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Microbial communities in sandy beaches from the three domains of life differ by microhabitat and intertidal location

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Abstract

The microbial communities of sandy beaches are poorly described despite the biogeochemical importance and ubiquity of these ecosystems. Using metabarcoding of the 16S and 18S rRNA genes, we investigated the diversity, microhabitats (with or between sand grains) and intertidal distributions of microorganisms (including meiofauna) from pristine sandy beaches in British Columbia, Canada, and hypothesized that abiotic variations due to microhabitat or intertidal gradients influence the distribution of microorganisms on local scales. Bacterial, archaeal and protistan communities of the sand were clearly distinct from interstitial communities, and from planktonic communities of the overlying seawater, which correlated with differences in function and lifestyle (e.g., sulphur reduction and gliding motility). In contrast, meiofaunal communities could not be distinguished by sample type, suggesting that they are more frequently mobilized between these microhabitats. Across intertidal zones, high intertidal, mid intertidal and low intertidal/swash communities were distinct and correlated with moisture, organic carbon and phosphate content, implying that the distribution of microorganisms is influenced by intertidal abiotic gradients. However, few taxa at the genus or species level individually contributed to this zonation pattern; rather, a unique combination of multiple microbial taxa was probably responsible. Although significant differences in microbial community composition on sandy beaches can be attributed to microhabitat and intertidal gradients, further investigations are needed to assess community assembly processes, the consistency of these distributions, and the functions of the majority of the microorganisms observed in the sand and their effects on the biogeochemistry and ecology of sandy beaches.

KEYWORDS
beach, community assembly, intertidal, meiofauna, metabarcoding, microbiome, protists, sand, zonation

1 | INTRODUCTION

Sandy beaches occupy about two thirds of unfrozen marine coastlines worldwide and are ecologically and geologically important environments that provide food and habitat to many organisms, buffer coastlines from physical impacts of the sea, and alter terrestrial run-off and groundwater (McLachlan & Defeo, 2018; Nel et al., 2014). Sandy beaches form a complex ecosystem at the intersection of marine and terrestrial environments and contain animals with diverse modes of feeding, such as scavengers, predators, filter-feeders and deposit-feeders, that are in turn supported by primary producers (phytoplankton) and allochthonous inputs (e.g., seaweeds, carrion; Dugan et al., 2011; Liebowitz et al., 2016; Schlacher et al., 2008). In addition, many important biogeochemical

processes, including photosynthesis, decomposition of organic matter, nitrogen cycling and sulphur cycling, are accomplished by an active community of microorganisms (Bacteria, Archaea and protists) within sandy beach ecosystems (de Beer et al., 2005; Santoro et al., 2006, 2008; Wu et al., 2018). Microorganisms also affect the physical structure and stability of the sand through the production of extracellular polymeric substances and biofilm formation (de Brouwer et al., 2005; Lubarsky et al., 2010; Stal, 2003). Despite their ecological and biogeochemical importance, the diversity of microorganisms inhabiting sand has yet to be fully characterized, especially at the molecular level. This is all the more imperative considering that coastal sandy ecosystems are imminently threatened by increasing coastal development as well as sea-level rise, more frequent storms, and other perturbations due to a rapidly changing global climate (Barnard et al., 2015; Defeo et al., 2009; Nel et al., 2014).

Sandy beaches are diverse in their geomorphology, and are classified based on the degree of the shore slope, ranging between reflective (with the steepest slopes) to dissipative (with flattest slopes). The type of beach is dependent on tidal range, wave energy and other hydrodynamic processes, which also affect the composition of sediment (coarse sand to silt) deposited intertidally (Wright & Short, 1984). In addition, similar to other intertidal environments, daily tidal cycles also result in wide variations in abiotic conditions such as moisture, wave exposure, temperature and salinity.

Beach type, together the currents from inshore to the surf zone, are known to affect the abundance of animal larvae and zooplankton that are transported to the shoreline (Morgan et al., 2017). For example, dissipative beaches with long, flat intertidal zones typically have greater macrofaunal richness, compared to the reflective beaches with a shorter and steeper shore face (Barboza et al., 2012; Defeo & McLachlan, 2005). Microbial diversity in sandy beaches is also influenced by beach morphodynamics. Sand grain size, shape and permeability have been shown to influence microbial richness and abundance in sediments (Barboza & Defeo, 2015; Lallias et al., 2015; Probandt et al., 2017).

Across the intertidal beach environment, zonation patterns are often observed for animals, where transitions in the distribution of species result in distinctive communities. Macrofaunal diversity and their distribution across the intertidal are strongly influenced by shifts in abiotic factors (e.g., moisture, wave exposure, temperature and salinity), but also biological factors including competition and predation (Defeo & McLachlan, 2005; McLachlan, 1990; Schlacher & Thompson, 2013). The distribution of meiofauna (i.e., microscopic animals; typically defined as between 0.45 and 1 mm in size) across sandy beaches is also governed by a combination of abiotic and biotic factors (Giere, 2009), though the specific factors affecting their distribution are distinct from macrofauna because of their limited motility and greater passive transportation (Giere, 2019). For example, macrofaunal diversity tends to increase with lower intertidal elevations (Armonies & Reise, 2000; Degraer et al., 1999), whereas meiofaunal diversity is often at its maximum in the middle of the intertidal zone, which is consistent with the intermediate disturbance hypothesis (Armonies & Reise, 2000; Gheskiere et al., 2004; Gingold et al., 2010). These patterns, however, are affected by beach geomorphology, chemistry, seasonality and other local variations (Baia & Venekey, 2019; Degraer et al., 2003; Gheskiere et al., 2005; Hua et al., 2016; Kotwicki et al., 2005; Maria et al., 2013).

For prokaryotes (Bacteria and Archaea) and protists, several studies have investigated their distribution patterns within sandy beaches using molecular approaches, in contrast to meiofaunal investigations that typically do not incorporate molecular data. Recent analyses have demonstrated differences between bacterial communities from high- and low-tide environments (Cui et al., 2013; Halliday et al., 2014; Staley & Sadowsky, 2016), though the probable transitions in composition through mid-intertidal zones and the factors contributing to these changes in distribution have yet to be elucidated. Moreover, the different ways that microorganisms exist in sand can affect their distribution and dispersal across the intertidal zone and beyond. For example, sessile bacteria that are firmly attached to sand grains (often via extracellular polysaccharides or in biofilms) are probably mobilized and dispersed by the movement of sand grains typically driven by wind and waves; by contrast, interstitial microorganisms, which either exist in the water between sand grains or are loosely associated with sand grains, can also be dispersed by porewater flow driven by waves and groundwater flow (Boehm et al., 2014; Gobet et al., 2012; Meadows & Anderson, 1966). Dispersal of the microorganisms via groundwater has been investigated as a route of faecal bacterial contamination via sewage intrusion to recreational beaches (Cui et al., 2013; Fresia et al., 2019; Halliday et al., 2014; Romão et al., 2017; Yamahara et al., 2007). Otherwise, little information exists on the diversity and distribution of microorganisms from sandy beaches, the occurrence of zonation patterns as for larger organisms, and the factors that influence the distribution and dispersal of beach microorganisms, such as living attached to sand versus within interstitial spaces.

To help fill the large gap in our current understanding of microbial and meiofaunal diversity of sandy beaches and community assembly processes, we surveyed five pristine beaches of the North Pacific coast of British Columbia, Canada. We investigated whether microbial or meiofaunal communities differ due to microhabitat (i.e., sessile vs. interstitial) or local environmental gradients (i.e., zonation patterns across the intertidal area). To do so, we characterized microbial diversity across the three domains of life (Bacteria, Archaea and Eukaryota) using high-throughput metabarcoding from seawater and interstitial samples for all five beaches and from whole sand samples for two beaches. Our survey provides insights into the distribution and ecological functions of these microorganisms in sandy beaches and establishes a baseline for evaluating potential impacts of pollution, climate change and other perturbations that are particularly threatening to sandy beach ecosystems.

2 | MATERIALS AND METHODS

2.1 | Sand sampling

Sand and seawater samples were collected from June 12 to 16, 2014 from five beaches (North Beach, West Beach, 2nd Beach, 3rd Beach and 7th Beach) on Calvert Island, British Columbia, Canada (Figure 1). All the beaches experience mixed semidiurnal tides, and samples were collected during one of the largest annual spring tides where the tidal range was ~4.3–4.7 m. At each beach, a transect line across the intertidal zone was established from high tide to low tide, generally located near the centre of the beach or corresponding to locations of remote cameras mounted to examine dune formation.

Just prior to the lowest tide of the day, we measured shore face morphology with a range finder, and visually identified high-tide, mid-tide and low-tide zones of the beach along the transect line based on observations of changes in sand moisture, distance from the shoreline and the slope of the beach (Figure 1). At low tide, samples were collected from the overlying seawater and subtidal sand in the swash zone and progressed towards the high tide line ahead of the flooding tide. This ensured that each sample had experienced most of the possible evaporation and air exposure of an intertidal cycle prior to collection. For intertidal sand samples, we collected surface sand (to a depth of 1 cm) using a sterile Petri dish at sampling sites along the transect line roughly corresponding to the middle of the identified zones. Additional sampling sites along the transect were added corresponding to prominent features on the beach: seawater tide pools at 3rd Beach and 7th Beach, sand bar at 7th Beach, and the emergence of groundwater at West Beach. Three replicate samples were taken at each sampling site, spaced ~1–5 m apart, perpendicular to the transect line.

We also sampled sand from these intertidal zones from West Beach in June 2015, July 2015, September 2015 and January 2016 to investigate seasonal changes in distribution patterns. For January 2016, samples were collected during the low tide that occurred during the daytime, which was not the lowest tide of the day and only exposed the upper half of the entire intertidal zone.

2.2 | Abiotic measurements: Temperature, pH, nutrients, organic matter, moisture and sand grain composition

At each sampling site, in situ temperature and, where possible, pH were measured with an ExStikII (Extech Instrument Corporation) and salinity was measured from porewater with a refractometer. Moisture content of the sand was measured as the change in mass after drying sand samples at 110°C for 2 h or until visibly dried per gram of wet sand. Ammonium (NH_4^+), nitrate (NO_3^{2-}), phosphate (PO_4^{3-}) and organic carbon concentrations (per kg dry sand) were also measured from air-dried sand using standard procedures (with 2 M KCl extraction, BrayP-1 extraction and loss of ignition, respectively, at the Analytical Chemistry Services Laboratory, British Columbia Ministry of Environment and Climate Change Strategy, Victoria, BC). The relative composition of sand grain sizes at each site was determined by shaking dried sand through a series of sieves of decreasing mesh sizes (355, 250,106 and 45 μm), and weighing the mass of sand in each sieve.

2.3 | Sand and seawater processing for DNA and chlorophyll extractions

For DNA and chlorophyll extractions from whole sand samples, which included interstitial organisms, 1 and 5 g of sand was directly stored at −20°C, respectively. Whole sand was processed for West Beach and 2nd Beach only.

All sand samples were processed for examination of interstitial communities, as a subset of the sand samples. Ten grams of sand was shaken vigorously by hand with 20 ml of 0.22-μm filtered seawater. This 0.22-μm filtered seawater probably contained extracellular and viral DNA that added some diversity to the samples if this DNA attached to particles or the filter membranes in the subsequent filtration step, but probably in relatively negligible amounts. For DNA extractions, 4 ml of the seawater from shaken sand was prefiltered through a 150-μm filter then collected onto a 0.22-μm Supor filter (Pall Corporation), and stored at −20°C. For chlorophyll extractions, 10 ml of the seawater from shaken sand was prefiltered through a 150-μm filter and then collected onto a GF/F filter (Whatman), and stored at −20°C.

For seawater samples, 100 ml was prefiltered through a 150-μm filter then collected onto a 0.22-μm Supor filter for DNA analysis or onto a GF/F filter for chlorophyll analysis.

2.4 | Chlorophyll concentration

Chlorophyll *a* was extracted by incubating whole sand or GF/F filters in 90% acetone overnight at 4°C. Chlorophyll *a* fluorescence was measured from the acetone before and after acidification with 10% hydrochloric acid (10-AU fluorometer; Turner Designs), and these fluorescence measurements were converted to chlorophyll concentrations using a calibrated standard curve.

2.5 | DNA extraction and PCR amplicon sequencing (metabarcoding)

DNA was extracted using the PowerSoil DNA Isolation kit (MO BIO) from whole sand and from interstitial and seawater samples collected onto 0.22-μm filter membranes following the manufacturer's protocol. The DNA was used to assess diversity from three different marker regions: the V4 region of the 16S rRNA gene (16S-V4) for Bacteria and Archaea, V4 region of the 18S rRNA gene (18S-V4) for Eukaryota, and the V9 region of the 18S rRNA gene (18S-V9) also for Eukaryota. More variable marker regions, such as the cytochrome

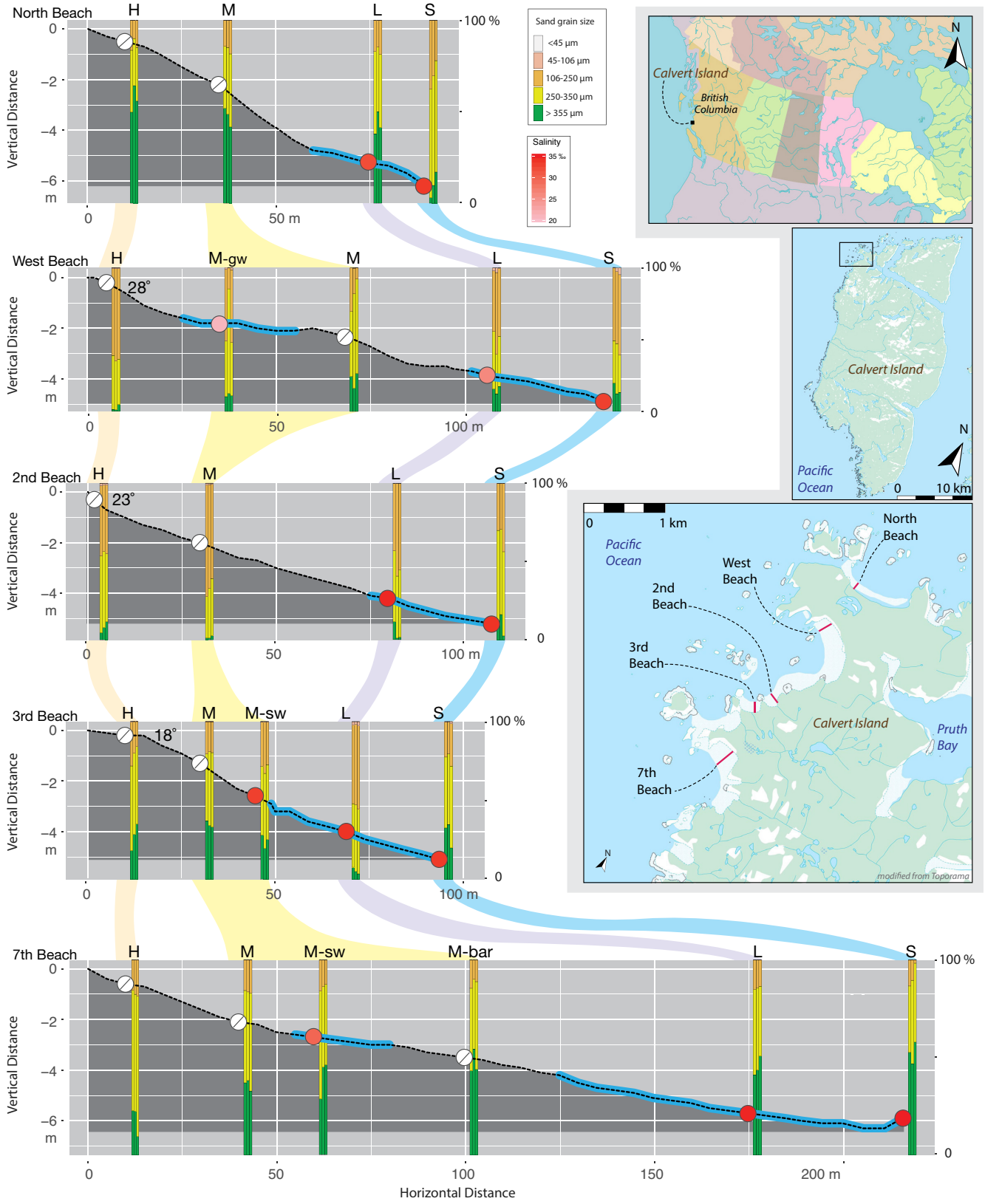


FIGURE 1 Maps indicating the location of the beaches and transects (red lines) sampled from Calvert Island, British Columbia, Canada. Graphs indicate the slope of the beach face as the vertical distance (height) relative to high tide (0 m) vs. the horizontal intertidal location along the sampled transect. The composition of sand grain sizes (bar plots) is shown for the sampled sites (in triplicate) of the transect, as well as salinity (red-shaded circles) where porewater was available (as indicated by the blue line along the shoreface). Coloured ribbons connect beaches at sampling sites of equivalent intertidal zones: high (H), mid (M), low (L) and swash (S)

oxidase I gene (COI) or internal transcribed spacer regions (ITS), which are commonly used to identify animals and fungi to lower taxonomic levels, were not used to focus instead on a comprehensive assessment of eukaryotic diversity including protists and poorly characterized meiofauna.

The 16S-V4 and 18S-V9 regions were PCR (polymerase chain reaction)-amplified and sequenced following the standard protocol of the Earth Microbiome Project (Thompson et al., 2017). The PCR primers consisted of an Illumina adapter sequence, 12-bp sample barcode, 12-nucleotide pad and linker, and target-specific nucleotides to amplify the 16S-V4 region from Bacteria and Archaea: 515FB—GTGYCAGCMGCCGCGGTAA and 806RB—GGACTACNVGGGTWTCTAAT, or the 18S-V9 region from eukaryotes: Euk1391f—GTACACACCGCCCGTC and EukBr—TGATCCTTCTGCAGGTTCACCTAC. Single-end sequences were generated using a HiSeq 2500 instrument (Illumina), and then, using the QIIME 1.9.1 split_libraries_fastq.py script, the sequences were demultiplexed, primers and adapter sequences were removed, and sequences were minimally quality-filtered (Phred quality threshold of 3 for at least 75% of the read length).

The 18S-V4 region was PCR-amplified and sequenced following the standard protocol of the Integrated Microbiome Resource (Comeau et al., 2017). For this protocol, the PCR primers included the P5 Illumina adapter, 8-bp sample barcode, P7 Illumina adapter and target-specific nucleotides to amplify the 18S-V4 region from eukaryotes: E572F—CYGCGGTAATTCCAGCTC and E1009R—AYGGTATCTRATCRTCTTYG. The 18S V4 amplicons were sequenced with a MiSeq instrument (Illumina) generating 2 × 300 nucleotide reads, and paired reads of sufficient length after quality trimming (Phred score threshold of 15) were merged using PEAR (Zhang et al., 2014).

2.6 | Sequence analysis

16S-V4, 18S-V4 and 18S-V9 sequences were analysed separately, but following the same procedures using tools implemented in QIIME (version 1.8.0) (Caporaso, Kuczynski, et al., 2010). Chimeras were removed using USEARCH with the RDP trainset No15 and SILVA 119 databases as references for 16S and 18S sequences, respectively (Cole et al., 2014; Quast et al., 2013). Clusters of sequences with 97% similarity were generated using UCLUST (Edgar, 2010), which assigns the centroid sequence to represent the cluster as the operational taxonomic unit (OTU). All OTUs representing fewer than five sequences in total across all samples were removed from further analysis. Samples below a threshold of 8000 total sequences were also removed from further analysis.

The 16S rRNA OTUs were taxonomically identified using scikit learn in QIIME2 trained on the SILVA 16S rRNA database (version 132) (Yilmaz et al., 2014), and OTUs identified as "Chloroplast" and "Mitochondria" were removed from the 16S rRNA analysis. The 18S-V4 and -V9 rRNA OTUs were first taxonomically identified using UCLUST, and a combined database of 16S and 18S rRNA sequences (GreenGenes 97otus from version 13.8 and PR2 version 4.5, respectively) was used to identify and remove bacterial, archaeal and organellar sequences (Guillou et al., 2013; McDonald et al., 2012). The taxonomic identification of the remaining eukaryotic sequences was updated using sci-kit learn and the PR2 version 4.12 database. However, for all organisms identified as belonging to Hacrobia, a highly contentious taxonomic group, we have labelled these as Cr yptophyta + Haptophyta + Centroheliozoa. Following taxonomic identification, 18S-V4 and -V9 data were split into Metazoa OTUs (i.e., animal) and nonmetazoan OTUs (i.e., protists) and analysed separately.

OTUs were aligned using PYNAST (Caporaso, Bittinger, et al., 2010) against a GreenGenes 16S rRNA reference alignment or a PR2-V9 reference alignment for 16S-V4 OTUs and 18S-V9 OTUs, respectively. 18S-V4 OTUs were aligned using MAFFT (Katoh & Standley, 2013). From these alignments, sites with gaps in >99% of sequences were removed, and phylogenetic trees were built using FASTTREE (Price et al., 2010), which were used to calculate UniFrac distances (see below).

2.7 | Diversity and community analyses

Diversity and community analyses were performed in R implementing the PHYLOSEQ, VEGAN, METAGENOMESEQ and DESEQ2 packages (Love et al., 2014; McMurdie & Holmes, 2013; Oksanen et al., 2019; Paulson et al., 2013). Analysis of the OTUs was initially examined by grouping the data into sand, interstitial and seawater samples. Rarefaction was used to normalize sampling depth for all samples (rarefying to the number of reads in the sample with the lowest sampling depth) and alpha diversity was measured using the Shannon diversity index. Statistically significant differences in alpha diversity were evaluated using one-way analysis of variance (ANOVA) and Tukey's honest significant difference post hoc tests. Principal coordinates analysis (PCoA) of unweighted UniFrac distances was used to examine differences in community composition between samples (beta diversity). PERMANOVA (using adonis from the VEGAN package) was used to test the statistical significance of the distances in community composition between sand, interstitial and seawater samples. To identify OTUs that significantly changed in abundance between sample types, DESeq from the DESEQ 2 package was used with an adjusted *p*-value threshold of .01 using read abundances for each OTU normalized for compositional analysis using cumulative sum scaling (CSS) implemented in the METAGENOMESEQ package. For higher taxonomic ranks, this differential abundance analysis was also performed using scaled read abundances for each OTU that were summed according to their classification.

Samples from individual beaches were analysed to examine changes in diversity across the intertidal zone. In addition to beta diversity and differential abundance analyses as described above, distance-based redundancy analysis using constrained analysis of principal coordinates (using capscale from the VEGAN package) was used to examine the variation in community composition explained

TABLE 1 Number of reads and OTUs analysed for Bacteria, Archaea, protists and Metazoa after processing and filtering metabarcode sequencing data

16S-V4			
Total number of reads / OTUs	19,074,892 / 84,025		
	Bacteria	Archaea	Poorly identified
Number of reads (% of reads)	18,833,309 (98.7)	240,662 (1.3)	298,997 (1.6)
Number of OTUs (% of OTUs)	81,806 (97.4)	2196 (2.6)	3414 (4.1)
18S-V4			
Total number of reads / OTUs	3,414,187 / 7142		
	Protists	Metazoa	Poorly identified
Number of reads (% of reads)	1,504,963 (44.1)	1,909,224 (55.9)	4176 (0.1)
Number of OTUs (% of OTUs)	5602 (78.4)	1540 (21.6)	146 (2.0)
18S-V9			
Total number of reads / OTUs	19,893,825 / 92,193		
	Protists	Metazoa	Poorly identified
Number of reads (% of reads)	9,876,406 (49.6)	10,017,419 (50.4)	814,692 (4.1)
Number of OTUs (% of OTUs)	55,104 (59.8)	37,089 (40.2)	6171 (6.7)

Note: Percentage of the total number of reads or OTUs are shown in parentheses. "Poorly identified" refers to reads or OTUs that could not be identified below Bacteria, Archaea and Eukaryota.

by moisture content, sand grain size, percentage organic matter, and ammonia, nitrate, phosphate and chlorophyll concentrations.

3 | RESULTS AND DISCUSSION

3.1 | Geochemistry of sampled beaches

We sampled sand from at least four distinct zones (high, mid, low and swash) along intertidal transects of five beaches on Calvert Island, located in the central coast of British Columbia (Figure 1). West Beach and 7th Beach are dissipative beaches with long and flat shorefaces, and exposed to higher energy waves with groundwater seepage (West Beach) and seawater tide pools (7th Beach, probably residual from the last high tide) that were present in the mid intertidal zones and also sampled on the days of survey (Figure 1). North Beach, 2nd Beach and 3rd Beach are narrower and steeper beaches, and more reflective and sheltered from waves. Despite the differences in shoreface, all beaches consisted of relatively similar sized sediments, of well-sorted to moderately sorted sand, across the lengths of their intertidal areas (Figure 1; Table S1). Moisture content, which we hypothesized to be a primary factor affecting community composition, varied, as expected, from almost no moisture in high intertidal samples to complete saturation from swash zone samples. Salinity, nitrate, ammonia, phosphate and organic matter also tended to increase from high tide to low tide (Table S1).

3.2 | Overview of sequence data

Community composition from sand, interstitial and seawater samples were evaluated from metabarcode sequences from the 16S-V4 region for Bacteria and Archaea, and the 18S-V4 and V9 regions for protists and metazoans. For the 27 sand samples collected from West Beach and 2nd Beach, sequence quality and quantity thresholds were met for all samples except for 16S-V4 data from one 2nd Beach high tide replicate and 16S-V4, 18S-V4 and 18S-V9 data from one 2nd Beach low tide replicate, which were removed from further analysis (Table S2). Interstitial samples were collected from all beaches, but several samples did not meet quality and quantity thresholds, probably because the abundance of interstitial organisms was generally lower and more variable than from whole sand. In particular, only six out of 15 high tide samples had sufficient 18S-V4 data for analysis, suggesting a low abundance of eukaryotes at this intertidal elevation (Table S2). Most significantly, all of the 2nd Beach interstitial samples were removed from further analysis due to insufficient data, with the exception of 16S-V4 data from one high tide and one mid tide sample. In total, of the 72 interstitial samples collected across five

beaches, 46 16S-V4, 42 18S-V4 and 45 18S-V9 samples met quality and quantity thresholds (Table S2).

Bacteria comprised the vast majority of the 16S-V4 data, and because Archaea were a minor component, we analysed the 16S-V4 data of both taxa together as Bacteria + Archaea (Table 1). The 18S-V4 and 18S-V9 data comprised an approximately equal number of reads from protists and metazoans from mostly microscopic taxa (i.e., meiofauna), but OTU counts were much higher for protists (including fungi), compared to metazoans (Table 1). The 18S-V9 sequencing yielded many more reads and OTUs than the 18S-V4 data, which might be due to higher variability of the V9 marker region, or differences in the sequencing methodologies that were used. However, once the OTUs were taxonomically identified, the results were similar (see Appendix S1). The 18S-V4 OTUs were better identified because this region is represented more comprehensively across the diversity of eukaryotes in sequence databases compared to the V9 region, and therefore we have focused the main results on the 18S-V4 region with complementary analyses of the 18S-V9 data in Figures S11–S14. In addition, note that the number of rRNA gene copies per cell can be highly variable between taxa, and even intraspecifically, particularly among protists which can range from one to >500,000 copies per cell (Lavrinienko et al., 2021). Consequently, differences in the abundances of the 16S or 18S sequence reads were not interpreted to correspond with similarly sized changes in cell abundance or biomass but as indicators of changes in diversity and composition.

3.3 | Microbial diversity is highest in the sand

We investigated microbial community composition from three different sample types: whole sand from two beaches, and the interstitial extract and seawater from the swash zone from these and three additional beaches; we also assessed whether significant differences exist among these sample types that could influence distribution patterns or modes of dispersal.

Operational taxonomic unit diversity, as measured by the Shannon diversity index, was highest for Bacteria, followed by that of protists in all sample types (Figure 2a). For Bacteria and Archaea, OTU diversity was significantly higher in the sand than in seawater. Protists were also more diverse in the sand than in seawater, but diversity in the sand was highly variable. By contrast, metazoans were equally diverse in the sand and seawater.

Diversity from the interstitial samples was lower than that of the sand for all groups of organisms (Bacteria, Archaea, protists and Metazoa) (Figure 2a). This was expected because the interstitial community is a subset of the sand samples, and previous microscopic observations of sand grains indicate that the vast majority of microorganisms are associated with a particle (Gobet et al., 2012; Meadows & Anderson, 1966; Probandt et al., 2018). Diversity in the interstitial samples exhibited a greater degree of variation, which probably reflects inconsistency in the strength of associations with sand grains (Figure 2a). However, many OTUs from the interstitial samples were not found from the sand samples, suggesting that they were rare in the sand community but a relatively more abundant component in porewater, contributing to a distinct interstitial community (Figure S1).

3.4 | Bacteria, Archaea and protists have distinct sand, interstitial and seawater communities, but metazoans do not

The microbial communities associated with seawater and with sand are known to be distinct from each other despite the mixing of seawater and sand, as well as the interstitial porewater (Gobet et al., 2012; Massana et al., 2015; Newton et al., 2013). We also observed the equivalent separation of these communities for Bacteria + Archaea and for protists (Figures 2b,c and 3a,b; Table S3). In addition, the interstitial communities of these microbial groups were also distinct from either the sand or seawater communities, representing a subset of beach microorganisms that are more likely to be dispersed by porewater flow, including Bacteria from the Alphaproteobacteria, Chlamydiae, Cyanobacteria, Firmicutes, Fusobacteria, Patescibacteria, PAUC34f and Tenericutes, Archaea from the Euryarchaeota and Thaumarchaeota, and protists from the Alveolata, Cryptophyta + Haptophyta + Centroheliozoa, and Rhizaria which were significantly enriched in the interstitial community (Figure 3a,b; Figures S2 and S4). While the microbial communities of the sand and seawater formed tight clusters in the PCoA, the interstitial microbial communities did not cluster closely together (Figure 2b,c), indicating high variation in community composition, which is consistent with the high variation in alpha diversity (Figure 2a).

In contrast to the bacterial, archaeal and protistan communities, the metazoan sand communities were more variable in composition and were not distinct from the interstitial communities. This indicates that metazoans are probably more readily released from the sand so that there are greater similarities in the composition of organisms between the interstitial and sand samples (Figures 2d and 3c; Figure S1). These results are reasonable given that sessile meiofauna are not known to exist in soft sediments.

3.5 | Beach microorganisms: Possible functions and lifestyles

The most abundant bacterial, archaeal, protistan and metazoan sequences in the sand and interstitial samples are from the Gammaproteobacteria, Euryarchaeota, Alveolata and Nematoda, respectively (Figure 3a–c). The relative abundance of major taxonomic groups, as well as differential abundance analyses of specific OTUs, are presented in more detail in Figures S2–S7, and in the Supplementary Text we also describe other general trends in the composition of taxa in the sand, interstitial and seawater communities. Here, we focus our results on taxa that provide insight into

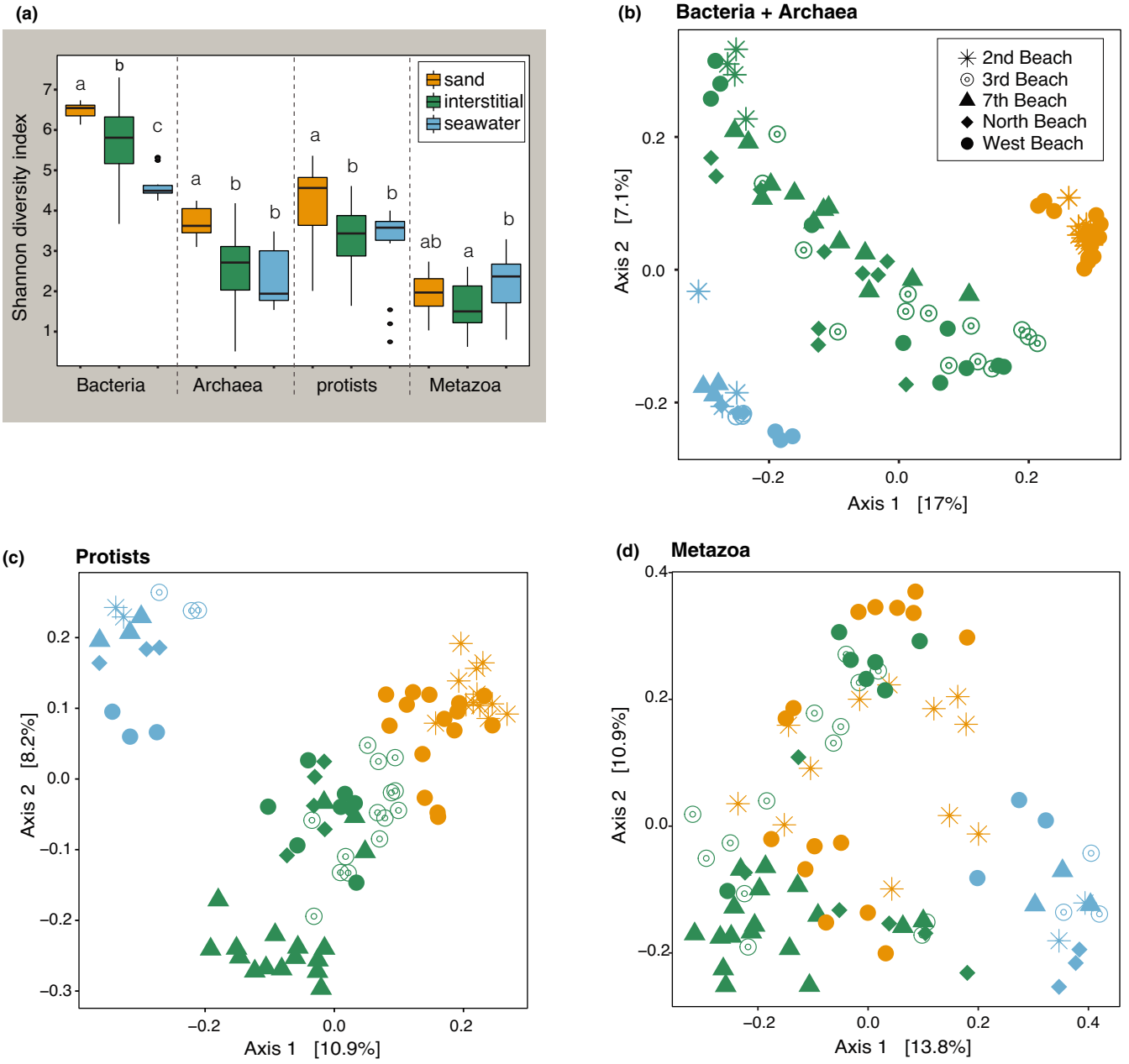


FIGURE 2 (a) Distribution of diversity, as measured by the Shannon diversity index, for different sample types and microbial groups. The solid line within the boxes is the median value. The lower and upper edges of the boxes indicate the 25% and 75% quartiles, respectively. The whiskers indicate the range of values extending at most 1.5 times the interquartile range with values outside of this range indicated by square points. Boxes are coloured based on the sample type. Different letters above the boxes indicate significantly different distributions of diversity for each type of sample based on post hoc tests ($p < .001$) for each microbial group. (b–d) Principal coordinates analysis of unweighted UniFrac distances of OTU composition. Samples are coloured based on the type of sample as in (a), and different symbols are used for each sampled beach

possible functions and ecological roles of microorganisms in beach sand.

Among the bacterial OTUs, those from the Deltaproteobacteria were significantly more abundant in the sand compared to interstitial and seawater samples, consistent with previous studies (Gobet et al., 2012; Halliday et al., 2014). Of these OTUs, 46% are from families that are known to reduce sulphur (Desulfobulbaceae, Desulfobacteraceae, Desulfarculaceae and Desulfuromonadaceae) (Figure 3a,d; Figures S2 and S3). OTUs from the Rhizobiales, an Alphaproteobacteria group comprising nitrogen-fixing symbionts with plant roots, were also significantly more abundant in sand (Figure 3d; Figure S3). Other bacteria with probable roles in the nitrogen cycle include two Nitrospira OTUs (nitrite-oxidizers) that were significantly more abundant in the sand (Figure 3d; Figure S3). In contrast, OTUs from the phylum Thaumarchaeota (ammonia-oxidizers) were generally more abundant in interstitial and seawater

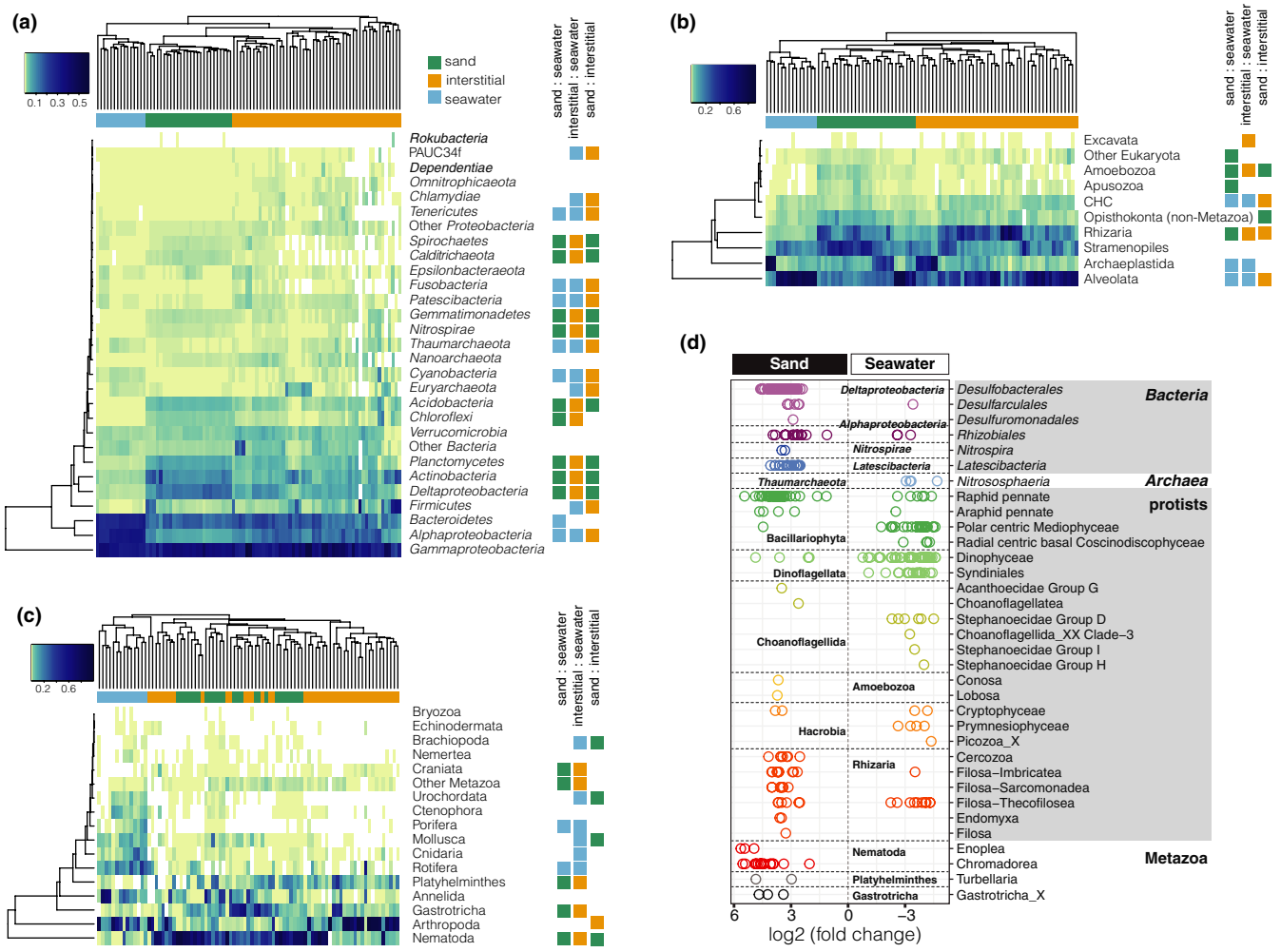


FIGURE 3 Hierarchical clustering and heat maps of OTU relative abundance for high-ranking taxonomic groups of (a) Bacteria + Archaea (16S-V4), (b) protists (18S-V4) and (c) Metazoa (18S-V4). The top graph shows clustering based on unweighted UniFrac distances of the OTU composition between samples. The left-side graph shows clustering based on the relative abundance of OTUs for each taxonomic group. On the right, coloured squares indicate taxa that were significantly higher in relative abundance in the sand, interstitial or seawater samples based on pairwise comparisons of these sample types. Samples are labelled by colour indicating the type of sample, either sand (green), interstitial (orange) or seawater (blue). These colours also indicate the sample type from which a taxon was significantly more abundant. For Bacteria + Archaea, only taxa comprising >1% of the total reads are shown. CHC = Cryptophyta + Haptophyta + Centroheliozoa. (d) Fold change in abundance of select taxa of OTUs found to be significantly different based on compositional analysis comparing sand and seawater samples. Taxa selected have known functional roles or lifestyles that may be significant in differentiating the microbial ecology of sand and seawater habitats. Positive fold changes indicate higher abundances in sand, while negative fold changes indicate higher abundances in seawater. The full list of all OTUs with significantly different abundances by habitat are shown in Figures S3, S5 and S7

samples, suggesting they are perhaps more probably dispersed by porewater (Figure 3d; Figures S2 and S3). Other possible ammonia-oxidizers, such as Nitrosomonas, were identified from the sand and interstitial samples but were not significantly different in abundance compared to seawater. Of the commonly detected Bacteroidetes that are known degraders of polysaccharides, many Bacteroidia OTUs were significantly more abundant in sand and others less abundant, indicating uncharacterized habitat preferences for these taxa (Figure S3). Among the rarer taxa, OTUs from the recently described Latescibacteria (saprophytic degraders of large cellular molecules; Farag et al., 2017) were also enriched in the sand (Figure 3d; Figure S3). Based on the typical functions of these bacterial taxa, sulphate reduction, polysaccharide degradation, nitrogen fixation and nitrite oxidation may comprise characteristic biogeochemical roles of the bacterial communities firmly associated with sand at beaches, while ammonia-oxidation is probably a characteristic process occurring in porewater. However, these and other potential functional roles need confirmation, in addition to identifying the functions of poorly known taxa which were more abundant in sand, such as the Thermoanaerobaculia (Acidobacteria), Acidimicrobiia (Actinobacteria) or Anaerolineae (Chloroflexi) (Figure S3).

In the case of protists, differences in lifestyles often correlated with the differential abundance of taxa in the sand. For example, Bacillariophyta (i.e., diatoms of the Stramenopiles), which are photoautotrophs, were abundant in all sample types, yet upon closer inspection, OTUs of pennate diatoms were significantly more

abundant in the sand, while centric diatoms were more abundant in seawater (Figure 3d; Figure S5). The former tend to glide or firmly attach to the surface of sand grains while the latter tend to be planktonic (Kooistra et al., 2009; Mitbavkar & Anil, 2002). At the supergroup taxonomic level, Amoebozoa and Apusozoa, which are mostly known to attach to and glide on surfaces, were also more abundant in the sand (Figure 3b; Figure S4) while Cryptophyta + Haptophyt a + Centroheliozoa and Rhizaria were significantly enriched in the interstitial community (Figure 3b; Figure S4), reflecting their ability to swim in porewater (Okamoto et al., 2009), but at the OTU level, several specific OTUs were more abundant in sand (Figure 3d; Figure S5). As expected, many OTUs from known planktonic taxa, such as Dinoflagellata (both Dinophyceae and Syndiniales) and choanoflagellates (including Stephanoecidae), were more abundant in the seawater (Figure 3d; Figure S5). These differences in lifestyle that are correlated with habitat probably also contribute to functional differences, but the ecological roles of many protists are often only broadly known and more detailed investigations are needed (e.g., nitrogen use or extracellular polysaccharide production of pennate vs. centric diatoms).

Very few metazoan groups were distinctly enriched solely in the sand or in the interstitial spaces, which would identify sandy beach-specific animals that tend to be either strongly attached or unattached to sand grains. Arthropoda, however, were one of the most abundant taxa in the interstitial spaces and seawater but not in whole sand (implying that they are not tightly associated with sand grains); Gastrotricha, Nematoda and Platyhelminthes were more abundant in whole sand and specifically interstitial spaces but not in seawater (Figure 3c; Figure S6). These results probably reflect the ability of these organisms to actively move between these habitats (Giere, 2009) and is consistent with our observation that the sand and interstitial communities of metazoans could not be clearly distinguished (Figures 2b–d and 3a–c). However, in detecting meiofauna using environmental DNA, the relative abundance of meiofauna may potentially be overestimated due to remnant DNA that persists after an animal has left. The enrichment of gastrotrichs, nematodes and platyhelminths in sand (either attached or in the interstitial spaces) was confirmed at the OTU level (Figure 3d; Figure S7). In addition to these differentially abundant OTUs, we uncovered a large diversity of nematodes from sand and interstitial samples (441 out of 1540 metazoan OTUs), of which 62% could not be identified to the genus level. These nematodes might be better identified by sequencing a different marker region. However, species-level identifications are possible with 18S rRNA metabarcode data (Macheriotou et al., 2019), so these unidentified OTUs either indicate the lack of closely related reference sequences in 18S rRNA databases, or they could represent new, undescribed taxa. These meiofauna, especially nematodes, are often abundant in marine sediments and contribute to the remineralization of nutrients (Nascimento et al., 2012; Schratzberger et al., 2019; Schratzberger & Ingels, 2018), and identifying them more specifically could fill a large gap in our knowledge of meiofaunal diversity and benthic ecology.

3.6 | Local variation and intertidal zonation in the sand and interstitial communities

While community differences by sample type were consistently observed across the five sampled beaches (Figures 2b–d and 3a–c), we also investigated whether microbial communities could be distinguishable on local scales, such as by beach or by intertidal location within a single beach. We compared whole sand communities at various intertidal elevations from West Beach and 2nd Beach, which are adjacent to one another, yet of contrasting beach types (relatively dissipative and reflective, respectively).

For these sand communities, we observed significant local differences for all organisms between West Beach and 2nd Beach, and with high sampling resolution across the intertidal elevations (Figure 4a–c; Table S3). As a result, for all groups of organisms, a zonation pattern is evident across the intertidal from high tide to swash zones on both beaches, with the high intertidal sand communities being particularly distinct. Community turnover is primarily and consistently correlated with moisture content as we had hypothesized; organic carbon and phosphate concentrations were also correlated though to a lesser degree (Figure 4a–c).

In comparing interstitial communities, we examined samples from North, West, 3rd and 7th beaches (most samples from 2nd Beach lacked sufficient data). For the interstitial bacteria and archaea (Figure 4d), neither local differences by beach nor by intertidal zone were clearly discerned, indicating that interstitial bacteria and archaea are more easily mixed or dispersed across the intertidal elevations, or respond similarly to abiotic changes. For interstitial protists and metazoans (Figure 4e,f), local differences (among beaches) and intertidal zonation patterns were identifiable, although not as clearly as for the sand communities. 7th Beach protist and metazoan communities were particularly distinct from the other beaches, which correlated with lower phosphate concentrations, but greater grain size and nitrate concentration (Figure 4e,f). Geographically, 7th Beach is distinct from the other beaches in that it directly opens to southerly winds and currents affecting exposure to storms, resulting in a distinct local geomorphology affecting grain size and probably the composition of dispersed organisms.

We also observed a slight difference between the metazoan and protistan communities in that the metazoan communities from mid-tide sand bar samples clustered more closely with the swash communities, while that of protists clustered with mid-tide communities. This difference suggests that the distribution of interstitial metazoans in the intertidal zone is more greatly influenced by the overlying seawater during high tide, probably due the ability of meiofauna to mobilize using tidal currents to disperse to the intertidal area; by contrast, the distribution of interstitial protists is more dependent on the abiotic conditions of the sediment at each elevation owing to their limited mobility.

From the beaches sampled in this study, we demonstrate distinct microbial communities in the mid-intertidal zone and similar microbial communities in the low tide and swash zones. By sampling the

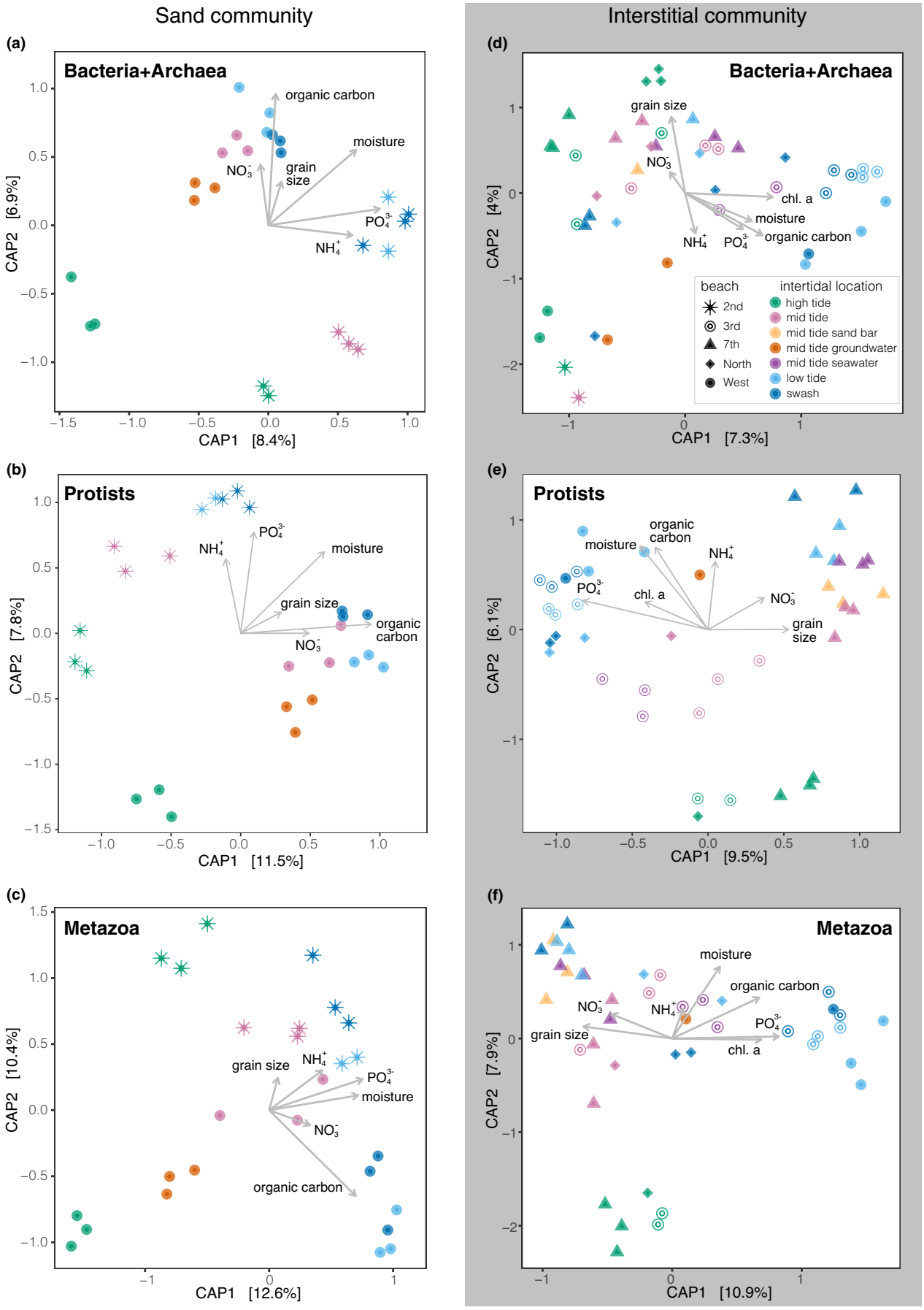


FIGURE 4 Distance-based redundancy analysis constrained by environmental variables of sand (a–c) and interstitial (d–f) samples. Using constrained analysis of principal components (CAP), (a) and (d) are biplots from Bacteria + Archaea (16S-V4), (b) and (e) are from protists (18S-V4) and (c) and (f) are from Metazoa (18S-V4) with arrows indicating the magnitude and direction of the variation in community composition based on unweighted UniFrac distances explained by each environmental variable. For all plots, point shapes indicate the sampled beach and colours indicate the intertidal location as indicated in (d). "chl. A" refers to chlorophyll *a*

intertidal zone at a finer scale, we have provided higher resolution in understanding the intertidal distribution of sandy beach microorganisms missed by previous investigations that addressed regional or local habitats at coarser scales (i.e., only comparisons of high tide sand and swash zone sand) (Cui et al., 2013; Gong et al., 2015; Halliday et al., 2014; Staley & Sadowsky, 2016). This is also the first demonstration of intertidal zonation of sandy beach protistan and meiofaunal communities based on environmental DNA sequencing.

Intertidal zonation of selected meiobenthos has also been demonstrated from other sandy beaches (Degraer et al., 1999; Gheskiere et al., 2004; Kotwicki et al., 2005; Maria et al., 2013), though based entirely on morphological identifications of taxa. Unfortunately, morphological characters are not necessarily reliable for identifying these small animals, especially to the species level, hence causing the "meiofauna paradox," where many meiofauna species (identified by morphology, which could actually be assemblages of cryptic species) have cosmopolitan distributions despite their limited reproductive and dispersive potential (Giere, 2009). By utilizing a high-throughput molecular approach, a comprehensive and consistent measure of diversity could be applied in assessing meiofaunal distribution patterns from these sandy beaches. Although our samples focused on the top 1 cm of the sediment, expanding surveys to greater depths would offer us further insight on the effect of burrowing behaviours, anoxic gradients or other depth-related factors on microbial and meiofaunal distributions.

3.7 | The development of zonation patterns with diverse high intertidal communities

The observation of zonation in the microbial communities of these mid- to high-energy beaches was surprising given the dynamic movement of sand associated with daily tidal cycles. How does this distribution pattern emerge on these sandy beaches? The zonation pattern was correlated with moisture content, but also organic carbon and phosphate concentrations. This is an interesting contrast from microbial zonation patterns known from other soft sediment intertidal environments (e.g., mangroves and sand flats), where chemical and redox gradients have been shown to be the most significant abiotic factors (Zhu et al., 2018; Zhu, Wang, Shi, Zhang, et al., 2018). In our study, however, all samples were collected from the surface sediment (top 1 cm), so anoxic layers and redox gradients were not sampled or examined. Instead, we hypothesized that greater abiotic stress in the high intertidal zone (e.g., desiccation and high heat) during the low tide period would select for microorganisms that are tolerant of these conditions (environmental filtering), which would result in a loss of diversity from low to high tide zones. This hypothesis is compatible with the fact that metabarcode sequencing was not successful for many high intertidal interstitial samples, where moisture levels were probably too low to retain an abundant interstitial microbial community (Table S2).

To further test this hypothesis, we examined the correlation of alpha diversity with the moisture content of the samples. Contrary to expectations, diversity for all groups of organisms was not significantly lower in the high intertidal samples than to wetter samples lower in the intertidal (Figure S8). In the case of protists, diversity of high intertidal samples was the highest of all the samples, but diversity was generally consistent across the intertidal zone (with the exception of mid tide groundwater seepage samples that had particularly low diversity perhaps due to the lowered salinity). These observations indicate that the distinct microbial communities from the high intertidal zones are not predominantly the result of selective culling due to extremes in abiotic factors; instead, they are the result of community turnover favouring microorganisms that thrive in the drier, hotter environment. These results corroborate results from Hawaiian and Californian beaches where dry, high tide sands were more or equally diverse in bacteria than sand in the swash zones (Cui et al., 2013; Halliday et al., 2014). In addition, our analyses did not support the intermediate disturbance hypothesis (i.e., where diversity is greatest at intermediate levels of disturbance), unlike observations from other beaches (Gingold et al., 2010; Hua et al., 2016).

In dynamic environments, the distribution of microorganisms is known to be influenced jointly by microbial growth rates and residence time (Crump et al., 2004). In the case of sand-associated communities, the residence time of the surface sand will vary across the intertidal zone, lasting up to several hours between two high tides. Because our samples were taken in mid-June, when conditions were probably favourable for rapid growth, microorganisms that preferred a particular intertidal environment may have replicated at a high rate, contributing to the observed zonation pattern. Also, although we did not observe visible biofilms on the beaches we examined, actively growing biofilm microbial communities could have stabilized the sediments and lengthened residence time beyond the tidal cycle by restricting the redistribution of sand during the low tide, and thus may have contributed to microbial zonation patterns. Biofilms are also known to help structure complex communities, even on the surface of single sand grains (Probandt et al., 2018), and might have contributed to the high diversity of organisms observed from the high intertidal elevations. Additionally, in the interstitial communities, water flow adds another degree of complexity (Boehm et al., 2014). Although it was beyond the scope of our study to inspect for biofilms and biological interactions, or to measure residence times and in situ growth rates of the microbial communities in the sand, investigation of these factors would give us further insight

into the development of local microbial distribution patterns, as well as microhabitat communities at sandy beaches (i.e., attached and interstitial communities).

3.8 | Few OTUs are strongly different in abundance between intertidal elevations

High intertidal communities are clearly distinct and diverse, and at a broad phylogenetic scale, several taxa were significantly different in relative abundance compared to low tide and swash samples (Figure 5). For example, of the prokaryotes, Fibrobacteres, Nanoarchaeota and Spirochaetes increased from high tide to the swash zone, whereas Actinobacteria and Firmicutes generally decreased (Figure 5a,d). For protists, CONTH_3 (a poorly characterized ciliate group) and a Centroheliozoa group from sand samples were higher in relative abundance from high tide elevations and then consistently decreased across the intertidal zone, but from interstitial samples Chrysophyceae (Ochrophyta), Filosa-Sarcomonadea (Cercozoa) and Filosa-Thecofilosea (Cercozoa) were the taxa that decreased (Figure 5b,e). In addition, several taxa (e.g., Bangiophyceae and Karyolectia from sand; Chrysophyceae and Endomyxa from the interstitial zone) were distinctly lower from sand bar and groundwater samples collected at mid tide.

At the OTU level, however, only a small number of OTUs, all protists, were significantly more abundant in the high intertidal communities compared to the low intertidal and swash communities (Figure S9). These include protistan OTUs that belong to Labyrinthulomycetes (heterotrophic amoeboid stramenopiles), the Protaspa lineage of Filosa-Thecofilosea (heterotrophic gliding cercozoan flagellates) and Chytridiomycetes (water fungi, known to attack diatoms). These taxa are typically specialized to live in terrestrial or saline sediments and some are known to have a cyst stage with a thick cell wall (Scholz et al., 2017; Ueda et al., 2015) that probably develops to tolerate stressful environments.

Operational taxonomic units that were more abundant in the low intertidal and swash zones compared to the high intertidal zone include diatoms (Bacillariophyta), dinoflagellates (Dinophyceae), Chlorodendrophyceae and metazoans (Figure S9). For metazoans, gastrotrichs and flatworms were significantly more abundant in the low tide and swash communities at the OTU level, including *Turbanella* (Gastrotricha) and *Parotoplana* (Platyhelminthes), as well as nematodes (Chromadorea, *Daptonema* and Oncholaimidae) (Figure 5c,f; Figure S9).

Interestingly, we did not find any bacterial or archaeal OTUs that were significantly different in abundance in the high intertidal compared to the low intertidal and swash zones even though community composition analyses indicated clear differences. The differences in community composition could be due to many OTUs with small changes in abundance that did not pass the significance threshold in the differential abundance analysis. There were also many more bacterial OTUs than for protists and metazoans so that the threshold for statistical significance is higher due to corrections for multiple testing (Love et al., 2014).

In summary, a limited number of OTUs were significantly different in abundance between samples from different intertidal locations. This is consistent with the distance-based redundancy analyses where samples clustered by intertidal location, but only a small percentage of the variation in community composition in sand was explained by the first two coordinates (Figure 4). The remaining variation in community composition is probably due to other factors beyond what were examined in this study, which have obscured the detection of significant patterns in OTU distribution based on intertidal location.

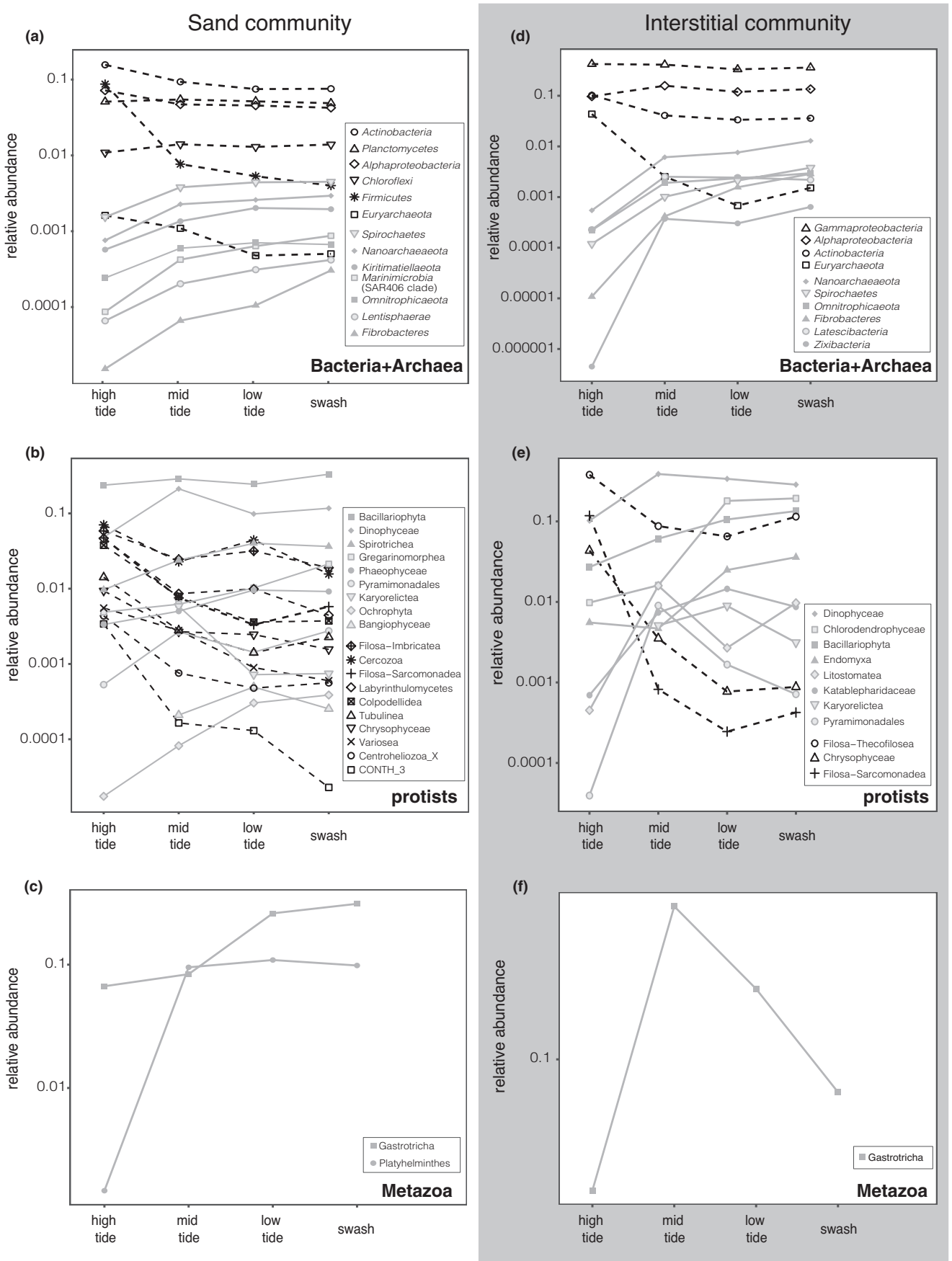
3.9 | Seasonality

To assess the consistency of intertidal zonation patterns over time, we analysed protists and metazoans from sand samples of West Beach during different seasons in 2015 and 2016. Consistent zonation of high tide and low/swash microbial communities was observed in spring (June) and summer (July) samples, and interannually. A striking difference, however, was the winter communities (January 2016), which were distinct and devoid of zonation (Figure S10).

During the winter months, microbial growth rates are slower due to shorter day lengths and lower temperatures. In addition, at the study sites, the lowest tides occur during the night so that the beaches are rarely exposed to full sunlight. Moreover, these beaches are exposed to strong storms during winter, which significantly alters beach morphology during each storm cycle. Together, these factors contribute significantly to a distinct sandy beach community in winter when zonation patterns are disrupted. However, despite disrupted zonation in winter, low tide and swash communities return to a similar composition in the summer months interannually, but mid tide and high tide communities were more distinct (Figure S10).

Although zonation patterns were evident from these beaches, further investigations are needed to assess the consistency of zonation for specific microbial taxa on sandy beaches, especially in considering the processes that re-establish zonation after disruption during the winter months, including environmental filtering,

FIGURE 5 Relative abundance of selected taxa across intertidal zones from sand (a–c) and interstitial (d–f) samples. Selected taxa are those that were significantly different in abundance when comparing high intertidal to low intertidal and swash samples at relatively high taxonomic levels. Dotted black lines and solid grey lines indicate taxa that were significantly more and significantly less abundant in high intertidal samples, respectively. Relative abundance is plotted on a log scale. To observe trends across all beaches, the plots do not include abundances from mid tide zones that were not present in all beaches (i.e., mid tide groundwater, mid tide sand bar, mid tide seawater)



residence time and biotic interactions. A significant percentage of the variation in community composition could not be attributed to intertidal location, but implying that factors unrelated to intertidal gradients, such as variations in geochemistry or stochastic effects, also need to be considered in understanding the diversity and dynamics of intertidal microbial communities (Chase & Myers, 2011; Cisneros et al., 2011; Richter-Heitman et al., 2020).

4 | CONCLUSIONS

Beach sand is dominated by microorganisms and our metabarcoding study provides a comprehensive overview of their diversity and distribution across the three domains of life from pristine sandy beaches on the central coast of British Columbia. The distribution and diversity of Bacteria, Archaea, protists and Metazoa differed, with the typically smaller and unicellular microorganisms (Bacteria, Archaea and protists) demonstrating more consistent compositions in the sand and seawater than metazoans. Although many meiofauna species are known to associate with sand grains, we infer that most meiofauna are motile or loosely associated with sand grains, so they disperse more easily between sand, interstitial spaces, and seawater across the beaches than Bacteria, Archaea and protists.

Despite tidal flow that disrupts and redistributes sand in the intertidal zone twice daily, the microorganisms and meiofauna are distributed in distinct communities that transition in composition across intertidal zones, correlating with moisture and nutrient concentrations. Although several high-level taxa were shown to contribute to this zonation pattern, only a small number of OTUs (none for Bacteria and Archaea) were recognized as differentially distributed as community composition changed across the intertidal zone. Nonetheless, by establishing that a zonation pattern exists, sandy beaches should be investigated further to elucidate the community assembly processes that shape these patterns and form these distinct microbial and meiofaunal communities.

We have shown that the distinct communities of sandy beach microorganisms exhibited different potentials for dispersal, whether attached to sand grains or carried by water within the interstitial spaces. Together, these microorganisms probably perform key biogeochemical functions in their microhabitats, but a large fraction of microbial taxa in sandy beaches are yet to be characterized at this level. Even though sandy beaches are the dominant habitat along coastlines worldwide, much remains to be discovered concerning the diversity and role of these microorganisms in the ecology and biogeochemistry of beaches.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

This study was conceived by N.O., V.T., B.S.L. and P.J.K. N.O. and V.T. designed and carried out the field and laboratory research, as well as analysed the data. V.T. and N.O. wrote the manuscript, edited by B.S.L. and P.J.K.

DATA AVAILABILITY STATEMENT

The 16S-V4 and 18S-V9 sequence data have been deposited in the European Nucleotide Archive (ENA, www.ebi.ac.uk/ena) under project accession no. PRJEB14727 with the 16S-V4 data deposited under run accession nos. ERR1519910 to ERR1520023 and 18S-V9 data deposited under run accession nos. ERR1520036 to ERR1520147. These data are also available in QIITA (qiita.ucsd.edu) under study no. 10145 as part of the Earth Microbiome Project. The 18S-V4 sequence data have been deposited in the ENA under project accession nos. PRJEB24000 (for samples collected in June 2014) and PRJEB40353 (June 2015 to January 2016).

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