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The Effects of a Typhoon on the Dynamic of Microbial Community Structure and Water Quality of the Marine Bathing Beach

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Abstract: Dalian Jinshitan beach was chosen to evaluate the impact of a typhoon on the bacterial community structure and water quality of a marine bathing beach. The concentration of enterococci was determined by the cultivation method. The bacterial community structure and abundance were analyzed using the 16S rDNA next-generation sequencing and qPCR methods. Results showed that the abundance of cultivable enterococci both in alongshore and offshore seawater increased, while it decreased in dry, wet and submerged sand. The water quality deteriorated immediately after the typhoon, and nearly recovered one month after the typhoon. The typhoon event also decreased the bacterial abundance and changed the bacterial community of the beach. Sphingomonadaceae and Rhodobacteraceae significantly increased in seawater and decreased in dry sand immediately after the typhoon. Human and other fecal taxa increased in water and sand. One month after the typhoon, the diversity and many dominant bacterial taxa nearly recovered in seawater and wet sand. Our work shows that the typhoon changed the bacterial dynamics, deteriorated the water quality and proved the transportation of bacterial taxa and input of fecal pollution between water and beach sand or land. Apart from the impact of the typhoon, the geographical location was another important factor in the changed bacterial community.

Keywords: 16S rDNA next-generation sequencing; bacterial community dynamics; water quality; enterococci; marine bathing beach; transportation

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1. Introduction

About 80% of global tourism and 50% of international tourism is located around coastal areas [1]. International travel, especially coastal tourism, has skyrocketed during the past half-century, and public health risk events caused by pathogenic microorganisms have increased year by year. Human exposure to polluted bathing beach environments by pathogenic bacteria can increase the risk of relevant gastroenteritis. The illness rate of tourists in seawater is about two times higher than in freshwater [2]. An epidemiological survey showed that considerable numbers of gastroenteritis and respiratory diseases are related to the contamination of pathogens in seawater [3]. The risk of infectious diseases caused by bacterial pollution on coastal bathing beaches has become a significant global problem [4].

Recently, reports have shown that pathogenic bacteria abundance in the beach sand is commonly much higher than in seawater [4,5]. The beach can provide shelter for

pathogens [6], resulting from moderate moisture, temperature, biofilm formation [7], and relatively few predations and competition effects [8]. Epidemiological surveys show that tourists exposed to the beach have a greater risk (20–50%) of suffering from gastrointestinal diseases compared with non-beachgoers and are significantly associated with concentrations of *Enterococci* (fecal indicator bacteria of seawater) in beach sand [9]. Furthermore, beach sand as a reservoir of pathogenic bacteria causes transmission of non-fecal origin pathogens into the seawater, which may impact water quality and public health [10].

However, the current understanding of bacterial contamination and transportation between beach sand and water is lacking. Extreme weather such as typhoon and rainstorm accelerate the interaction between beach sand and seawater interface, which provides an excellent opportunity to study the impact of beaches on seawater quality [11]. Heavy rainfall can also increase land-based pollution inputs (nutrients and fecal pathogens) [12] and change the transport pathways of fecal indicator bacteria (FIB) and pathogens [8]. Furthermore, the rapid influx of freshwater can change the bacterial community composition of beach sand and seawater, which could be a potential water quality monitoring tool [13]. High-throughput sequencing approaches have observed the restructure of an aquatic bacterial community caused by superstorm sand in stream water [12] and the bacterial restoration of a surfing beach caused by a strong typhoon event in the United States and Japan [4]. However, bacterial pollution on different beaches has pronounced geographical heterogeneity, and the reconstruction process of bacterial community structure depends on hydrological and topographic features.

In order to evaluate the effects of beach sand on the water quality in China, Dalian Jinshitan bathing beach, the largest bathing beach in north China, was chosen and a typical typhoon event was followed to (1) analyze if the pathogenic transformation happened between the beach and seawater using enterococci quantification; (2) evaluate the potential impact of the typhoon on water quality by bacterial community structure; (3) assess the restructure of normal bacterial flora in the seawater and beach.

2. Materials and Methods

2.1. Sample Collection

Jinshitan bathing beach is located in the North Yellow Sea of China, the largest and only natural light bath in northern China, and attracts nearly 30,000 to 100,000 visitors each day during peak tourist season. This beach is separate from the city of Dalian and easily impacted by typhoons. On 20 August 2018, typhoon "Wombia" of the international number 1818 passed through Dalian (CMA 25 m/s), accompanied by torrential rains (rainfall ranged from 100 to 250 mm). The survey started immediately after the typhoon (21 August 2018) and lasted nearly one month (15 September 2018). Samples were collected on 11 August as a reference before the typhoon.

The sampling site was set in the west area of the beach because there was no sewage outlet, and only the Putaogou river located upstream of this area (W3 station) was used for storm drainage. Therefore, when there was no rainfall, this beach experienced little or no influence from urbanized pollution on water quality. Three stations (W1, W2, and W3) were evenly distributed (about 650 m apart) and covered the area of routinely gathered bathers (Figure 1). During the typhoon event, two water depths were set, alongshore (AS) (0.5 m) and offshore sites (OS) (1.0 m). Three different beach sand zones were chosen: dry sand, wet sand, and submerged sand. In detail, dry sand (DS) (5–15 cm) was collected at 2 m above the high tide line (backshore sand) and wet sand (WS) (5–15 cm) from the intertidal zone (foreshore sand), while submerged sand (SS) (0–10 cm) was collected at 0.5 m water depth (nearshore sand). Four samples of the surrounding beach sand and seawater were set within a 0.5 m radius; 500 g surface sand and 5 L surface seawater (from the depth of 30 cm) were collected and mixed.

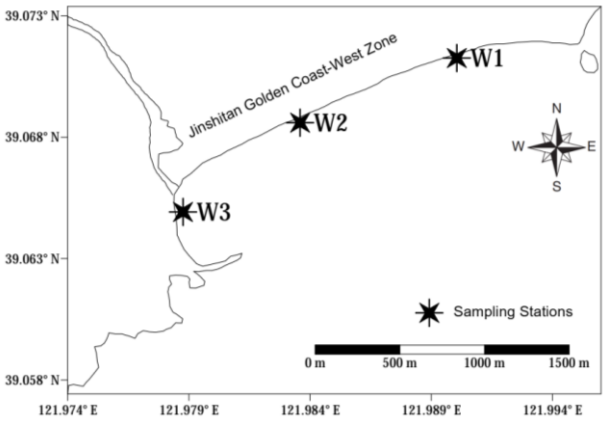


Figure 1. Sampling sites of Jinshitan Bathing Beach.

2.2. Physical, Chemical, Meteorological, and Hydrological Parameters Analysis in Water and Beach Sand

Physical, chemical, meteorological, and hydrological parameters were measured. The water temperature, dissolved oxygen, conductivity, salinity, pH, and redox potential were measured by ProQuatro Multiparameter Meter (YSI, Rye Brook, NY, USA) in situ. The sand particle size was analyzed using the LS13320 laser diffraction particle size analyzer (BECKMAN COULTER, Brea, CA, USA) according to the Youden-Windworth equal ratio φ value particle level standard, and the particle size parameters were calculated using the Fokker–Ward graphical formula. The sediment particle size was determined according to the marine monitoring code of China [14]. Ammonium nitrogen (NH_4^+), nitrate (NO_3^-) and nitrite (NO_2^-) were analyzed according to the soil monitoring specification of environment of China [15]. Total nitrogen (TN) and total phosphorus (TP) were analyzed according to previous methods [16,17]. Total organic carbon (TOC) and the water content of the sand were analyzed according to the marine monitoring specification of China [18].

2.3. Enterococci Cultivation

All seawater and beach sand samples were analyzed for cultivable enterococci by membrane filtration method according to ISO 7899–2–2000 (E) and further identified according to the patent [19]. Briefly, 10 g sand samples were mixed with 90 mL sterilized phosphate balanced solution (PBS) and filtered using a 0.45 μm nitrocellulose membrane filter, and 100 mL seawater samples were filtered directly. The filter membrane was attached to Slanetz and Bartley medium (Hopebio Biotechnology Co., Ltd., Qingdao, China) and incubated at 37 °C for 44 ± 4 h. The membrane was transferred to 44 °C pre-heated enterococci agar plate with sterile forceps and incubated at 44 °C for 2 h. Typical black or brown colony on the back of the counting plate was identified using the contact enzyme experiment, and the bacteria with a negative contact enzyme was enterococci. All samples were tested in triplicate.

2.4. 16S rRNA Gene Quantification

The bacterial abundance in seawater and beach sand samples was quantified using the qPCR method. The 16S rRNA gene V4 region and primers 515F 5′-GTGCCAGCMGCCGCGGTAA-3′ and 806R 5′-GGACTACCAGGGTATCTAAT-3′ were chosen, and a standard curve was created. The qPCR reaction system was 20 μL and consisted of 10 μL TB Green Premix, 0.4 μL upstream and downstream primers, 0.4 μL ROX II, 6.8 μL ddH_2O, 2 μL template DNA. The PCR reaction conditions were set as follows: pre-denaturation at 94 °C for 5 min, denaturation at 94 °C for 30 s, annealing at 62 °C for 30 s, extension at 72 °C for 32 s, for 30 cycles, collecting fluorescent signals.

2.5. 16S rRNA Gene Sequencing

The V4-V5 region of the 16S rRNA gene in each sample was amplified using the primers 515F: 5′-GTGCCAGCMGCCGCGG-3′ and 907R: 5′-CCGTCAATTCMTTTRAGTTT-3′. The PCR reaction solution consisted of 4 μL of 5× fastpfu buffer, 2 μL of 2.5 mmol Deoxynucleotide Triphosphates (dNTPs), 0.8 μL of 5 μm primers, 0.4 μL of FastPfu DNA Polymerase, 10 ng template DNA, and double-distilled water (ddH_2O) was supplemented to 20 μL. The PCR reaction conditions were as follows: pre-denaturation at 95 °C for 5 min, denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 45 s, 30 cycles, and final elongation at 72 °C for 10 min. The PCR products were purified using AxyPrepDNA Purification Kit (AXYGEN (Hangzhou) Biotech Co., Ltd., Hangzhou, Zhejiang, China), detected using QuantiFluor™-ST blue fluorescence quantitative system (PROMEGA, Promega (Beijing) Biotech Co., Ltd., Beijing, China), and mixed in proportion according to the sequencing requirements. The Illumina PE250 library was constructed, sequenced, and the raw sequences in seawater and beach sand samples were deposited in the Sequence Read Archive of the National Center for Biotechnology Information under the accession number srr10177426-srr10177449.

2.6. Data Analysis

The concentration of enterococci was transformed to log 10 (X + 1) to achieve normality. SPSS software V21.0 (IBM, New Orchard Road, Armonk, NY, USA) was used to process the data, including ANOVA and Pearson's correlation analysis between the different groups, where $p \leq 0.05$ was considered significant. The distribution of enterococci and fecal flora were shown using Origin 9.1 (OriginLab 2019b, Northampton, MA, USA).

Sequences with 97% similarity were clustered into a single operational taxonomic unit (OTU) using Ultra-fast sequence analysis (USEARCH) software V10 (Robert Edgar). The taxonomy of each OTU by alpha diversity indices (Chao1, Shannon, Simpson) and beta diversity was assigned against the Silva database using the Quantitative Insights into Microbial Ecology (QIIME) V1.9.1. Principal coordinate analysis (PCoA) and unweighted pair group method with non-metric multidimensional scaling (NMDS) were applied to present the distance among different samples, Redundancy analysis (RDA) was conducted to analyze the relationship between samples and environmental factors, and analysis of similarities (ANOSIM) was used to analyze the differences in the microbial community structure using the 'vegan' packages in R version v4.0.2 (R Foundation for Statistical Computing, Vienna, Austria).

Fecal flora was analyzed to evaluate the impact of the typhoon on the water quality. OTUs classified as *Bacteroidaceae*, *Porphyromonadaceae*, *Clostridiaceae*, *Lachnospiraceae*, *Ruminococceae*, *Rikenellaceae*, or *Prevotellaceae* [20,21] were considered as human fecal taxa. OTUs classified as *Erysipelotrichaceae*, *Peptostreptoococcaceae*, *Enterobacteriaceae*, *Enterococcaceae* [22] were considered other fecal taxa (OFT). Total fecal taxa were human plus other fecal taxa.

3. Results

3.1. The Physical-Chemical Factors of the Beach during the Typhoon Event

Water quality parameters, physical properties of seawater, and beach samples of Jinshitan bathing beach were shown in Tables 1 and 2. Water temperature ranged from 23.4 to 29.4 °C, DO from 6.18 to 10.26 mg/L, conductivity ranged from 27,905 to 52,546 μS/m, salinity ranged from 16.95 to 31.52, pH ranged from 7.87 to 8.22, and oxidative redox potential (ORP) ranged from 67 to 265 mV. During the typhoon event, salinity, pH, and ORP ($p < 0.05$) decreased in all three stations, and the salinity and conductivity in the W3 station changed significantly ($p < 0.01$), from 31.47 to 16.95.

Table 1. Water quality parameters and bacterial concentration of alongshore seawater.

Samples	Water Temperature (°C)	DO (mg/L)	Conductivity (μS/m)	Salinity	pH	ORP (mV)
11 August 2018 W1AS	27.7	7.97	50,798	31.43	8.09	206
21 August 2018 W1AS	25.8	6.57	48,317	30.94	7.94	149
15 September 2018 W1AS	23.7	6.46	46,530	31.07	8.08	217
11 August 2018 W1OS	27.2	8.63	50,444	31.49	8.13	265
21 August 2018 W1OS	25.7	6.30	48,265	30.96	7.91	185
15 September 2018 W1OS	23.6	6.43	46,535	31.17	8.09	210
11 August 2018 W2AS	27.0	7.40	50,122	31.40	8.03	144
21 August 2018 W2AS	25.5	6.91	46,919	30.15	7.92	125
15 September 2018 W2AS	23.6	6.31	46,650	31.20	8.07	204
11 August 2018 W2OS	26.8	7.88	50,049	31.47	8.06	244
21 August 2018 W2OS	25.5	6.95	47,163	30.29	7.93	151
15 September 2018 W2OS	23.6	6.18	46,614	31.19	8.07	207
11 August 2018 W3AS	29.4	7.57	52,546	31.46	8.02	182
21 August 2018 W3AS	25.8	7.17	27,905	16.95	7.87	67
15 September 2018 W3AS	23.7	6.60	46,528	31.05	7.99	190
11 August 2018 W3OS	29.0	10.26	52,180	31.52	8.22	213
21 August 2018 W3OS	26.2	6.67	39,792	24.72	7.89	101
15 September 2018 W3OS	23.4	6.22	46,420	31.20	8.07	205

Note: AS represented alongshore seawater; OS represented offshore seawater.

3.2. The Effect of the Typhoon Event on Water Quality According to Enterococci Concentration

Jinshitan bathing beach was not an urban beach, and there was no pollution source point in the west area except for the heavy storm. Therefore, the nutrient concentration was relatively low, as shown in Table 2. Nitrate, ammonium, total phosphorus, total nitrogen, and TOC were (0.90~2.54) $\times 10^{-3}$ μg/g, (7.34~39.20) $\times 10^{-3}$ μg/g, 0.10~0.27 μg/g, (8.13~45.90) $\times 10^{-3}$ μg/g, and (0~0.593) $\times 10^{-3}$ μg/g, respectively, with nitrite concentration below the minimum detection limit. ANOVA analysis showed that only ammonium concentration in submerged sand was significantly higher than in wet sand ($p < 0.05$). In addition, there was no significant difference in the nutrients during the typhoon event ($p > 0.05$).

There were some differences in the particle size of beach sand at different regions of the same station, and the particle size changed after the typhoon. Before the typhoon, the particle size of dry sand and submerged sand were sand. However, the particle size of wet sand was different: W1 and W2 stations were sand (>50%) and gravel (>25%), while the W3 station was gravel (>50%) and sand (>25%). Immediately after the typhoon, wet sand of W2 changed into sand (>75%); the submerged sand of W1 changed into gravel (>50%) and sand (>25%). Until one month after the typhoon, this trend did not change, except for the dry sand of the W1 station.

Table 2. Physical-chemical factors of beach sand.

Samples	Sediment Grain Size (%)				Median Size (Mdφ)	Average Particle Size (Mzφ)	Water Content (%)	NO_3^- (10^{-3} μg/g)	NH_4^+ (10^{-3} μg/g)	TP (10^{-3} μg/g)	TN (10^{-3} μg/g)	TOC (10^{-3} μg/g)
	Gravel	Sand	Silt	Clay								
11 August 2018 W1DS	0.00	99.90	0.10	0.00	0.67	0.68	0.96	1.744	15.4	140	16.7	0.059
21 August 2018 W1DS	20.05	79.95	0.00	0.00	0.32	0.05	1.19	1.668	10.8	209	12.5	0.027
15 September 2018 W1DS	0.00	99.55	0.28	0.17	0.99	1.01	0.18	2.458	9.1	185	10.7	0.057
11 August 2018 W2DS	0.00	99.50	0.29	0.21	1.17	1.22	3.22	1.693	12.5	131	13.7	0.593
21 August 2018 W2DS	0.00	99.91	0.09	0.00	0.81	0.82	1.26	1.617	13.1	103	15.8	0.210
15 September 2018 W2DS	0.00	100.00	0.00	0.00	0.94	0.94	0.02	1.234	9.7	204	10.4	0.210
11 August 2018W3DS	0.00	99.58	0.26	0.16	0.79	0.80	2.20	1.923	14.9	204	16.0	0.026
21 August 2018 W3DS	0.00	100.00	0.00	0.00	0.51	0.51	1.55	1.617	12.5	149	13.8	0.262
15 September 2018 W3DS	0.00	100.00	0.00	0.00	0.96	0.96	0.19	1.081	13.1	170	37.4	0.223
11 August 2018W1WS	24.71	75.29	0.00	0.00	−0.08	−0.23	2.02	1.744	13.1	138	14.6	0.040
21 August 2018 W1WS	0.00	100.00	0.00	0.00	0.31	0.30	15.74	1.489	10.8	189	11.1	0.044
15 September 2018 W1WS	0.00	99.60	0.23	0.17	1.10	1.17	16.86	2.535	10.8	243	13.9	0.303
11 August 2018W2WS	42.66	57.16	0.11	0.07	−0.09	−0.60	3.70	1.642	9.1	268	13.5	0.436
21 August 2018 W2WS	0.00	100.00	0.00	0.00	0.95	0.95	7.57	1.489	7.34	129	8.13	0.222
15 September 2018 W2WS	0.00	99.63	0.21	0.17	1.07	1.09	9.09	1.132	10.2	197	11.3	0.225
11 August 2018W3WS	64.75	35.25	0.00	0.00	−1.22	−1.24	4.75	1.719	12.5	242	13.2	0
21 August 2018 W3WS	0.00	100.00	0.00	0.00	0.94	0.83	9.55	1.591	10.8	221	11.2	0.241
15 September 2018 W3WS	0.00	99.67	0.18	0.16	1.06	1.06	10.89	1.336	9.1	160	19.4	0.228
11 August 2018W1SS	0.00	98.87	0.71	0.42	2.20	2.07	7.63	1.770	39.2	177	45.9	0.070
21 August 2018 W1SS	63.72	36.26	0.02	0.00	−0.97	−0.86	9.66	1.668	9.7	213	13.3	0
15 September 2018 W1SS	52.96	46.84	0.14	0.06	1.86	1.72	19.77	1.540	14.3	262	15.9	0.263
11 August 2018 W2SS	0.00	98.58	0.99	0.43	2.10	1.94	20.28	1.668	7.3	169	13.7	0.324
21 August 2018 W2SS	0.00	100.00	0.00	0.00	0.12	0.12	19.11	1.489	21.8	200	11.6	0.268
15 September 2018 W2SS	0.00	99.23	0.45	0.31	−1.04	−0.85	13.91	1.795	11.4	256	11.9	0.180
11 August 2018 W3SS	0.00	100.00	0.00	0.00	1.57	1.57	13.59	1.566	14.3	158	44.0	0.045
21 August 2018 W3SS	0.00	100.00	0.00	0.00	1.19	1.19	21.34	1.821	11.4	252	13.4	0.196
15 September 2018 W3SS	0.00	100.00	0.00	0.00	1.15	1.14	22.75	0.903	16.6	152	22.7	0.227

Note: DS represented dry sand; WS represented wet sand; SS represented submerged sand.

The abundance of cultivable enterococci was estimated to assess the microbiological water quality during the typhoon event, and the average concentrations of cultivable enterococci were 162 CFU/100 mL and 21,134 CFU/100 g in seawater and sand samples, respectively (Figure 2). However, the concentration of cultivable enterococci between alongshore and offshore seawater had no significant difference ($p > 0.05$). The concentration of cultivable enterococci in beach sand was significantly higher (10–100 fold) than in seawater samples ($p < 0.05$). The cultivable enterococci in alongshore and offshore seawater ($p < 0.01$), dry and wet sand ($p < 0.05$), submerged sand and seawater (alongshore and offshore) ($p < 0.05$), as well as submerged and wet sand ($p < 0.01$), all showed significant correlations with each other.

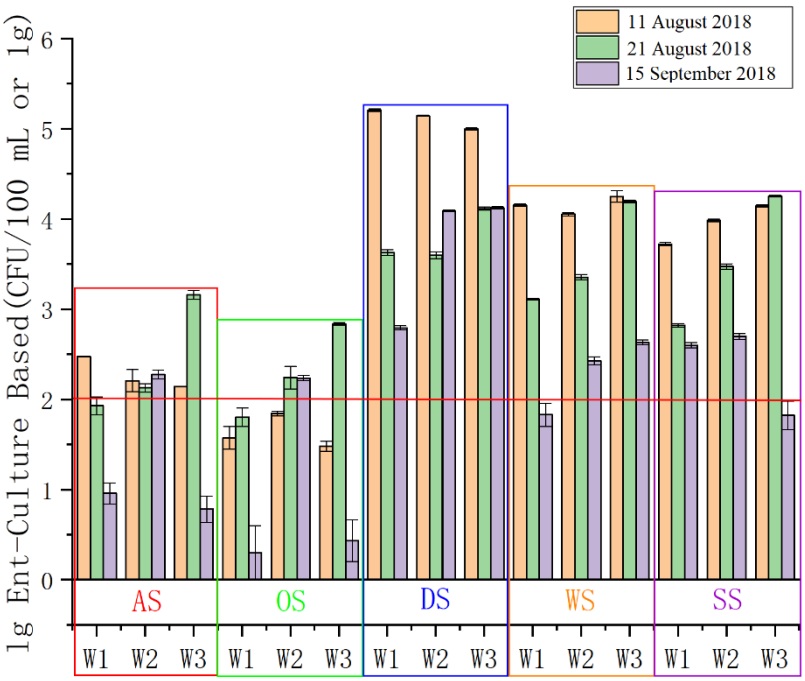


Figure 2. The concentration distribution of enterococci during the typhoon event, in which DS represents dry sand; WS represents wet sand; SS represents submerged sand; AS represents alongshore seawater; OS represents offshore seawater.

Immediately after the typhoon, the abundance of cultivable enterococci in alongshore ($p > 0.05$) and offshore seawater ($p < 0.05$) increased, while it decreased in the dry sand ($p < 0.01$), wet sand and submerged sediment samples ($p > 0.05$). In addition, its abundance in alongshore seawater of W1 and W2 stations decreased immediately after the typhoon while it significantly increased in the W3 station ($p < 0.01$). The enterococci abundances increased immediately after the typhoon in offshore seawater samples, which was also evident in the W3 station. About 33.3%, 66.7%, and 33.3% of samples exceeded the US EPA recreation criterion before, immediately after, and one month after the typhoon (>104 CFU/100 mL). However, the abundance of enterococci in dry sand, wet sand, and submerged sand decreased, except for the W3 submerged station. After one month of the typhoon, enterococci abundance decreased to lower than before the typhoon, except at the W2 seawater station.

3.3. Abundance and Diversity of the Bacterial Community on the Beach

Real-time PCR technology was used to analyze the bacterial abundance of the beach using the 16S rDNA gene. The results showed that the bacterial abundance was 3.3×10^3~8.9×10^6 copies/ mL and 2.4×10^7~3.5×10^9 copies/g in seawater and sand samples (dry sand, wet sand, and submerged sediment), respectively. The bacterial concentration in sand samples was significantly higher (100–1000 fold) than that of seawater

samples ($p < 0.01$), the same as enterococci. The bacterial concentration in seawater and sand samples decreased immediately after the typhoon, especially in the alongshore seawater and dry sand samples ($p < 0.01$). One month after the typhoon, there was some increase in the bacterial concentration, but it did not recover to the level before the typhoon (Figure S1).

Illumina sequencing was carried out on seawater and sand samples of Jinshitan bathing beach. The sequences of each sample ranged from 30,483 to 53,302. Approximately 17,182 OTUs were obtained from seawater and 76,873 OTUs from sand samples (1220 to 3536 OTUs per sample), respectively (Table S1). Alpha diversity showed that OTUs ($p < 0.01$), Chao ($p < 0.01$), and Shannon index in sand samples ($p < 0.01$) were significantly higher than in the seawater samples, and the Simpson index in beach sand was significantly lower ($p < 0.01$) (Figure 3). Alpha diversity gradually increased from the dry sand to wet sand and submerged sand ($p < 0.05$).

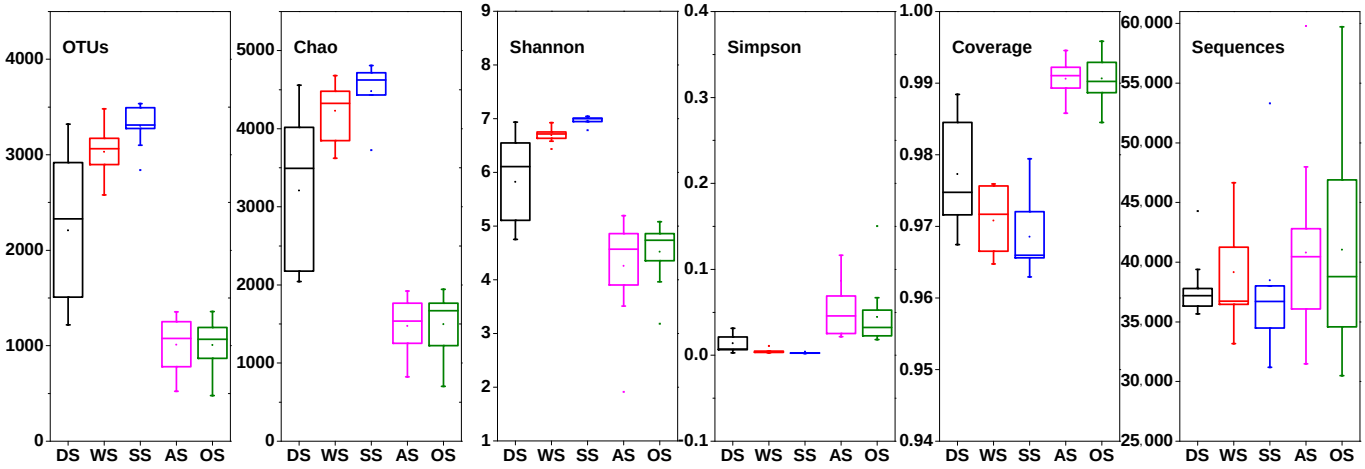


Figure 3. Boxplot of sample: α diversity in seawater and beach sand in Jinshitan bathing beach. DS represents dry sand; WS represents wet sand; SS represents submerged sand; AS represents alongshore seawater; OS represents offshore seawater.

By ANOVA, OTUs and Shannon index significantly increased in seawater samples immediately after the typhoon event ($p = 0.010, 0.024$). In contrast, the Chao index did not significantly increase ($p > 0.05$), and there was no significant change in the diversity indexes (OTUs, Shannon, and Chao index) one month after the typhoon ($p = 0.543, 0.262, 0.957$).

All alpha diversity indexes (OTUs, Chao, and Shannon) of the dry sand samples significantly increased immediately after the typhoon ($p = 0.004, 0.007, 0.026$) and then significantly decreased one month after the typhoon ($p = 0.000, 0.000, 0.002$), to even lower than before the typhoon event ($p = 0.013, 0.003, 0.042$). The OTUs and Chao indexes of wet sand samples also significantly increased immediately after the typhoon ($p = 0.011, 0.020$) but did not change after one month ($p = 0.102, 0.106$). There was no significant change in alpha diversity indexes in submerged sand before and after the typhoon ($p > 0.05$).

3.4. Bacterial Community Structure and Composition in Seawater and Sand Samples of the Bathing Beach

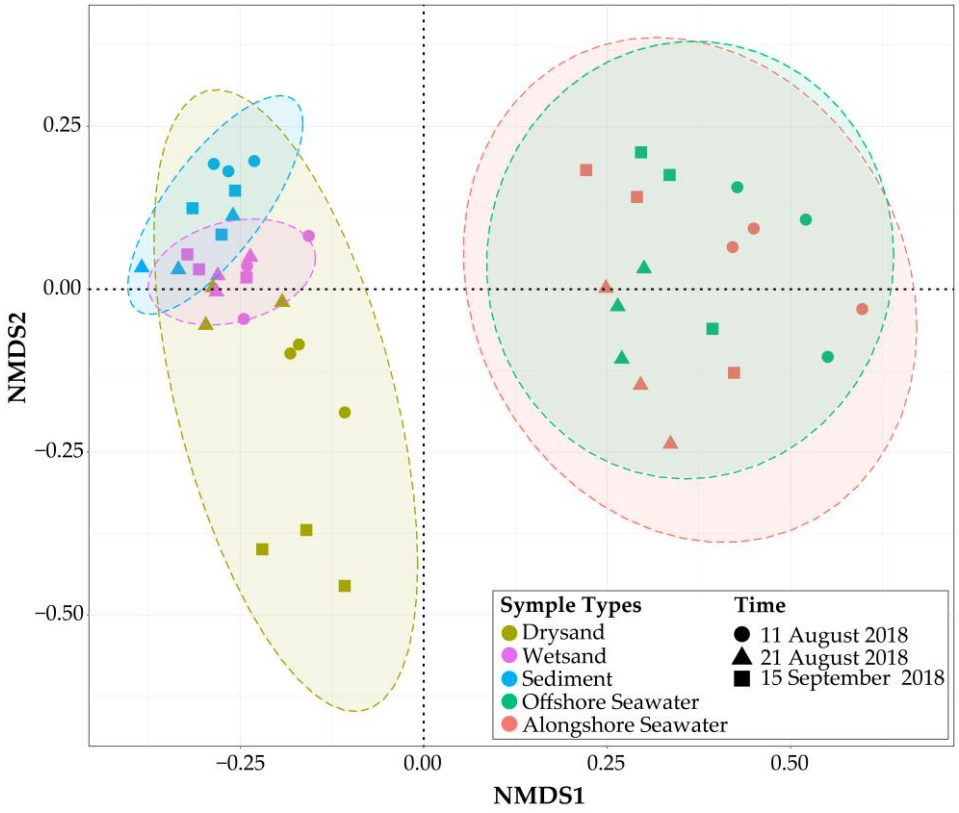
All the detected OTUs were classified into 55 phyla, 144 classes, 363 orders, 627 families, and 2256 genera. Although Proteobacteria and Bacteroidetes were dominant in seawater (accounting for 73.21% and 15.18%, respectively) and sand samples (accounting for 41.98% and 17.17%, respectively), the bacterial communities at the phylum level in seawater and sand were different (Figure S2).

NMDS also showed that the seawater samples' bacterial community was completely separated from beach sand, even experiencing the typhoon event (Figure 4a). In addition, the bacterial community structure in alongshore and offshore seawater samples was

clustered at the OTU level. ANOSIM analysis showed no difference in the bacterial communities at the phylum level between alongshore and offshore seawater samples ($p > 0.05$). In contrast, there was a significant difference between before and immediately after the typhoon ($p < 0.05$). Nearly one month after the typhoon, the bacterial community in seawater approached the cluster before the typhoon.

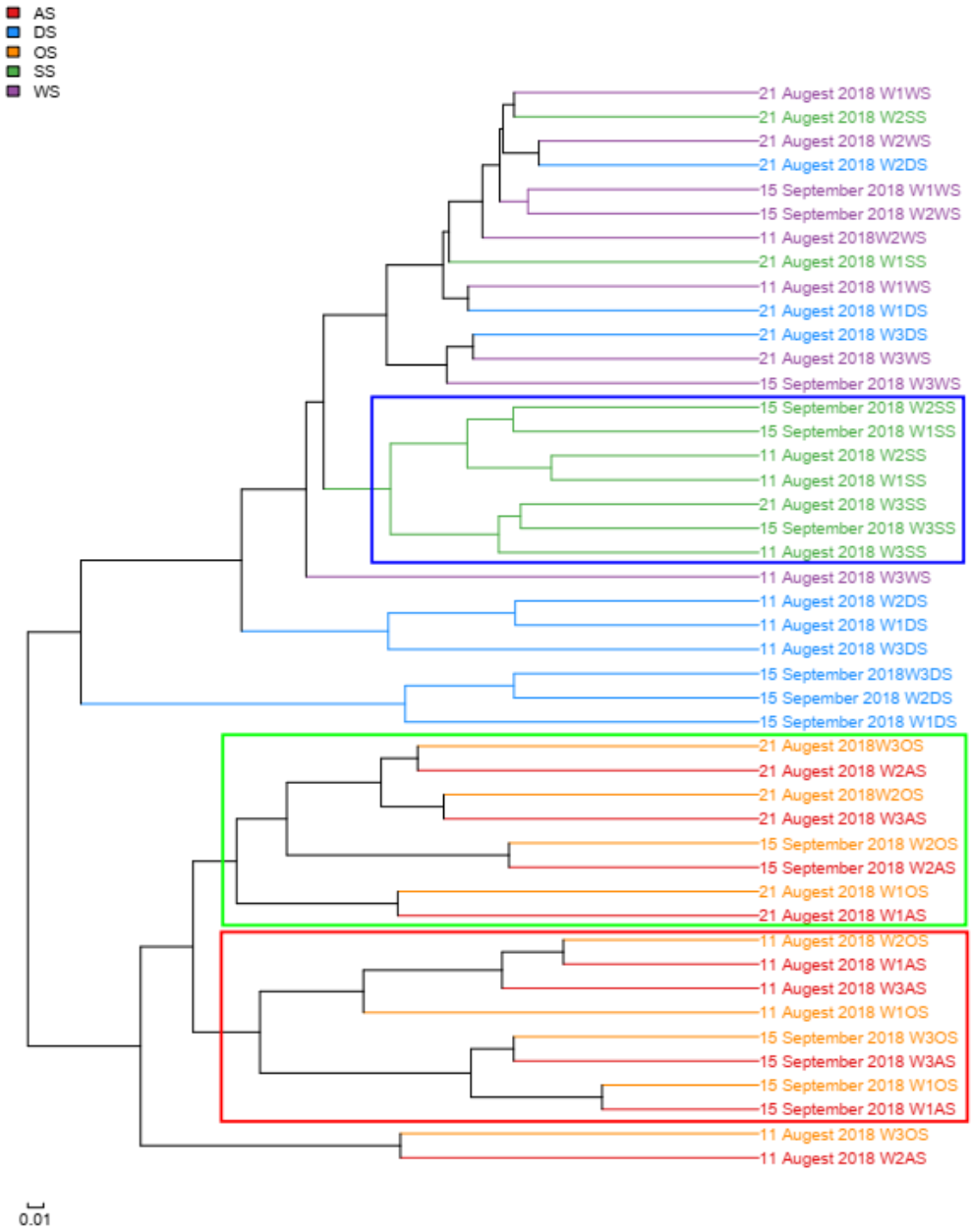
NMDS analysis also showed that most bacterial communities in the dry sand were separate from wet and submerged sand, while there was some overlap among dry sand, wet sand, and submerged sand immediately after the typhoon event (Figure 4a). ANOSIM analysis showed no difference between wet sand and submerged sand samples at the phylum level ($p > 0.05$). Furthermore, the bacterial community structure in dry sand samples was significantly different before, immediately after, and one month after the typhoon event ($p < 0.01$).

Hcluster_tree was performed to quantify the homologies, which showed that the bacterial community before (11 August 2018) and nearly one month after the typhoon (15 September 2018) clustered in seawater, while most bacterial communities at different stations clustered immediately after the typhoon (21 August 2018), except for W2 station nearly one month after the typhoon (15 September 2018). Most bacterial communities clustered immediately after the typhoon in beach sand (dry, wet, and submerged sand), concordant with the NMDS analysis. Before and one month after the typhoon, the bacterial community in subtidal sediment (SS) clustered, while this trend was not evident in dry sand (DS) and wet sand (WS) (Figure 4b).



(**a**)

Figure 4. *Cont.*



(**b**)

Figure 4. NMDS and Hcluster_tree based on weighted_unifrac_otu and bray_curtis, respectively, in Jinshitan bathing beach during typhoon events. (**a**) NMDS; (**b**) Hcluster_tree.

3.5. The Bacterial Community Structure Transition Due to the Typhoon Event

In alongshore seawater (AS), before and immediately after the typhoon, the dominant bacteria (more than 10%) did not change at the phylum level, i.e., Proteobacteria and Bacteroidetes. In offshore seawater (OS), before the typhoon, the dominant bacteria were Proteobacteria (73.1%), Bacteroidetes (11.8%), and Cyanobacteria (11.1%), while immediately after the typhoon, the dominant bacteria changed into Proteobacteria (78.4%) and Bacteroidetes (10.7%), and Cyanobacteria decreased to 2.4%. Immediately after the typhoon (21 August 2018), the dominant bacteria in the dry sand changed from Proteobacteria (46.9%) and Bacteroidetes (29.5%) to Proteobacteria (42.1%), Bacteroidetes (15.6%), Acidobacteriota (11.1%) and Planctomycetota (10.5%). In comparison, the dominant bacteria in the wet sand changed from Proteobacteria (46.0%) and Bacteroidetes (25.0%) to Proteobacteria (49.3%), Bacteroidetes (12.4%) and Planctomycetota (10.9%) (Figure 5).

Alphaproteobacteria and Gammaproteobacteria abundance fluctuated during the typhoon. Gammaproteobacteria was highest before the typhoon and further decreased from 61.0% to 44.2% in seawater, while Alphaproteobacteria increased from 15.4% to 44.2% immediately after the typhoon. In dry sand, Alphaproteobacteria decreased from 19.2% to 10.8%, while Gammaproteobacteria increased from 27.6% to 31.3%. In wet sand, Alphaproteobacteria decreased from 14.8% to 8.0%, while Gammaproteobacteria increased from 31.1% to 41.2%.

The dominant Sphingomonadaceae of the class Alphaproteobacteria significantly increased in seawater samples but significantly decreased in dry sand samples immediately after the typhoon and recovered nearly one month after the typhoon event (15 September 2018). *Rhodobacteraceae*, the largest family of Alphaproteobacteria, decreased in dry sand but increased in seawater. *Woeseiaceae* and *Marinobacteraceae* from Gammaproteobacteria increased in dry sand and wet sand, respectively. *Flavobacteriaceae* from the class Bacteroidetes, the largest family of this phylum, significantly decreased in the beach sand (dry and wet sand) and seawater samples immediately after the typhoon. *Saprospiraceae* (Bacteroidetes) also decreased. *Thermoanaerobaculaceae* from Acidobacteroita increased in dry and wet sand. All these taxa nearly returned to the former level or showed a recovery trend nearly one month after the typhoon event.

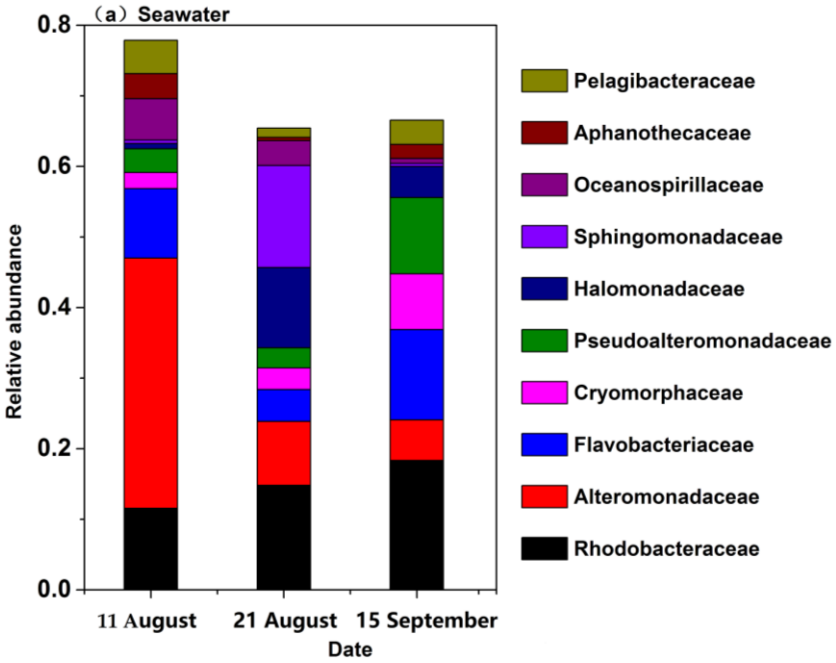


Figure 5. *Cont.*

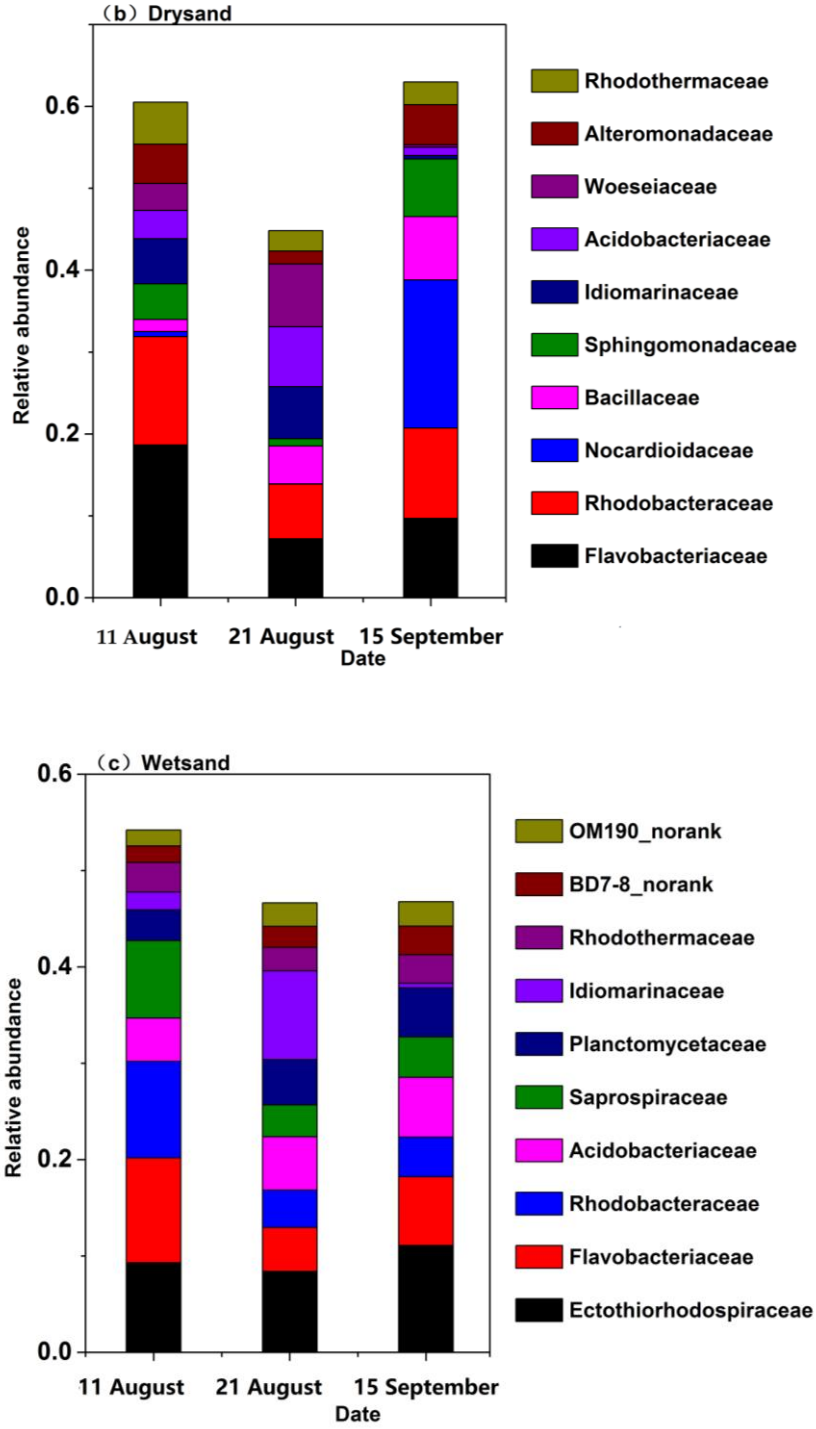


Figure 5. Changes in community structure at the family level (top 10) before and after the typhoon events in seawater (**a**), dry sand (**b**), wet sand (**c**), in which each column represents the parallel of three samples.

3.6. Fecal Contamination Based on DNA Sequencing during the Typhoon Event

The fecal taxa were analyzed to evaluate the fecal input caused by typhoon and heavy storm. Sequences associated with human fecal taxa (*Bacteroidaceae*, *Prevotellaceae*, and *Rikenellaceae*) and other fecal taxa (*Enterococcaceae* and *Enterobacteriaceae*) were found significantly more abundant immediately after the typhoon (Figure 6). Therefore, the total fecal taxa significantly increased at the family level.

The genera of *Enterococcus* was further analyzed and were not detected using high-throughput sequencing technology in most samples. *Enterococcus* was mainly detected in sand samples (1–12 OTUs), especially immediately after and one month after the typhoon. *Enterococcus* was also detected in wet sand after the typhoon event. Only 1 OTU of *Enterococcus* was detected immediately after the typhoon event, respectively, in the alongshore seawater, offshore seawater, and submerged sand samples.

3.7. The Main Environmental Factor Affecting Bacterial Community Structure

RDA was conducted to identify specific environmental drivers of community structure in seawater and sand of Jinshitan bathing beach. The detected water quality parameters explained 46.50% of the total variance (Figure 7a), and the permutation test further showed that pH ($p < 0.01$) and ORP ($p < 0.05$) had the highest correlation with microbial community structure, followed by salinity ($p > 0.05$) and water temperature ($p < 0.05$). Furthermore, total bacteria (B16S) gene abundance ($p < 0.05$) and enterococci concentration (ENT-C) ($p > 0.05$) in seawater were important biological factors for community structure variance.

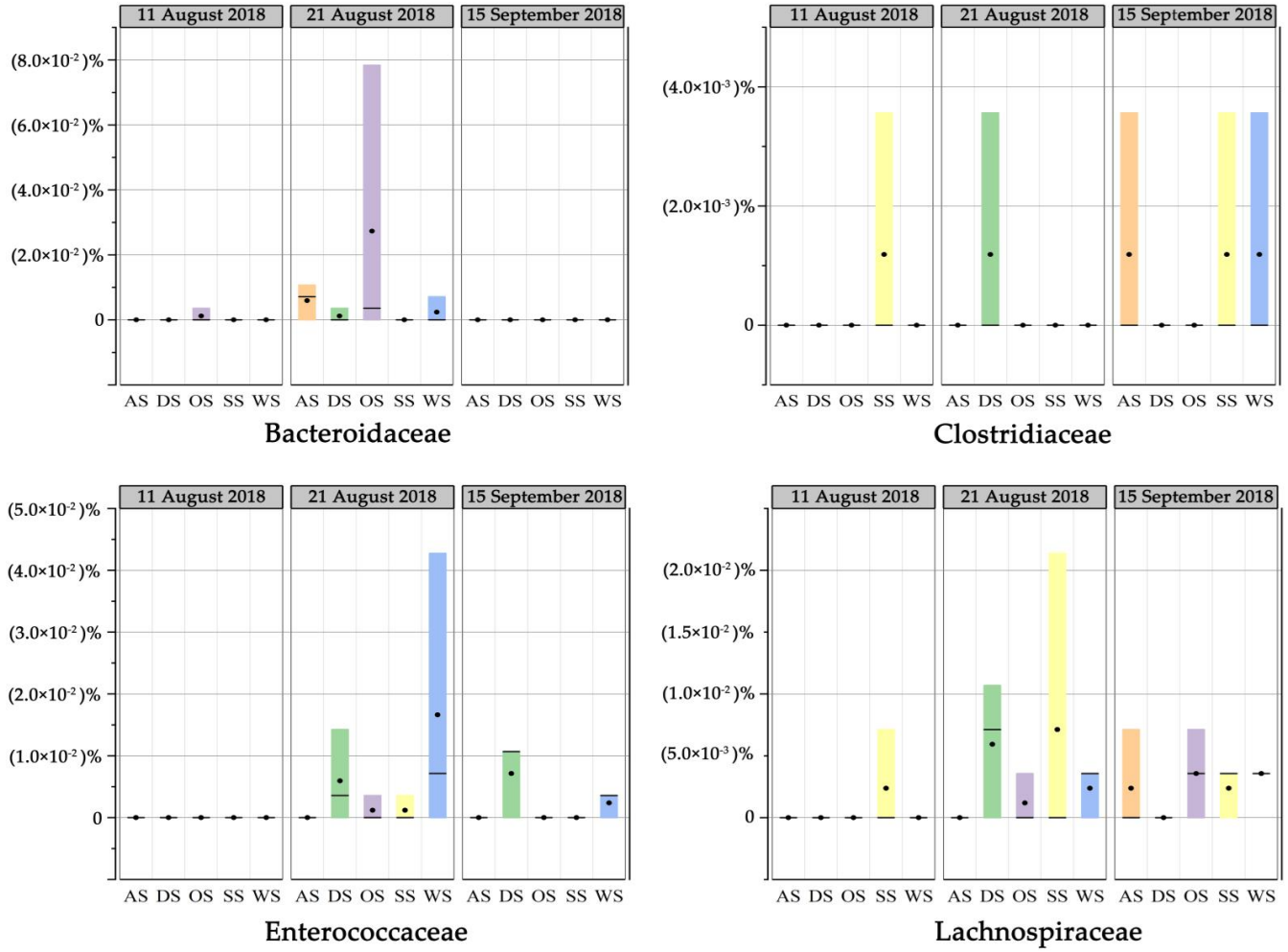


Figure 6. *Cont.*

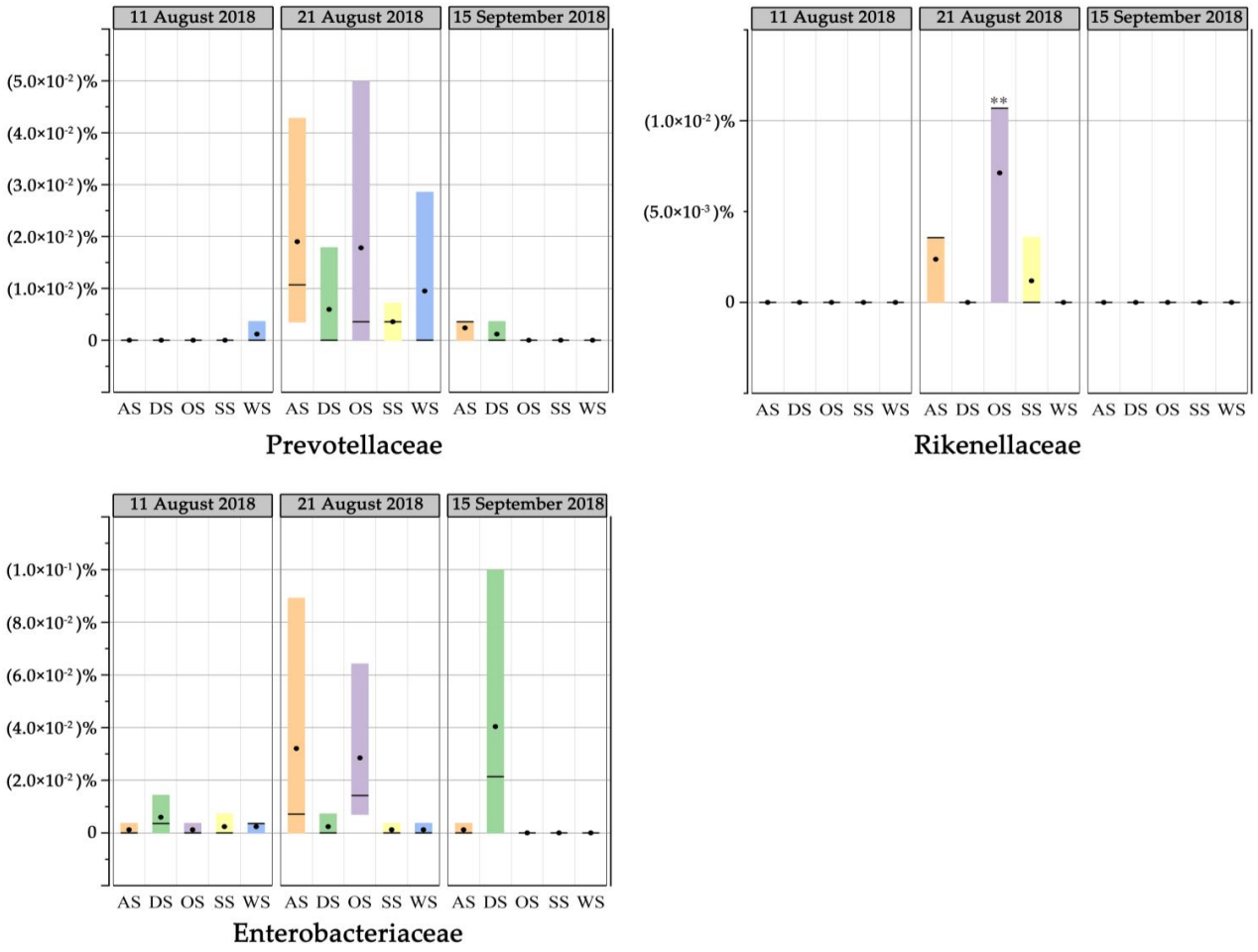
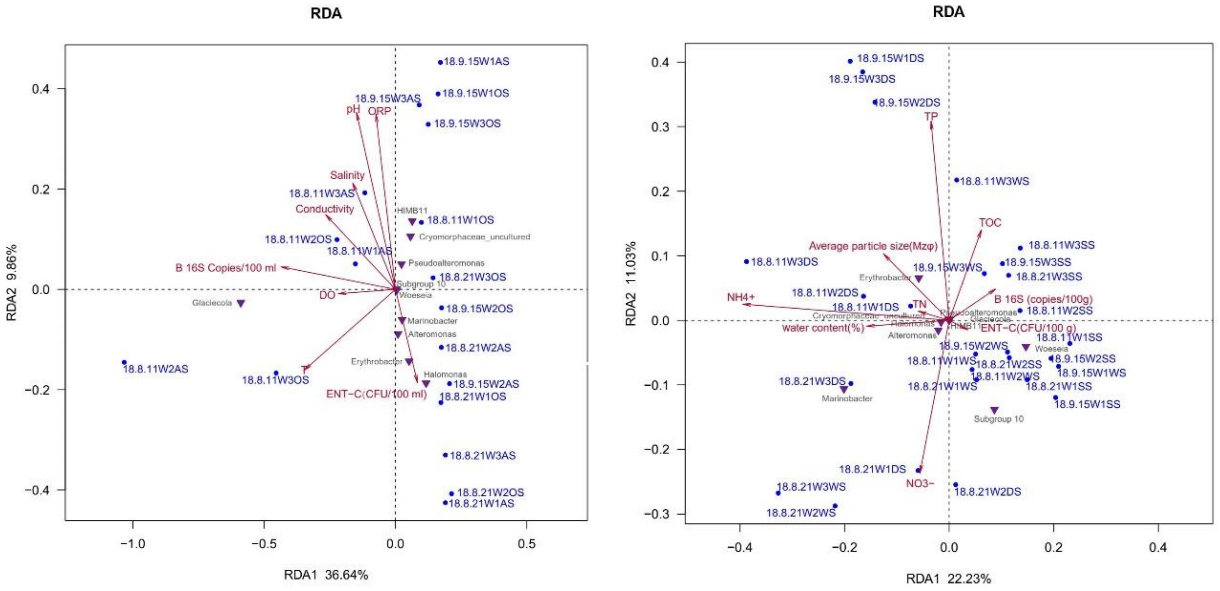


Figure 6. The percentage of sequences associated with human and other fecal taxa during the typhoon events in Jinshitan bathing beach.



(**a**) (**b**)

Figure 7. RDA showed the relationship between physical-chemical parameters and the top ten bacterial genera in Jinshitan Bathing Beach. (**a**) Seawater; (**b**) beach sand.

RDA showed that nutrient and sand particle size explained 33.26% of the total variance of the bacterial community in beach sand (Figure 7b). The permutation test showed that total nitrogen ($p > 0.05$), NH_4^+ ($p < 0.05$), and NO_3^- ($p > 0.05$) were the main factors impacting bacterial community structure, followed by TOC and sand particle size ($p > 0.05$). The abundance of total bacteria and cultivable enterococci contributed little to the bacterial community structure variance in beach sand.

4. Discussions

The impact of typhoon and heavy storm on the water quality of marine bathing beaches has not been well studied due to the apparent geographical heterogeneity of bacterial pollution. In this study, one typical typhoon event in 2018 was tracked, and the change in bacterial community structure, FIB abundance, and specific taxa of fecal pollution was evaluated in seawater and beach sand samples. Furthermore, the restructuring of the bacterial community and driven factors were also assessed.

4.1. Effects of the Typhoon on Water Quality

The different typhoon impacts on water quality were observed based on FIB of enterococci and fecal taxa. The cultivable enterococci increased in seawater (alongshore and offshore seawater) but reduced significantly in dry sand after the typhoon ($p < 0.01$). Meanwhile, exceeding ratios of recreation water quality (enterococci > 104 CFU/100 mL, US EPA criterion) doubled, from 33.33% to 66.7%. This result may prove the transportation of the enterococci from dry sand into seawater [10,23] or that they were washed deep into the sand beach caused by the surface runoff after the typhoon event. Suzuki proved that enterococci could be detected through the surface to 85 cm deep in dry sand after experiencing a typhoon [4]. Most probably, new land-based fecal pollution was transported during a rainstorm, proved by the increase in human (*Bacteroidaceae*, *Prevotellaceae*, and *Rikenellaceae*) and other fecal taxa (*Enterococcaceae* and *Enterobacteriaceae*) after the typhoon (Figure 6), and which posed a potential threat to water quality of the bathing beach and public health [24].

The impact of the typhoon on water quality was different in all stations and metrics. In general, enterococci increased during the typhoon; however, the increase was observed only in alongshore seawater of the W3 station ($p < 0.01$), while enterococci decreased in W1 and W2 stations immediately after the typhoon. This could be due to the direct input of the Tupaogou river, located upstream of the W3 station. The enterococci also had a slight increase in the sample of W3 submerged sand due to a possible sediment resuspension [25], which decreased in the beach sand (dry sand, wet sand, and submerged sand) of other stations. Correspondingly, the salinity and conductivity in the W3 station declined substantially ($p < 0.01$), while TOC in beach sand of this station increased, representing a significant influence of terrestrial nutrient flux from river to beach [26]. Rudic et al. proved the strong positive correlations between enterococci and organic matter [27]. Therefore, although the concentration of enterococci both in dry sand and wet sand of W3 station decreased due to the rainstorm, the decline was significantly less than that in W1 and W2 stations. Besides, on the same vertical gradient, the water depth of the W3 station was shallow, and the wave energy was influenced by the beach bottom slope [28]; therefore, it was more easily affected by the typhoon. Except for the input of the river and water depth, the swimmers were another impacting factor of enterococci in bathing beach. The former report showed that for swimmers exposed to seawater for 15 min, 600,000 CFU enterococci would be shed into the water [29]. Therefore, with the sharp decrease of swimmers after the typhoon, the concentration of enterococci decreased in seawater of W1 and W2 stations.

4.2. Typhoon-Induced Redistribution of Bacterial Community

Studies show that dry sand's richness and diversity in freshwater and seawater beaches are generally higher than in seawater and wet sand [30]. In the absence of severe weather changes, the community structure of dry sand is more stable than wet and submerged

sand [31]. In this study, the alpha diversity gradually increased from seawater to dry sand, wet sand, and submerged sand under the influence of typhoons. Cluster results showed that the community structure in the beach sand samples changed drastically due to the impact of the typhoon. NMDS further showed the transportation of the bacteria during the typhoon from dry sand into wet and submerged sand (Figure 4). The evidence showed that many bacterial taxa were washed into the seawater [32] or deep sand [33], which can be proved by the changed sediment particle size. The increase in the nutritional load could increase bacterial richness and diversity in the seawater [34]. Although bacteria's abundance decreased in seawater and beach sand immediately after the typhoon, richness and diversity significantly increased. The reason for the increase of bacteria richness and diversity was environmental pressure.

The change in the bacterial community during the typhoon was analyzed further. Compared with before the typhoon, Cyanobacteria decreased in seawater after the typhoon, while Acidobacteriota and Planctomycetota increased. Bacteroidetes decreased both in seawater and sand after the typhoon. Although proteobacteria were the dominant bacteria at the phylum level during the typhoon, Alphaproteobacteria and Gammaproteobacteria fluctuated. *Sphingomonadaceae* (Alphaproteobacteria), probably derived from soils and surface waters, significantly increased in seawater but decreased in dry sand immediately after the typhoon, representing the bacteria's transportation from land or sand to seawater [35]. Both *Woeseiaceae* in dry sand and *Marinobacteraceae* in wet sand within Gammaproteobacteria increased. *Flavobacteriaceae* from the pyhlum Bacteroidetes significantly decreased in the beach sand and seawater samples immediately after the typhoon, and *Saprospiraceae* within the phylum Bacteroidetes also decreased. Comparatively, *Thermoanaerobaculaceae* from Acidobacteroita, mainly sources related to freshwater hot springs and sediments [36], increased in dry and wet sand, which represented the land-based pollution.

4.3. The Recovery of Bacterial Community Structure after the Typhoon

Previous studies showed that heavy storm events significantly restructure instream bacterial communities [12,37]. Our results further confirmed the transportation or exchange of bacterial community in seawater and beach sand after the typhoon, potentially caused by sediment resuspension, surface runoff, and riverine input. Previous reports have observed the restructuring of in-stream bacterial community structure after a storm event [12], and the bacterial community of surface sand recovered within one month on one recreational beach [4]. In this work, cluster analysis showed that the bacterial community was restored in seawater and wet sand after one month of the typhoon. However, the bacterial community of dry sand did not recover. The bacterial community change in dry sand was probably impacted by other factors independent of the typhoon, such as geographical location [12].

Studies showed that the more complex the microbial diversity, the stronger its ability to resist environmental disturbance and pollution [38], thus recovering quickly [4], even if disturbed by force majeure [39]. In this study, the bacterial community in the wet sand showed the highest species richness and diversity; therefore, its bacterial community structure was stable and recovered quickly. The bacterial community in the dry sand was more easily impacted by disturbance than in wet and submerged sand; thus, the bacterial community did not recover in dry sand one month after the typhoon. NH_4^+ and NO_3^- were the main factors impacting bacterial community structure in beach sand, while there was no significant nutrient change during the typhoon event. Furthermore, the geographical location and natural properties of the sand were also the impacting factors [40]. Although the bacterial community in seawater had the lowest species richness and diversity, the bacterial community was nearly restored. The seawater possibly had a strong cacheability compared with beach sand, and the change of physical parameters such as temperature was comparably stable. In general, processes of accumulation/transportation of compounds were faster [31]. RDA analysis further showed that pH, ORP, and water temperature were the significant factors of bacterial community structure variance in seawater, followed by bacteria abundance.

5. Conclusions

(1) The concentration of enterococci in sand samples decreased while it increased in seawater, and the water quality significantly deteriorated after the typhoon and heavy storm. Human and other fecal taxa increased immediately after the typhoon caused by runoff or riverine input. The transportation of fecal bacteria was observed from sand or runoff to seawater on the bathing beach.

(2) There were significant differences in microbial community structure between seawater and beach sand samples even after the typhoon. Immediately after the typhoon, the bacterial community diversity commonly increased. One month later, the bacterial diversity in seawater and dry sand decreased, while there was a significant difference between before and one month after the typhoon in the dry sand. However, in wet or submerged sand, the diversity nearly recovered.

(3) The bacterial community in Jinshitan bathing beach changed after the typhoon. Cyanobacteria decreased in seawater, while Acidobacteriota and Planctomycetota increased. Bacteroidetes in seawater and sand decreased. However, Alphaproteobacteria and Gammaproteobacteria fluctuated during the typhoon event.

(4) The water quality parameters of seawater had a high degree of interpretation for their community structure, among which pH, ORP, and water temperature were the most significant factors. Bacteria abundance was another vital factor. NH_4^+ was the most driven factor of the bacterial community in the sand. Apart from the impact of the typhoon, the geographical location was another important factor in the changed bacterial community.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w14101631/s1, Figure S1: The concentrations of total bacteria in seawater and sand samples of Jinshitan bathing beach. Figure S2: The bacterial community at phylum level in Jinshitan bathing beach during typhoon events of 2018. Table S1: 16S rRNA gene sequencing information of bacteria in beach seawater and sand.

Author Contributions: H.M. designed experiments and wrote the manuscript; Y.W. carried out this experiment and prepared the original draft, who had the same contribution to this article as H.M.; J.S. participated in designing experiments; Y.F. participated in experiments; J.X. analyzed experimental results; T.S. revised the manuscript; K.R. was responsible for formal analysis; Y.J. participated in experiments, J.F. designed and supported the study. All authors have read and agreed to the published version of the manuscript.

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