmicutes*, the Pacific Ocean beach sands harbored higher proportions of *Gammaproteobacteria* (e.g., *Thiotrichaceae*) and *Planctomycetes*.

Analysis of the abundant families, which were those that had a mean relative abundance of at least 1% over the entire data set, revealed that their abundances differed significantly by region, as determined using ANOVA (Table 3), with greater differences between the abundances of these families in Great Lakes sands versus sands of the two marine regions. Among the less abundant families for which results are not depicted in Fig. 2, regional differences were also observed, where Great Lakes beaches had significantly greater abundances of families within the *Alphaproteobacteria*

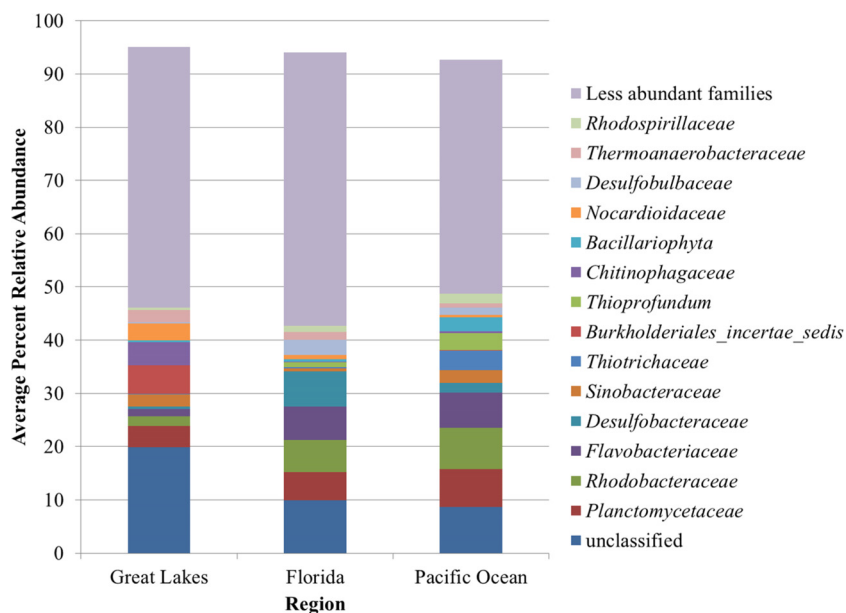


FIG 2 Family-level distribution of OTUs found to differ significantly by the Kruskal-Wallis test at an α level of 0.05.

TABLE 3 Relative abundance of families from all regions

Family ^a	Mean relative abundance \pm SD ^b (%)		
	Great Lakes	Florida	Pacific Ocean
Unclassified	20.51 \pm 6.26 ^A	10.54 \pm 3.74 ^B	9.48 \pm 2.60 ^B
<i>Planctomycetaceae</i>	4.04 \pm 1.69 ^A	5.51 \pm 1.65 ^B	7.53 \pm 2.92 ^C
<i>Rhodobacteraceae</i>	1.90 \pm 0.91 ^A	6.05 \pm 4.66 ^B	7.98 \pm 2.71 ^C
<i>Flavobacteriaceae</i>	1.40 \pm 1.12 ^A	6.49 \pm 4.00 ^B	6.77 \pm 4.50 ^B
<i>Desulfobacteraceae</i>	0.46 \pm 0.48 ^A	6.87 \pm 4.86 ^B	1.87 \pm 1.01 ^C
<i>Sinobacteraceae</i>	2.36 \pm 1.54 ^A	0.61 \pm 0.51 ^B	2.5 \pm 1.51 ^A
<i>Thiotrichaceae</i>	0.13 \pm 0.09 ^A	0.19 \pm 0.17 ^A	3.67 \pm 2.37 ^B
<i>Burkholderiales incertae sedis</i>	5.33 \pm 2.35 ^A	0.07 \pm 0.12 ^B	0.11 \pm 0.20 ^B
<i>Chitinophagaceae</i>	4.81 \pm 2.28 ^A	0.26 \pm 0.44 ^B	0.36 \pm 0.30 ^B
<i>Thiopfundum</i>	0.01 \pm 0.06 ^A	0.84 \pm 1.26 ^B	3.23 \pm 1.98 ^C
<i>Bacillariophyta</i>	0.58 \pm 0.84 ^A	0.60 \pm 0.57 ^A	2.92 \pm 3.62 ^B
<i>Desulfobulbaceae</i>	0.25 \pm 0.25 ^A	3.07 \pm 2.16 ^B	1.51 \pm 0.64 ^C
<i>Thermoanaerobacteraceae</i>	2.25 \pm 1.41 ^A	1.46 \pm 0.80 ^B	0.88 \pm 0.36 ^C
<i>Nocardioideaceae</i>	3.22 \pm 3.37 ^A	0.74 \pm 1.08 ^B	0.48 \pm 1.55 ^B
<i>Rhodospirillaceae</i>	0.61 \pm 0.37 ^A	1.25 \pm 0.92 ^B	1.98 \pm 0.95 ^C
<i>Geobacteraceae</i>	0.59 \pm 0.31 ^A	0.96 \pm 0.36 ^B	2.04 \pm 0.91 ^C
<i>Comamonadaceae</i>	3.84 \pm 1.01 ^A	0.16 \pm 0.21 ^B	0.13 \pm 0.22 ^B
<i>Sphingomonadaceae</i>	3.41 \pm 2.98 ^A	0.24 \pm 0.36 ^B	0.22 \pm 0.36 ^B
<i>Verrucomicrobiaceae</i>	1.43 \pm 0.78 ^A	1.04 \pm 0.77 ^B	1.15 \pm 0.52 ^B
<i>Syntrophobacteraceae</i>	0.95 \pm 0.51 ^A	1.66 \pm 2.21 ^B	1.19 \pm 0.69 ^A
<i>Chromatiaceae</i>	0.20 \pm 0.22 ^A	1.77 \pm 0.90 ^B	1.56 \pm 0.54 ^B
<i>Saprospiraceae</i>	0.32 \pm 0.24 ^A	0.92 \pm 1.01 ^B	1.64 \pm 0.65 ^C

^a Only families present at a mean level of >1% over the entire data set are shown.

^b The relative abundance in samples sharing the same superscript letter (A, B, or C) do not differ significantly by ANOVA at an α value of 0.05.

(e.g., *Sphingomonadaceae*), *Betaproteobacteria* (e.g., *Comamonadaceae* and *Rhodocyclaceae*), and *Verrucomicrobiaceae*. Sands from Florida and Pacific Ocean beaches had in common at comparable abundances several low-abundance families (e.g., *Geobacteraceae*, *Syntrophobacteraceae*, and *Chromatiaceae*) that were present at significantly greater abundances than they were in sands from Great Lakes beaches. However, sands from Florida beaches harbored families within the *Bacteroidetes* (e.g., *Cytophagaceae*) at abundances greater than those in sands from Pacific Ocean beaches. Furthermore, families within the *Firmicutes* differed in abundance; for example, Florida sands had greater relative abundances of *Veillonellaceae*, while Pacific sands had greater abundances of *Bacillaceae*.

Ordination of samples by PCoA revealed the separation of samples by region (Fig. 3), and this clustering was significant by AMOVA ($P < 0.001$). Families within the *Proteobacteria* were the predominant drivers of separation of the communities among beaches regionally (Fig. 3). Furthermore, differences in bacterial community compositions (beta diversity) between regions were significant by ANOSIM ($P < 0.001$). Evaluation of both unweighted and weighted UniFrac distances also revealed significant differences in the phylogenetic structures of bacterial communities among regions ($P < 0.001$ for all analyses; see Fig. S3 in the supplemental material).

Regional variation in bacterial communities. Interrogation of taxonomic shifts among the major phyla and *Proteobacteria* classes on the basis of sample depth and distance from the shoreline revealed little variation due to depth (discussed in greater detail below) and regionally specific variation with distance from the shoreline by ANOVA. Among the Great Lakes sand samples, distance from the shoreline was associated with an expansion of the abundance of *Alphaproteobacteria* ($P = 0.026$) and *Actinobac-*

teria ($P < 0.001$) and a decline in the abundance of *Bacteroidetes* ($P < 0.001$), *Planctomycetes* ($P < 0.001$), *Betaproteobacteria* ($P = 0.009$), and *Verrucomicrobia* ($P < 0.001$) (Fig. 4). The *Verrucomicrobia* ($P = 0.001$) showed higher relative abundances at a greater distance from the shoreline among the Florida beaches, with a corresponding reduction in *Deltaproteobacteria* ($P = 0.028$) and *Acidobacteria* ($P < 0.001$). Among the Pacific Ocean beaches, *Firmicutes*, *Actinobacteria*, and *Betaproteobacteria* had higher relative abundances at a distance of 10 m from the shoreline ($P < 0.001$ to 0.021) than at the other distances, with reductions in *Deltaproteobacteria*, *Planctomycetes*, *Acidobacteria*, and *Verrucomicrobia* being detected ($P < 0.001$ to 0.043). Notably, *post hoc* tests revealed no significant differences in the relative abundances of phyla between the samples taken at the shoreline and those taken 1 m from the shoreline ($P \geq 0.670$).

Ordination of samples within a given region revealed a significant separation of communities on the basis of the beach sampled by AMOVA ($P < 0.001$ for all analyses; Fig. 5 to 7). Differences in beta diversity were also significantly different among beaches within a region by ANOSIM ($P \leq 0.009$, $P < 0.001$, and $P < 0.001$ for the Great Lakes, Florida, and Pacific Ocean regions, respectively). Similarly, weighted UniFrac distances indicated significant differences in the abundance-based phylogenetic structure of communities among beaches within each region ($P < 0.001$). Evaluation of unweighted UniFrac distances revealed a greater phylogenetic similarity of communities within the Great Lakes, with no significant differences between the communities in samples from 63rd Street Beach in Chicago, IL (USA), and those in the other samples tested ($P = 0.458$ to 0.553). However, the communities in sands from all other freshwater beaches differed significantly ($P = 0.031$ to 0.041). The phylogenetic structures of the communities in sands from the two beaches sampled in Florida

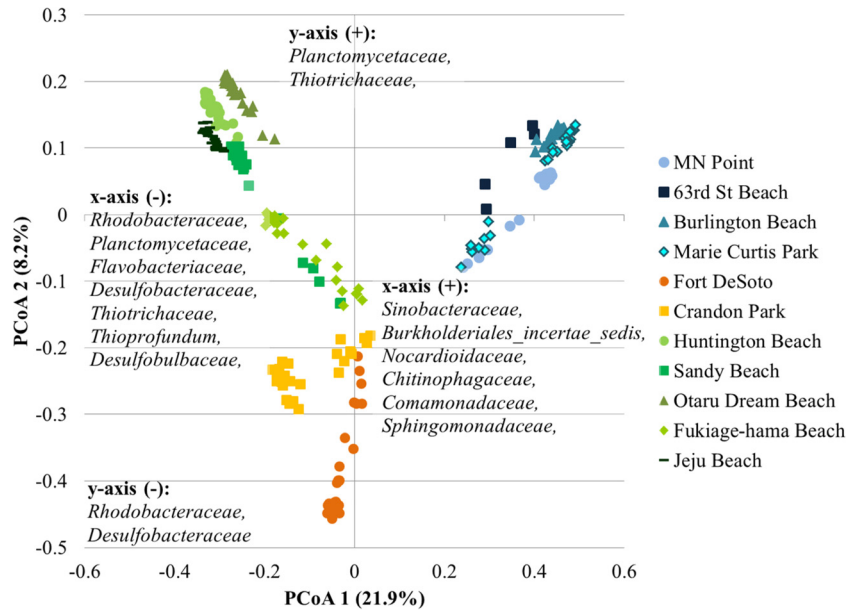


FIG 3 Principal coordinate analysis of microbiota from all sand samples. The relationship between the ordination plot and the distance matrix had an r^2 value of 0.57. The families listed represent the taxonomic assignments of OTUs that significantly affected positioning along either axis ($P < 0.05$) and were present at a mean relative abundance of $>1.0\%$ among all samples. Families are listed in order of declining abundance.

were not significantly different using unweighted UniFrac analyses, although differences in UniFrac metrics approached significance ($P = 0.050$). In contrast, the differences in the unweighted phylogenetic structures of the communities in the sands of all beaches in the Pacific Ocean region were significant ($P \leq 0.018$).

Local variation in bacterial communities. The local variation in sand communities was assessed with respect to sample depth (10, 20, and 30 cm) and distance from the shoreline (0, 1, and 10 m) for all locations except the 63rd Street Beach, where replicate

samples could not be amplified or too few sequence reads were obtained for inclusion in the analysis. As a result of insufficient replication, data for this beach were excluded from local analyses.

Ordination of samples from individual beaches revealed slightly different trends in the local variation by region (see Fig. S4 to S13 in the supplemental material). For the Great Lakes beaches, depth did not result in significant clustering of bacterial communities ($P \geq 0.633$). There was, however, significant clustering of samples by distance from the shoreline ($P \leq 0.03$), with each dis-

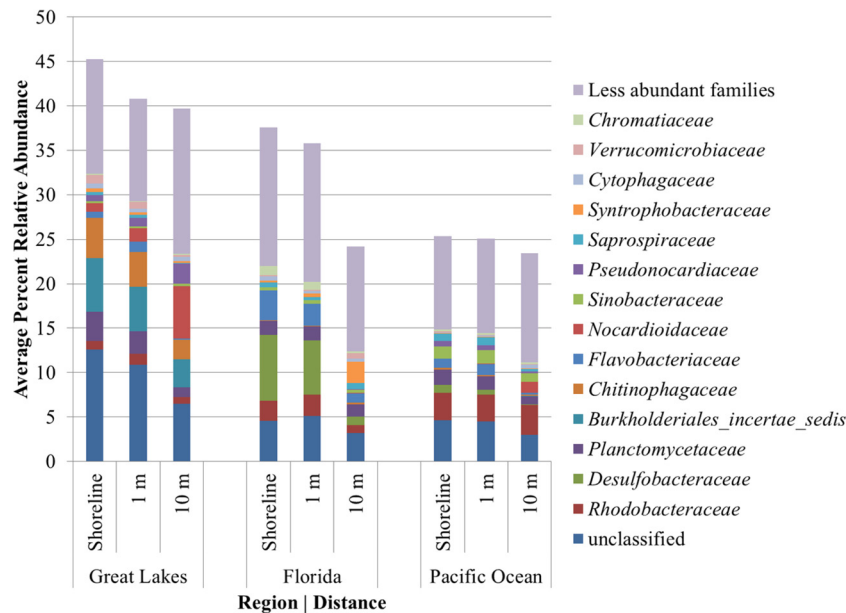


FIG 4 Family-level classification of OTUs for each region that differed significantly in relative abundance by distance from the shoreline by the Kruskal-Wallis test ($P < 0.05$).

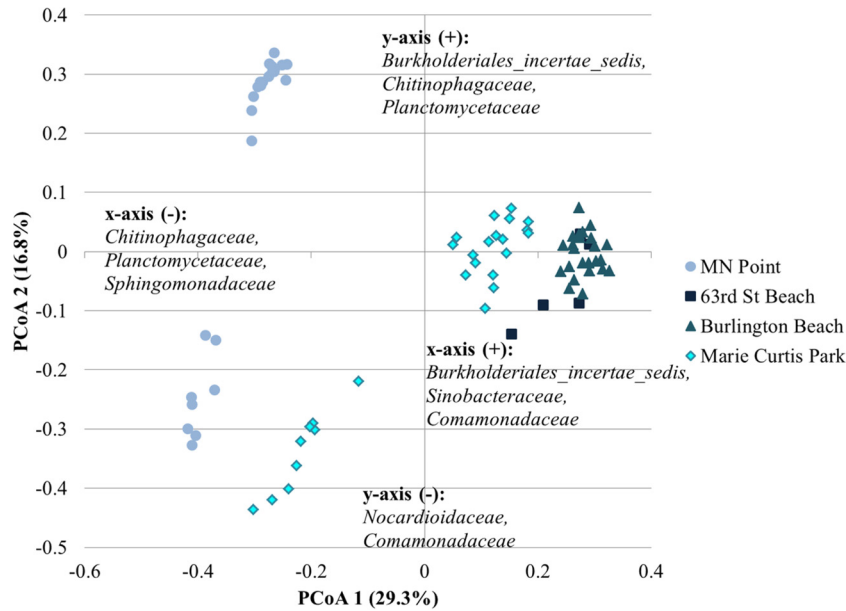


FIG 5 Principal coordinate analysis of microbiota from sand samples collected from Great Lakes beaches. The relationship between the ordination plot and the distance matrix had an r^2 value of 0.76. The families listed represent the taxonomic assignments of OTUs that significantly affected positioning along either axis ($P < 0.05$) and were present at a mean relative abundance of $>1.0\%$ among all samples. Families are listed in order of declining abundance.

tance clustering separately. Furthermore, distance from the shoreline was associated with decreases in bacterial groups commonly associated with freshwater environments, e.g., *Planctomycetaceae*, *Burkholderiales*, and *Chitinophagaceae* (Fig. 4).

At the Florida beaches, communities characterized from the Crandon Park beach also followed a trend similar to that for communities characterized from the Great Lakes beaches and clustered by distance from the shoreline ($P < 0.001$) but not by depth

($P = 0.092$). In contrast, communities from Fort DeSoto saw significant clustering by both depth and distance from the shoreline ($P = 0.035$ and 0.003). At this beach, the sample taken 10 m from the shoreline clustered independently ($P \leq 0.005$) and there was separation of the samples collected at 10-cm and 20-cm depths ($P = 0.023$). At both sites, distance from the shoreline corresponded to significant decreases in families, such as *Desulfobacteraceae* and *Flavobacteriaceae*, that may be associated with the water

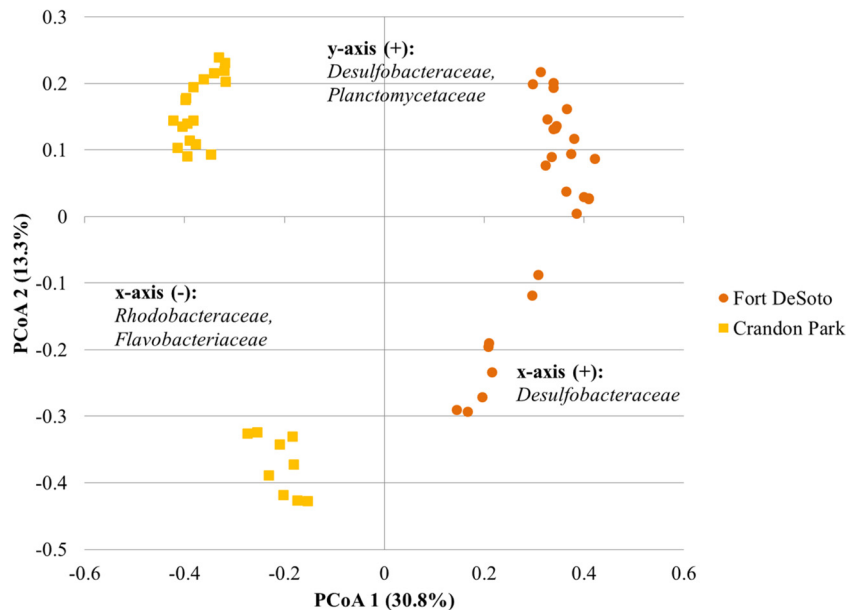


FIG 6 Principal coordinate analysis of microbiota from sand samples collected from Florida beaches. The relationship between the ordination plot and the distance matrix had an r^2 value of 0.74. The families listed represent the taxonomic assignments of OTUs that significantly affected positioning along either axis ($P < 0.05$) and were present at a mean relative abundance of $>1.0\%$ among all samples. Families are listed in order of declining abundance. No families showed significantly higher relative abundances with a decrease in the y -axis coordinate.

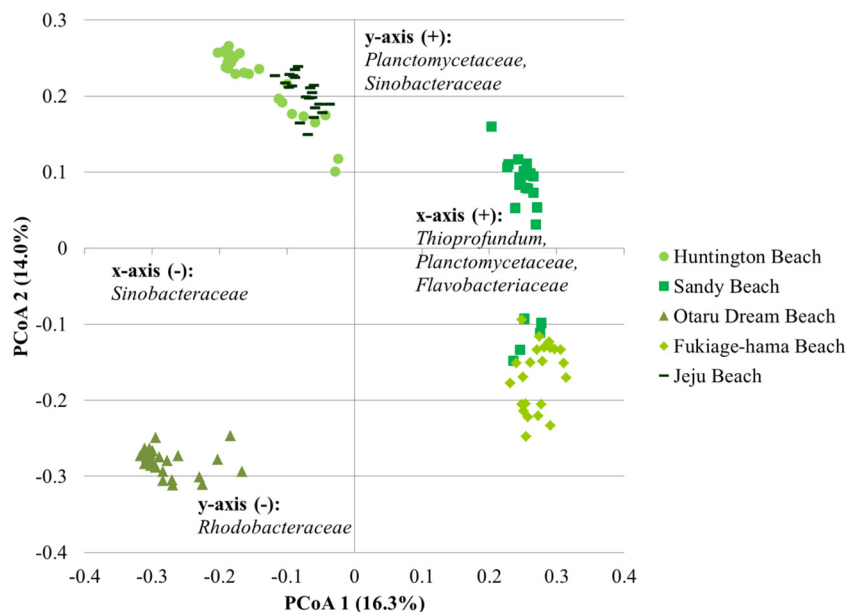


FIG 7 Principal coordinate analysis of microbiota from sand samples collected from Pacific Ocean beaches. The relationship between the ordination plot and the distance matrix had an r^2 value of 0.44. The families listed represent the taxonomic assignments of OTUs that significantly affected positioning along either axis ($P < 0.05$) and were present at a mean relative abundance of $>1.0\%$ among all samples. Families are listed in order of declining abundance.

column (Fig. 4), but at Fort Desoto, where differences in communities by depth were observed, variation in the abundances of bacterial groups showed no consistent trends by depth.

Among the marine beaches, Huntington Beach and Sandy Beach communities showed independent clustering at all distances from the shoreline ($P \leq 0.042$), with no clustering by depth detected ($P \geq 0.458$). Similarly, clustering by depth was not significant among the remaining marine beaches ($P \geq 0.171$), but communities in samples collected 10 m from the shoreline were significantly separated from those collected 1 m from or at the shoreline ($P \leq 0.002$). Similar to the findings for the Florida beaches, *Desulfobacteraceae* and *Flavobacteriaceae*, as well as *Planctomycetaceae*, were among the most abundant families that showed significant shifts in abundance with distance from the shoreline. With few exceptions, these trends were also observed among differences in beta diversity, as assessed by ANOSIM (Table 4).

DISCUSSION

Factors shaping the bacterial community structure in intertidal beach sands have only recently begun to be examined (3, 17, 32–35), and these studies have been limited to either local or regionally scaled study areas and primarily marine beaches. Data presented here provide novel insights into the bacterial community composition of freshwater beaches determined using next-generation sequencing. Furthermore, due to the consistency in the methodology used, OTU-level statistical comparisons among geographically isolated beaches in the Northern Hemisphere were possible. As originally hypothesized, each beach harbored a community with a unique composition, even among those beaches in the same region that were closer in proximity relative to the rest of the beaches in the data set. These differences are likely due to variations in factors, such as wave action, sand grain size, and nutrient content, which were previously suggested to be associ-

ated with community homogeneity (3). Furthermore, the local variation at each beach showed similar patterns among the beaches, with a significant variation in community structure between backshore and nearshore locations being detected, as reported elsewhere (32, 33).

One of the key findings of this study was the observation of regional similarity among beaches, as was observed, for example, in the close clustering and taxonomic similarity between the microbiota at Huntington and Jeju Beaches, even though they are separated by a linear distance of nearly 10,000 km. Previously, a distance-taxon relationship among intertidal sands sampled from sites at distances over 1,350 km apart was observed and was related to sand grain size, wave climate, and nutrient content (3). Thus, the regional similarity of these parameters may explain the similar distributions of bacteria. Importantly, in this study beaches were broadly classified into regions primarily on the basis of salinity, as other metadata were not collected, but more specific groupings on the basis of physicochemical parameters may reveal more robust trends shaping bacterial communities. Unfortunately, only moisture content data and no corresponding data regarding nutrient concentrations, wave climate, or sand grain size were collected as metadata here. Significant differences in moisture content suggest corresponding variations in physicochemical parameters. These differences likely affect species distributions at a finer taxonomic resolution, thus resulting in the observed differences in beta diversity.

The taxonomic distribution observed for marine samples in this study is similar to that reported elsewhere, where the predominant phyla and classes were the *Gammaproteobacteria*, *Alphaproteobacteria*, and *Bacteroidetes* (34). Family-level classifications revealed greater diversity among marine beaches. The abundant, cosmopolitan community identified at California beaches was comprised of *Alteromonadaceae*, *Bacillaceae*, *Flavobacteriaceae*, *Halomonadaceae*, *Planococcaceae*, *Pseudoalteromonadaceae*, and

TABLE 4 Summary of local differences in beta diversity (ANOSIM statistics) among all beaches

Region	Beach	Beta diversity		Post hoc results
		Depth	Distance from shoreline	
Great Lakes	Minnesota Point	0.982	<0.001	The results for all distances from the shoreline were significantly different from each other ($P < 0.001$)
	Burlington Beach	0.597	<0.001	The result for 10 m from the shoreline was significantly different from the results for the other distances ($P < 0.001$)
	Marie Curtis Park	0.677	<0.001	The results for all distances from the shoreline were significantly different from each other ($P \leq 0.012$)
Florida	Fort DeSoto	0.055	0.001	The result for 10 m from the shoreline was significantly different from the results for the other distances ($P \leq 0.003$)
	Crandon Park	0.029	<0.001	The difference between depths of 10 cm and 30 cm was significant ($P = 0.013$); the results for all distances from the shoreline were significantly different from each other ($P \leq 0.035$)
Pacific Ocean	Huntington Beach	0.763	<0.001	The result for 10 m from the shoreline was significantly different from the other distances ($P < 0.001$)
	Sandy Beach	0.577	<0.001	The results for all distances from the shoreline were significantly different from each other ($P < 0.001$)
	Otaru Dream Beach	0.604	<0.001	The result for 10 m from the shoreline was significantly different from the results for the other distances ($P < 0.001$)
	Fukiage-hama Beach	0.354	<0.001	The result for 10 m from the shoreline was significantly different from the results for the other distances ($P < 0.001$)
	Jeju Beach	0.324	<0.001	The result for 10 m from the shoreline was significantly different from the results for the other distances ($P \leq 0.001$)

Rhodobacteraceae (3), of which only *Flavobacteriaceae* and *Rhodobacteraceae* were identified among the abundant families found in the present study. Members of the *Flavobacteriaceae*, *Planctomycetaceae*, *Saprospiraceae*, and *Sinobacteraceae* were abundant in intertidal sands in Florida (35), and, in addition to *Flavobacteriaceae*, the *Planctomycetaceae* and *Saprospiraceae* were observed among the abundant families found in the present study. Similarly, *Paracoccus*, within the *Rhodobacteraceae*, was among the most abundant genera identified in sands in Hawaii (32). Notably, these families, some of which are typical marine taxa (51), were found at a significantly greater abundance in marine sands than in freshwater sands.

Several of the abundant taxa in freshwater beaches, such as the *Alphaproteobacteria*, including *Sphingomonadaceae*, *Actinobacteria*, and *Betaproteobacteria*, were previously found to be abundant in sands of Lake Michigan (17), and these lineages have been reported to be abundant and ubiquitous in freshwater (52). Therefore, it is likely that interactions between waterborne and sand communities are major drivers of low-resolution taxonomic diversity, especially among abundant taxa. Furthermore, the less frequent and less intensive water-sand interactions with distance from the shoreline likely explain why water-associated taxa show decreases in abundance in backshore sands. Subsequent variations in sand communities are then likely to occur in response to physicochemical parameters, especially among less abundant taxa, as has been previously suggested (2, 3, 17, 31).

In addition to environmental drivers of diversity and differentiation among the communities sampled, there are several methodological explanations that must be considered. As samples were collected at each beach at only a single time point, temporal variation is unlikely to be apparent in the current data set, but time has previously been reported to account for 34% of the variation in

bacterial communities in subtidal sands (31). Differences in sampling dates were likely to be masked in the current work by other meteorological parameters, such as prior rain events, which are more likely to disturb bacterial communities. Furthermore, the inclusion of 10 labs in the sampling strategy used here almost certainly resulted in technical variability in the interpretation and implementation of the sampling strategy. For example, differences in moisture content at the shoreline may reflect differences among labs regarding the degree of water saturation allowed in samples, which would also have resulted in differences in the communities characterized. This discrepancy may explain why differences due to depth, when observed, showed no trends in the relative abundances of taxa. Moreover, comparisons to previous studies, as described above, may show imperfect relationships due to biases introduced by the sequencing method used and the primers selected (36).

The local variations in community structure between backshore and intertidal samples found in the current study are similar to those described in a previous report (32), but the differences in diversity observed in the current study were opposite those observed previously. Here we observed lower Shannon indices for backshore samples, associated with a decline in moisture content. Another study reported no significant difference in the Shannon index between wet and dry sand (33). These discrepancies may be explained by characteristics in the water column, as both prior studies were evaluating bacterial communities in relation to fecal indicator bacteria and impaired water quality status, and impairment has been associated with a decrease in bacterial diversity (33). Interestingly, in freshwater beaches that were not subject to strong tidal influences, the community composition in samples obtained at each distance from the shoreline tended to be unique, while the community compositions in samples obtained at the

shoreline and 1 m from the shoreline were generally not distinct among marine beaches. This is similar to the findings of a previous study of the communities in freshwater sand, which saw a differentiation of the community structures between backshore and berm sand samples (17). These results may indicate that tide and wave actions serve to homogenize the bacterial communities of intertidal samples, while the more sporadic wetting of foreshore samples by wave action within the Great Lakes allows differentiation of these communities. These findings may be of particular importance to public health and, depending on the degree of tidal and/or wave activity, may affect the time required for beach sands to recover from the presence of pathogens or to achieve a decline in the level of risk from pathogens after a recent contamination event.

While sampling depth was previously shown to influence bacterial diversity and the community structure in subtidal sands (31), the differences in diversity and community composition found here did not vary. It is possible that in this study differences were not observed due to sampling during an outgoing tide, where intertidal sands would have been inundated and homogenized to the relatively shallow depth (30 cm) sampled. The finding that depth was a significant factor at Crandon Park is unusual, especially since differences were observed between 10- and 20-cm depths but not 10- and 30-cm depths. However, the differences in community structures by depth in the Fort DeSoto sands also approached statistical significance, suggesting that differences in community structure by depth may warrant further study, especially in Florida.

The results of this study expand our current understanding of bacterial communities in freshwater beach sands throughout the Great Lakes, as well as marine beach sands throughout the United States, Japan, and South Korea. This study is the first to assess geographic variability in beach sand bacterial communities on a global scale within the Northern Hemisphere. One of the key findings of this study is the high degree of regional taxonomic and, in some cases, phylogenetic similarity. This may be driven in part by interactions between the sand and water communities, since persistent core microbiomes for both freshwater and marine environments have been previously suggested (53, 54). We further show the presence of highly similar local community dynamics within the same beach, where moisture, most likely resulting from tidal cycles, is a major driver of the bacterial communities present. This study represents an important initial effort to characterize the bacterial communities in global beach sands and provides a fundamental basis for future efforts to determine factors affecting regional similarities in sand bacterial communities.

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