Seasonal variations and co-occurrence networks of bacterial communities in the water and sediment of artificial habitat in Laoshan Bay, China

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18 **Abstract**

19 Marine bacteria in the seawater and seafloor are essential parts of Earth's biodiversity, as they are

20 critical participants of the global energy flow and the material cycles. However, their spatial-

21 temporal variations and potential interactions among varied biotopes in artificial habitat are

22 poorly understood. In this study, we profiled the variations of bacterial communities among

23 seasons and areas in the water and sediment of artificial reefs using 16S rRNA gene sequencing,

24 and analyzed the potential interaction patterns among microorganisms. Distinct bacterial

25 community structures in the two biotopes were exhibited. The Shannon diversity and the richness

26 of phyla in the sediment were higher, while the differences among the four seasons were more

27 evident in the water samples. The seasonal variations of bacterial communities in the water were

28 more distinct, while significant variations among four areas were only observed in the sediment.

29 Correlation analysis revealed that nitrite and mud content were the most important factors

30 influencing the abundant OTUs in the water and sediment, respectively. Potential interactions and

31 keystone species were identified based on the three co-occurrence networks. Results showed that

32 the correlations among bacterial communities in the sediment were lower than in the water.

33 Besides, the abundance of the top five abundant species and five keystone species had different

34 changing patterns among four seasons and four areas. These results enriched our understanding

35 of the microbial structures, dynamics, and interactions of microbial communities in artificial

36 habitats, which could provide new insights into planning, constructing and managing these

37 special habitats in the future.

38 **Keywords:** Marine biodiversity, Habitat degradation, Artificial reefs, Potential interaction,

39 Keystone species

41 **Introduction**

42   Global marine biodiversity is threatened by habitat degradation, environmental pollution,

43   overexploitation, and other anthropogenic disturbances (Timothy et al., 2006; Pereira et al., 2010;

44   Todd et al., 2019). The decline of global biodiversity has resulted in changes in marine ecosystem

45   structures (Verity et al., 2002). In the coastal waters, habitat loss and degradation are especially

46   tricky problems that must be faced (Jackson et al., 2001; Koldewey et al., 2010). To recover

47   marine habitats that provide feeding, spawning grounds, and shelters, artificial habitats are

48   constructed to support the functions of coastal ecosystems (Janiak and Branson, 2021). Artificial

49   reefs (ARs) offer a series of ecological services like improving marine habitats, increasing fishery

50   resources, and manipulating assemblages of ocean organisms (Seaman and Sprague, 1993; Lima

51   et al., 2019), which is an important form of artificial habitat in marine fisheries. ARs facilitate

52   biological production and enhance fishery resources by creating additional habitats for marine

53   organisms (Ng et al., 2017). At present, ARs have been deployed worldwide by creating different

54   structures related to fishing, scuba diving and coastal recreation (Lima et al., 2019). In China, the

55   practice of deploying ARs to create artificial habitats has been proposed by the government since

56   the 1970s (Sun et al., 2017). The annual investment in ARs is about 100 million dollars in recent

57   years, and its primary purposes are conserving marine environment, enhancing fishery resources,

58   and developing recreational fishing (Yang et al., 2019; Xu et al., 2021). With the increasing

59   attention to ARs, most studies have focused on the behaviors of targeted species (Williams-Grove

60   and Szedlmayer, 2017), designs and engineering (Woo et al., 2014), ecological impacts (Shin et

61   al., 2014), fishery management (Lima et al., 2020) and others (Chen et al., 2013). However, few

62   studies focused on the bacterial community structure in ARs.

63   Microorganisms in marine ecosystems play crucial roles in global biogeochemical processes,

64   such as energy flow, carbon and nutrient cycles (Steele et al., 2011; Sunagawa et al., 2015).

65   Therefore, recognizing that the functions of microbial communities are essential to master the

66   restoration mechanism of artificial habitats. Scientific attempts have been made to isolate and

67   characterize particular microbiomes to explain the ecological roles of artificial habitats in the

68   1990s (Zentgraf et al., 1992). Then, the dynamics of bacterial communities associated with coral

69   reefs (natural habitats) and artificial habitats were compared to verify the ecological effects of

70   two habitats using terminal restriction fragment length polymorphisms (Soka et al., 2011).

71   Nowadays, high-throughput sequencing has dramatically facilitated the understanding of the

72   mechanisms of marine microbial ecology (Langille et al., 2013). Despite great advancements,

73   studies paying attention to the bacterial communities in the water and sediment of ARs are

74   limited. Wang et al. (2019c) assessed the impact of ARs on bacterial communities in the sediment

75   to reveal the changes of microbial structure and functions. Qin et al. (2019) and Zhu et al. (2020)

76   studied the shifts of community dynamics and interaction patterns of the protists in Daya Bay

77   after the deployment of ARs.

78   Microbial community dynamics have been observed at different time scales, from days

79   (Mangot et al., 2013), weeks (Berdjeb et al., 2018), months (Marquardt et al., 2016) to seasons

80   (Genitsaris et al., 2015), and years (Boras et al., 2010). Apart from seasonality, habitats are

81   important factors that influence microbial communities (Sun et al., 2019), especially between

82   water and sediment (Abia et al., 2017; Liu et al., 2018). After profiling the structures and

83   dynamics of microbial communities, co-occurrence network is a powerful tool to uncover the

84   potential ecological interactions among microorganisms (Barberan et al., 2012), and have been

85   applied to obtain a more integrated understanding of microbial communities (Mikhailov et al.,

86   2019; Zhang et al., 2020a).

87   Laoshan Bay is a representative semi-enclosed bay in northern China, with an area of

88   approximately 188 km^2. Laoshan Bay is an important marine culture area for sea cucumber

89   (*Apostichopus japonicus*) and oyster (*Crassostrea gigas*), and is a stock enhancement area for

90   shrimp and fish species (Sheng et al., 2018). The annual mean seawater temperature is

91   approximately 15°C and has significant seasonal changes (5°C-25°C; Wang et al., 2019b).

92   Fishery resources in Laoshan Bay have decreased rapidly because of habitat degradation and

93   overexploitation. To help reproductive success and support recruitment, two types of ARs were

94   deployed since 2005: (1) rock reefs were deployed with mean volumes of at least 0.04 m^3 (weight

95   100 kg); (2) concrete reefs were arranged with principal dimensions of 2 m × 2 m × 2 m.

96   For decades, ARs have been deployed worldwide around coasts to recover marine habitats, and

97   the ecological effects of ARs have been evaluated through periphytons, plankton, benthos, and

98   nekton (Aleksandrov et al., 2002; Scott et al., 2015; Ng et al., 2017; Chen et al., 2019). However,

99   few studies have examined the microbial communities. This study elucidated the bacterial

100   communities in the water and sediment of artificial habitat with two types of ARs using 16S

101   rRNA gene sequencing. The major objectives were: (1) to provide a comprehensive

102   understanding of the bacterial community structures and dynamics in the water and sediment of

103   ARs, (2) to reveal the influence of environmental factors on bacterial communities in the water

104   and sediment, respectively, and (3) to analyze the potential interactions among bacteria and

105   identify the keystone taxa in the ARs.

106

Materials and Methods

Study sites and sample collection

109   Triplicate water and sediment samples were collected from Laoshan Bay ARs in January

110   (winter), May (spring), August (summer), and November (autumn), 2020 (Fig. S1). Ninety-six

111   samples from four sampling areas were studied: rock reefs (RR), transition areas (TA), concrete

112   reefs (CR) and adjacent areas (AA). For the water samples, we used a plexiglass to collect 2 L

113   bottom water at every sampling area. For the sediment samples, grab sediment sampler was

114   applied to get about 1 kg surface sediment (0-10 cm) for analysis. Water and sediment samples

115   were stored at a cooler filled with ice and immediately transported back to the laboratory in two

116   hours. The methods for treating samples were followed as previously described in Fang et al.

117   (2021). All field experiment was permitted by "Measures for annual evaluation and

118   reexamination of national marine ranching demonstration areas", which was promulgated by

119   Ministry of agriculture and rural areas of China.

120   **Measurements of environmental factors**

121   The temperature (Temp), dissolved oxygen (DO), chlorophyll-a (Chla), sampling depth (Dep),

122   pH and salinity (Sal) of water samples were measured in situ using a YSI PRODSS multi-

123   parameter water quality analyzer (YSI, USA). Transparency (Trans) was obtained by a Secchi

124   disk. The turbidity (Turb) of each area was determined by a Turb 430 IR (Xylem Analytics,

125   Germany). The determination of the total organic carbon (TOC) was performed through a TOC-L

126   series total organic carbon analyzer (Shimadzu, Japan). Eight water environmental factor,

127   including suspended particulate materials (SPM), particulate organic matter (POM), chemical

128   oxygen demand (COD), ammonium (NH_4-N), nitrate (NO_3-N), nitrite (NO_2-N), active silicate

129   (SiO_3) and active phosphate (PO_4) were analyzed under the guidance of GB/T 12763-2007 (State

130   Bureau of Quality and Technical Supervision of China, 2007).

131   For the measurement of sediment characteristics, Mastersizer 3000 (Malvern, UK) was applied

132   to measure the mean particle sizes (Par). Sediment bulk density (BD) was defined as the dry

133   weight of the sediment (drying at 105°C for 72 h) divided by the wet volumes. Water content

134   (WC) was the weight proportion of water in the sediment (drying at 70°C for 72 h). After burning

135   the sediment to ash totally (550°C for 4 h), organic matter content (OM) was obtained. The

136   method for mud content (MC, dry sediment) was following Eleftheriou (2013). As for the salinity

137   (Sal), electrical conductivity (EC) and pH of the sediment, a mixture of sediment and deionized

138   water (2:5, w/v) was used.

139   **DNA extraction, PCR amplification, Illumina MiSeq sequencing and Sequence analysis**

140   Genomic DNA of the whole samples was extracted using the FastDNA® SPIN Kit for Soil (MP

141   Biomedicals, Irvine, CA, USA) according to the manufacturer's instructions. Specifically, the

142   universal primers 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-

143   GGACTACHVGGGTWTCTAAT-3′) were used, which amplified 468 bp in V3-V4

144   hypervariable region of 16S rRNA gene. The procedures of the PCR amplification was based on

145   the manufacturer's instructions and the standard protocols of Majorbio Bio-Pharm Technology

146   Co. Ltd. (Shanghai, China). Raw sequence reads were analyzed using QIIME 1.9 (Caporaso et

147   al., 2010). The detailed processes were all described in Supp. File 1. The sequencing data have

148   been deposited in the National Center for Biotechnology Information (NCBI) Sequence Read

149   Archive database under the accession number PRJNA725051.

150   **Statistical analysis**

151   The number of OTUs and alpha diversity estimators (Shannon, Simpson, Ace, Chao 1 and

152   Good's coverage) were analyzed among four seasons and two habitats. The seasonal and spatial

153   variations of bacterial community compositions were compared by principal coordinate analysis

154   (PCoA) based on the Bray-Curtis distance matrix (Borcard et al., 2011). The effects of the

155   temporal and spatial factors on the bacterial communities were tested using permutation

156   multivariate analysis of variance (PERMANOVA) and analysis of similarity (ANOSIM)

157   (Anderson, 2001; Clarke, 1993). Statistical differences were analyzed using the Kruskal-Wallis

158   test, and the differences were considered significant at $P < 0.05$. The correlations between the

159   most abundant OTUs (relative abundance > 0.5%) and environmental factors, sampling areas

160   were determined by Spearman correlation analysis, and the visualization was achieved by

161   heatmaps (Borcard et al., 2011). The impacts of environmental factors on bacterial communities

162   were evaluated by the Mantel test and partial Mantel test (Smouse et al., 1986). Statistical

163   analyses were conducted in R 4.0.2 using the "*phyloseq*" and "*vegan*" packages (www.r-

164   project.org).

165   The visualization of the three co-occurrence networks was built using Cytoscape 3.8.2

166   (www.cytoscape.org) for the all bacterial communities in the water, sediment and both two

167 habitats. To improve the visibility and sensitivity of the networks, OTUs observed in more than

168 50% of all samples and the mean relative abundance higher than 0.5‰ were selected. Then, we

169 calculated the Spearman correlations and significances between OTUs in R. Significant edges (P

170 < 0.001) with high correlations ($|r| > 0.8$) were chose to construct the co-occurrence networks.

171 The network topology parameters were calculated using the Analyze Network plugin of

172 Cytoscape to identify the keystone OTUs in the networks (Cheung et al., 2018). Three topology

173 parameters were: (1) degree: the number of edges that a node has; (2) betweenness centrality: the

174 number of shortest paths between any two nodes in the graph passing through a node; (3)

175 closeness centrality: the average distance of a node to any other node. After that, min-max scaling

176 was applied to standardize degree, 1-betweenness centrality, and closeness centrality,

177 respectively. Last, keystoneness was defined as the average of degree, 1-betweenness centrality,

178 and closeness centrality after min-max scaling was calculated (Berry and Widder, 2014); and the

179 top 10 OTUs with highest scores were identified as the keystone species (Cheung et al., 2018).

180 Jaccard index was applied to analyze the similarities of OTUs among networks (Dmitry et al.,

181 2016).

182

183 **Results**

184 **Bacterial alpha diversities and community compositions**

185 A total of 5,427,513 high-quality bacterial 16S rRNA gene sequences and 17,207 OTUs with

186 97% similarity levels were identified from 96 water and sediment samples. Sediment samples

187 (15,010 OTUs) had higher OTUs than water samples (9,423 OTUs), and 7,226 OTUs were

188 shared between the two habitats. The Good's coverage was over 90% in all samples, indicating

189 the current sequencing depth was sufficient for this study. Alpha diversity indices showed

190 significant variations between the habitats and seasons (Table 1), while there were no differences

191 among four areas. The diversity indices (Shannon, Chao 1 and Ace) of water samples were

192 significantly lower than sediment, while the diversity indices (Simpson and Good's coverage) of

193 water were higher (Table 1). A seasonal pattern of alpha diversity indices (e.g., Shannon) could

194 be observed in the water samples, which varied from 4.09 to 5.16 (Fig. 1). However, no

195 significant seasonal differences were found in the sediment except for the Simpson diversity

196 index (Table 1).

197   Seasonal variations of the abundant phyla in the water were more evident than sediment, but

198 the differences among the four areas were low. The total abundance of the three most abundant

199 phyla was over 74% and around 50% for water and sediment samples, respectively. For the water

200 samples, the three most abundant phyla (> 10%) were Proteobacteria, Cyanobacteria, and

201 Actinobacteria, with mean relative abundances of 42.8%, 18.3% and 13.1%, respectively (Fig. 2).

202 Marked seasonal changes were observed in many bacterial phyla. The phylum Proteobacteria had

203 a relative abundance greater than 56.8% in autumn, while its abundance was 23.6% in summer;

204 the phyla Actinobacteria and Cyanobacteria had the highest abundances in summer (20.9% and

205 29.4%) and the lowest abundances in winter (6.6% and 13.2%). In the sediment, the bacterial

206 community was dominated (> 10%) by the phyla Proteobacteria, Desulfobacterota, and

207 Acidobacteria, which comprised approximately 28.4%, 10.8%, and 10.2% of the relative

208 abundances, respectively (Fig. 2). The relative abundance of the phylum Chloroflexi (mean

209 abundance was 8.6%, ranked 4th) had marked seasonal changes, whose abundance in autumn

210 (12.0%) was twice that in winter (6.7%).

211 **Bacterial community structure**

212 Two different bacterial groups were observed between the water and sediment samples (Fig. 3;

213 Table 2). Significant seasonal differences were both observed for the water and sediment

214 samples, and the bacterial communities in the water samples were more divergent and separated

215 than sediment (Fig. S2). The bacterial communities in the sediment varied among seasons, areas,

216 and their interactions based on PERMANOVA and ANOSIM (Table 2). However, there was no

217   significant difference among the four areas in the water. The Bray-Curtis dissimilarities among

218   four areas had similar seasonal changing trends in the two habitats; and the dissimilarities were

219   highest in autumn and lowest in summer (Fig. 4). The top 39 abundant water OTUs and 24

220   sediment OTUs (> 5‰), which affiliated to six and thirteen bacterial classes, showed varying

221   abundances among four areas (Fig. S3). According to the results of cluster trees, bacterial

222   communities in the areas RR and TA were different from areas CR and AA in the water samples

223   (Fig. S3a); communities in the area CR was separate from other three areas in the sediment (Fig.

224   S3b).

225   **Relationships between abundant OTUs and environmental factors**

226   The relationships among bacterial communities and environmental factors were significant both

227   in the water and sediment samples based on the Mantel test (Table 3, Table S1). For the water

228   samples, NO_2-N (r = 0.414) was the most correlating factor in shaping the community; Temp,

229   Trans, PO_4, DO, SiO_3, and COD were significant correlating factors. In the sediment, MC (r =

230   0.125) was the only factor that significantly impacted the bacterial community. The relative

231   abundances of the top abundant OTUs were noticeably related to the environmental factors (Fig.

232   5). For the water samples, thirty-three OTUs were significantly correlated with Temp, while only

233   two OTUs were related to Chla (Fig. 5a). For the sediment samples, thirteen OTUs were highly

234   related to EC, and four OTUs were related to WC and OM (Fig. 5b). Also, two bacterial groups

235   were observed that responded to the environmental factors, conversely. For example, class

236   Gammaproteobacteria and class Alphaproteobacteria were divided into two groups for the

237   sediment samples, correspondingly.

238   **Co-occurrence networks and keystone species**

239   One co-occurrence network containing total bacteria both in the water and sediment, and two

240   networks for the communities in the water and sediment, were constructed to analyze the

241   connections, stability, and complexity of bacterial communities in the ARs (Fig. 6; Fig. S4 and

242   Fig. S5). For the total bacteria network, 185 nodes belonging to 18 phyla and 6,832 edges were

243   detected. A complete distinction of nodes from water and sediment samples formed two modules

244   (Fig. 6a). The most abundant OTUs (top five OTUs with highest abundance) in the network were

245   OTU18562 (Firmicutes), OTU18460 (Cyanobacteria), OTU10090 (Proteobacteria), OTU17592

246   (Proteobacteria), and OTU18751 (Actinobacteria). Four OTUs mainly from water samples

247   exhibited seasonal variations in their abundances; OTU10090 was from sediment samples, which

248   had no seasonal change (Fig. 7a). Differences of five abundant OTUs among four areas were not

249   significant except OTU18562 (Fig. 8a). The bacterial network for the water samples comprised

250   189 nodes belonging to 15 phyla, while only 1,772 edges were observed (Fig. S4). OTU18562

251   (Firmicutes), OTU14763 (Cyanobacteria), OTU17592 (Proteobacteria), OTU18751

252   (Actinobacteriota), and OTU18053 (Proteobacteria) had the highest abundances in the network.

253   Similar seasonal changes of OTU18562 and OTU17592 in abundance were observed, and the

254   other three OTUs showed similar trends (Fig. 7b). OTU18562 showed a most apparent difference

255   among four areas compared with other four abundant OTUs (Fig. 8b). For the sediment bacterial

256   network, 274 nodes belonging to 24 phyla and 1,166 edges were observed (Fig. S5). The top five

257   most abundant OTUs in this network were OTU10090 (Proteobacteria), OTU3621

258   (Desulfobacterota), OTU13814 (Myxococcota), OTU13137 (Desulfobacterota), and OTU6550

259   (Chloroflexi). The seasonal variations of the five OTUs were not evident (Fig. 7c), and

260   OTU10090 showed lower abundances in areas RR and CR than in TA and AA (Fig. 8c).

261   The top keystone OTUs with relative abundances higher than 1‰ were identified as the

262   keystone taxa in the co-occurrence networks. In the total bacterial co-occurrence network, the top

263   10 keystone OTUs were affiliated to five phyla, Myxococcota, Proteobacteria,

264   Gemmatimonadota, Chloroflexi and Actinobacteriota (Table S2a). The keystone OTU with

265   highest keystoneness was OTU13814 (Myxococcota, keystoneness = 0.840), which ranked 15

266   among all OTUs with a relative abundance of 12.3‰; the abundance of OTU12321

267   (Proteobacteria, keystoneness = 0.836) ranked 150 with a relative abundance of 1.8‰. There was

268   no significant seasonal abundance variation for the top five keystone OTUs except OTU13814,

269   and no evident differences among the four areas were found (Fig. S6a, S7a). For the water

270   network, the top 10 keystone OTUs included members of the phyla Bacteroidota, Proteobacteria,

271   Desulfobacterota, Gemmatimonadota and Acidobacteriota (Table S2a). The top two keystone

272   OTUs (OTU525 and OTU7969) had abundances of 2.3‰ (ranked 90) and 1.4‰ (ranked 123),

273   respectively. Similar seasonal trends of the abundance for the top five keystone OTUs were

274   observed, which showed higher abundance in spring and autumn (Fig. S6b). For the sediment

275   network, the top 10 keystone OTUs belonged to three phyla, Proteobacteria, Firmicutes and

276   Chloroflexi (Table S2c). OTU6991 (abundance 2.3‰, ranked 114) and OTU3902 (abundance

277   8.8‰, ranked 24) were the only two keystone OTU with keystoneness greater than 0.7. The

278   changing patterns among the four seasons and four areas for keystone OTU17592, OTU16870,

279   and OTU17616 were consistent (Fig. S6c and Fig. S7c).

280

281   **Discussion**

282   **Bacterial community characteristics in the water and sediment of ARs**

283   The distributions of the marine bacterial communities were highly of indigenous and specific,

284   while the abundant taxa that represented in the communities were analogous (Pommier et al.,

285   2007). In this study, Proteobacteria, Cyanobacteria, and Actinobacteria were the most abundant

286   phyla, with a total relative abundance of 74% in the water samples (Fig. 2), which was consistent

287   with previous studies in coastal waters (Lee and Eom, 2016; Ye et al., 2016). For the differences

288   of bacterial communities between habitats, sixteen shared phyla were observed between the water

289   and sediment, and five phyla among sixteen phyla had higher relative abundances in the water

290   than those in the sediment. The absolute predominance of the phylum Proteobacteria in the water

291   samples may be the main reason. Because of the higher abundance of Proteobacteria (42.8%) in

292 the water samples, significantly restricting the population size of the other bacterial phyla (e.g.,

293 Sun et al., 2019). Higher diversities of the bacterial communities were observed in the sediment

294 than water (Fig. 1; Table 1), which was in accordance with the results of Feng et al. (2009) and

295 Abia et al. (2017). Some reasonable assumptions were proposed. Ye et al. (2009) stated that

296 sediments within aquatic environments formed more complex environments, which resulted in

297 more prosperous bacterial communities; Perkins et al. (2014) noted that sediment provided

298 shelters for bacteria, which helped to defend against the impacts of predation and sunlight; Liu et

299 al. (2018) explained that lower concentration of suspended sediment in the water would lead to

300 lower microbial diversity.

301   The heatmap of the abundant OTUs with four areas revealed that bacterial abundance in RR

302 was inconsistent with other areas, especially in the sediment (Fig. S3). Many studies showed that

303 the diet preference of *A. japonicus* can change the microbial communities in the sediment (e.g.,

304 Zhao et al., 2020). Thus, we assumed that the aquaculture activities of *A. japonicus* in RR might

305 result in these distinctions. Besides, rock reefs were deployed more intensive than concrete reefs,

306 changing the substrate and flow field surrounding ARs more significantly (e.g. Tang et al., 2022),

307 which might impact the bacterial communities strongly. As for the differences of bacterial

308 abundance among four areas, some inconsistencies were observed. For instance, OTU1283

309 (Actinobacteriota) showed significantly higher abundance in RR and CR than that in TA and AA,

310 which suggested that it favored ARs. The phylum Actinobacteria plays important roles in

311 mineralizing organic matter in marine sediment (Bell et al., 1998), indicating that the organic

312 matter content in the ARs was higher than that in non-intervened areas. OTU7413 (family

313 Rhodobacteraceae) was regarded as an important food resource for *A. japonicus* (Zhao et al.,

314 2020), which showed a lower abundance in RR than other three areas.

315   As for the beta diversity among the four areas, the highest and lowest beta diversities were

316 found in autumn and summer, respectively (Fig. 4). Some current studies highlighted that high

317   beta diversity of bacterial communities in a particular season was a response to environmental

318   heterogeneity (Fournier et al., 2020). While some studies addressed that the Bray-Curtis

319   dissimilarities among microbial communities did not significantly correlated with environmental

320   factors such as temperature and salinity (Balzano et al., 2015). These opposite conclusions may

321   result from the differences in spatial and temporal scales (Hatosy et al., 2013).

322   **Seasonal dynamics of bacterial communities in ARs**

323   Marine bacterioplankton exhibit pronounced seasonal succession patterns worldwide (Bunse and

324   Pinhassi, 2017), and follow with the changes of temperature, chlorophyll-a, and other nutrients

325   (Mohapatra et al., 2020; Pinhassi et al., 2006). In this study, seasonal changes in the relative

326   abundances of diversity indices, abundant species and community structures were observed. The

327   seasonal variations of α diversity between water and sediment were inconsistent (Fig. 1). In the

328   water samples, a more considerable fluctuation of α diversity was observed, and had the highest

329   Shannon diversity in spring. Similar seasonal patterns in the Shannon diversity have been

330   observed in other seawaters (Piwosz et al., 2018; Zhu et al., 2020). Previous studies indicated that

331   sudden decrease of microbial plankton communities in winter and new growing season in spring

332   preceding the spring bloom (Espinoza-González et al., 2012; Figueiras et al., 2020) could

333   promote these seasonal patterns. Therefore, higher diversity in spring can be explained by the

334   intrinsically high growth rate of bacterioplankton (Agawin et al., 2000). However, no significant

335   differences of bacterial diversity in the sediment were detected. Marine sediment is characterized

336   as a habitat type with high biodiversity, complicated community structures and spatial

337   heterogeneity (Brandt et al., 2016). The bacterial communities in the sediment had wider niche

338   ranges and sufficient nutrients, which helped maintain and facilitate prosperous diversities (Shu

339   et al., 2020).

340   The abundance of the abundant phyla also showed conspicuous varieties (Fig. 2). For the water

341   samples, our results showed that Actinobacteria had the highest abundance in summer, when the

342   salinity was lowest because of higher precipitation level. Shen et al. (2018) confirmed that

343   Actinobacteria is sensitive to the change of salinity. Meantime, the peak abundance of

344   Cyanobacteria occurred in summer. Martin et al. (2017) also stated that the autotrophic

345   Cyanobacterium, Synechococcus exhibited higher abundances during monsoons. For the

346   sediment samples, the seasonal variations of the abundant bacteria were not significant compared

347   with water samples. For instance, Chloroflexi was the most abundant in autumn. Chloroflexi is

348   regarded as having anaerobic and heterotrophic lifestyles, which are positively related to organic

349   matters (Wilms et al., 2006). In autumn, the seaweed cultivation in the adjacent sea could

350   probably explain their higher abundances of Chloroflexi.

351   Marked seasonality of bacterial community compositions in the water was widely observed.

352   Shu et al. (2020) found distinct seasonal patterns of core and non-core bacterial communities in

353   an urban river; Mohapatra et al. (2020) discovered that seasonal variations of environmental

354   drivers highly impacted the bacterial communities. However, the seasonal variations of microbial

355   community compositions in the sediment were controversial. We observed significant seasonal

356   changes of bacterial communities at OTU level (Table 2), while some similar studies have

357   presented different conclusions. Liu et al. (2015) revealed noticeable seasonal variations of

358   bacteria community because of environmental heterogeneity; Palit and Das (2020) reported a

359   comparatively low seasonal fluctuation of sediment bacterial communities in part of sampling

360   sites based on the culture-dependent method; Ming et al. (2021) indicated that no marked

361   seasonal difference was observed among the bacterial community compositions. Different

362   temporal and spatial scales may cause these inconsistent results, and more follow-up studies are

363   suggested.

364   **Environmental characteristics and bacterial communities**

365   Environmental factors significantly affected bacterial communities, such as nitrite, temperature in

366   the water (Fadeev et al., 2018; Zorz et al., 2019) and mud content in the sediment (Lee et al.,

367   2020). In the present study, the Mantel test revealed that nitrite, temperature, and transparency

368   significantly correlated with the communities in the water, while mud content was the only

369   related factor in the sediment (Table 3). Although N and P are limiting factors of eutrophication,

370   they are important nutrients affecting the proliferation of bacteria (Huang et al., 2017). Our

371   results revealed that dissolved inorganic nitrogen (NO_3-N, NH_4-N, NO_2-N) was correlated with

372   many abundant OTUs (Fig. 5a). Water temperature is an important factor that governs microbial

373   growth and activity (Margesin, 2009), and thirty-three abundant OTUs were significantly related

374   to temperature. Light intensity could also shape different microbial communities (Davies and

375   Evison, 1991), and our studies confirmed that the correlation between transparency and the

376   bacterial communities was significant. Different electrical conductivity levels can influence

377   bacteria (Silverman and Munoz, 1975), which was in accordance with the results that the

378   abundances of thirteen dominant OTUs were affected by EC. Mud content was a vital factor

379   influencing microorganisms from sediment, higher content could offer more nutrition substrates,

380   which was meaningful to support greater microbial biomass (Wei et al., 2014).

381   **Co-occurrence networks and keystone species of bacterial communities in ARs**

382   Although the physical and chemical properties between water and sediment were fundamentally

383   different, the microbial communities are connected through the sedimentation of organic matters

384   (Lucie et al., 2011). To reveal the potential interactions among the bacterial communities in two

385   habitats, co-occurrence networks were used to promote our understanding of the microbiomes in

386   marine ecosystems (Berry and Widder, 2014). Species similarity between the water and sediment

387   networks was low (Jaccard = 11.8%), and the similarity between the water network and total

388   bacterial network (33.1%) was equal to that between the sediment network and total bacterial

389   network (31.5%). These results indicated that habitats significantly affected the compositions of

390   microorganisms in the co-occurrence networks, which was consistent with Zhang et al. (2020a).

391   A total of 47 OTUs were simultaneously observed in the three networks, and some played

392   important roles in ecosystems. For instance, family Woeseiaceae (OTU10090) covers a broad

393   physiological spectrum (Mußmann et al., 2017), which may promote plant survival by

394   participating in nitrogen and carbon cycling (Zhang et al., 2020b); family Methyloligellaceae

395   (OTU3417) was reported as an important host related to antibiotic resistance and metal resistance

396   (Zhang et al., 2021). Significant seasonality of the top five abundant OTUs for the networks was

397   exhibited, while the dissimilarities of their abundance among the four areas were relatively low

398   (Fig. 7 and Fig. 8). OTU18562 (family Planococcaceae) was the most abundant OTU in the total

399   network and water network, which showed evident temporal and spatial differences,

400   concurrently. OTU18562 affiliates to family Planococcaceae, which was correlated with the

401   decomposition of fertilizers (Suzuki et al., 2021). OTU17592 (family Moraxellaceae) had higher

402   abundance in RR and CR than in TA and AA in the total network and water network, indicating

403   positive relationships between OTU17592 and ARs existed. OTU18751 (family Microtrichales),

404   which showed the highest abundance in summer and lowest abundance in spring, played crucial

405   roles in hydrolyzing and utilizing complex organic matters (Li et al., 2021).

406   Keystone species are highly connected taxa in networks that play vital roles in maintaining

407   microbial community structures (Faust and Raes, 2012). The top ten keystone OTUs in the three

408   co-occurrence networks were significantly different. As reported, differences of key microbial

409   groups at ARs and open waters have also been studied (Zhu et al., 2020). The ranks of relative

410   abundance for the top ten keystone OTUs in the three network were low (Table S2). Two factors

411   could explain this phenomenon. On the one hand, it was suggested that abundant bacteria

412   contribute mostly to the biogeochemical cycles, while rare microbiomes might act to stabilize the

413   community (Shade et al., 2014; Genitsaris et al., 2015). Moreover, rare species may be disturbed

414   by the rapid changes of the environment (Shade et al., 2014). On the other hand, rare taxa could

415   considerably increase in abundance to respond the environmental disturbances; and these

416 dynamics may be the reasons for their greater contributions to microbial communities (Caron and

417 Countway, 2009).

418   In general, relative to the important functions of keystone species in the community, the

419 discovery of these species evidently falls behind (Palit et al., 2020), which leads to a limited

420 understanding of microbial ecology. The abundance of the top five keystone OTUs in the water

421 network all showed similar seasonal variations (Fig. S6b), suggesting keystone species were

422 more consistent in response to the changes of environmental conditions than abundant species

423 (Fig. 7b). OTU525 belongs to family Flavobacteriaceae, which were reported as important

424 bacterial populations associated with algal blooms closely (Pavlovska et al., 2021). What's more,

425 the seasonality of the top five keystone OTUs in the sediment network was also significant (Fig.

426 S6c). Three keystone OTUs (OTU17592, OTU16870 and OTU17616) in the sediment network

427 had significantly higher abundance in RR than in other three areas (Fig. S7c), showing their

428 positive relationships with the deployment of rock reefs. These three keystone OTUs all affiliate

429 to order Pseudomonadales, class Gammaproteobacteria. As reported by Eswaran and

430 Khandeparker (2019), Pseudomonadales participated in mediating the degradation of

431 carbohydrates by producing β-Glucosidases in a tropical estuarine environment. Some other

432 keystone OTUs are also crucial in the particular ecological process. For instance, the abundance

433 of OTU7413 (family Rhodobacteraceae) ranked 137th in the water network (Table S2b), which

434 was regarded as the key member of the initial microbial biofilm in coastal seawater (Hila et al.,

435 2013). OTU11572 and OTU8614 belong to family Thermoanaerobaculaceae, may play crucial

436 roles in nitrogen transformation in water (Wang et al., 2019a).

437
438 Conclusions

439 In the present study, we simultaneously profiled the temporal and spatial variabilities and

440 potential interaction patterns of bacterial communities in the water and sediment of ARs for the

441 first time. Seasonal variations of bacterial community compositions were observed in the two

442 habitats, while spatial changes were only detected in the sediment. Bacterial communities in the

443 rock reef area were significantly different with other three areas, which indicating the deployment

444 of ARs impacted the bacterial communities in sediment. The potential correlations among

445 bacterial communities in the sediment were lowest in the three networks, suggesting less niche

446 overlap existed. The abundant and keystone species in the networks showed discordant variations

447 among four seasons and four areas, revealing that two types of species played different ecological

448 functions. As important artificial habitats in marine fisheries, our discoveries about ARs are

449 crucial to better comprehend the mechanisms of microbial ecology in artificial habitats, to

450 promote restoration efficiency, to improve biodiversity, and to recover the marine environment.

451 Particularly, marine microbial loops are comprehensive networks containing bacteria, archaea,

452 and eukaryotes, and further studies are suggested to reveal the interactions among these

453 microbiomes in artificial habitats.

454

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References

462 **Abia A, Alisoltani A, Keshri J, Ubomba-Jaswa E.** 2017. Metagenomic analysis of the bacterial
463 communities and their functional profiles in water and sediments of the Apies River, South
464 Africa, as a function of land use. *Science of the Total Environment* 616-617: 326-334. DOI
465 10.1016/j.scitotenv.2017.10.322
466 **Agawin N, Duarte CM, Agustí S.** 2000. Nutrient and temperature control of the contribution of
467 picoplankton to phytoplankton biomass and production. *Limnology and Oceanography* 45(3):
468 591-600. DOI 10.4319/lo.2000.45.3.0591
469 **Aleksandrov BG, Minicheva GG, Strikalenko TV.** 2002. Ecological aspects of artificial reef
470 construction using scrap tires. *Russian Journal of Marine Biology* 28(2): 120-126. DOI
471 10.1023/A:1015396512150
472 **Anderson MJ.** 2001. Permutation tests for univariate or multivariate analysis of variance and
473 regression. *Canadian Journal of Fisheries and Aquatic Sciences* 58: 626-639. DOI:
474 10.1139/cjfas-58-3-626

475 **Balzano S, Abs E, Leterme SC.** 2015. Protist diversity along a salinity gradient in a coastal
476   lagoon. *Aquatic Microbial Ecology* 74(3): 263-277. DOI 10.3354/ame0174
477 **Barberan A, Bates ST, Casamayor EO, Fierer N.** 2012. Using network analysis to explore co-
478   occurrence patterns in soil microbial communities. *The ISME Journal* 6: 343-351. DOI
479   10.1038/ismej.2011.119
480 **Bell KS, Philp JC, Aw D, Christofi N.** 1998. The genus Rhodococcus. *Journal of Applied*
481   *Microbiology* 85(2): 195-210. DOI 10.1046/j.1365-2672.1998.00525.x
482 **Berdjeb L, Parada A, Needham DM, Fuhrman JA.** 2018. Short-term dynamics and
483   interactions of marine protist communities during the spring-summer transition. *The ISME*
484   *Journal* 12: 1907-1917. DOI 10.1038/s41396-018-0097-x
485 **Berry D, Widder S.** 2014. Deciphering microbial interactions and detecting keystone species
486   with co-occurrence networks. *Frontiers in Microbiology* 5: 219. DOI
487   10.3389/fmicb.2014.00219
488 **Boras JA, Sala MM, Vázquez-Domínguez E, Weinbauer MG, Vaqué D.** 2010. Annual
489   changes of bacterial mortality due to viruses and protists in an oligotrophic coastal
490   environment (NW Mediterranean). *Environmental Microbiology* 11(5): 1181-1193. DOI
491   10.1111/j.1462-2920.2008.01849.x
492 **Borcard D, Gillet F, Legendre P.** 2011. Numerical Ecology with R. Springer. Gewerbestrasse,
493   Switzerland.
494 **Brandt LD, House CH.** 2016. Marine subsurface microbial community shifts across a
495   hydrothermal gradient in Okinawa Trough sediments. *Archaea* 2016: 2690329. DOI
496   10.1155/2016/2690329
497 **Bunse C, Pinhassi J.** 2017. Marine bacterioplankton seasonal succession dynamics. *Trends in*
498   *Microbiology* 25(6): 494-505. DOI 10.1016/j.tim.2016.12.013
499 **Caporaso J, Kuczynski J, Stombaugh J.** 2010. QIIME allows analysis of high-throughput
500   community sequencing data. *Nature Methods* 7: 335-336. DOI: 10.1038/nmeth.f.303
501 **Caron DA, Countway PD.** 2009. Hypotheses on the role of the protistan rare biosphere in a
502   changing world. *Aquatic Microbial Ecology* 57(3): 227-238. DOI 10.3354/ame01352
503 **Chen JL, Chuang CT, Jan RQ, Liu LC, Jan MS.** 2013. Recreational benefits of ecosystem
504   services on and around artificial reefs: a case study in Penghu, Taiwan. *Ocean & Coastal*
505   *Management* 85: 58-64. DOI 10.1016/j.ocecoaman.2013.09.005
506 **Chen Q, Yuan H, Chen P.** 2019. Short-term effects of artificial reef construction on the
507   taxonomic diversity and eco-exergy of the macrobenthic faunal community in the pearl river
508   estuary, China. *Ecological indicators* 98: 772-782. DOI 10.1016/j.ecolind.2018.12.001
509 **Cheung MK, Wong CK, Chu KH, Kwan HS.** 2018. Community structure, dynamics and
510   interactions of bacteria, archaea and fungi in subtropical coastal wetland sediments. *Scientific*
511   *Reports* 8: 14397. DOI: 10.1038/s41598-018-32529-5
512 **Clarke KR.** 1993. Nonparametric multivariate analyses of changes in community structure.
513   *Australian Journal of Ecology* 18: 117-143. DOI: 10.1111/j.1442-9993.1993.tb00438.x
514 **Davies CM, Evison LM.** 1991. Sunlight and the survival of enteric bacteria in natural waters.
515   *Journal of Applied Microbiology* 70(3): 265-74. DOI 10.1111/j.1365-2672.1991.tb02935.x.
516 **Dmitry P, Julian H, Edwin KS Marcello P, Markus MN, Christian D, Christoph L, Heide**
517   **LF.** 2016. Utilizing the Jaccard index to reveal population stratification in sequencing data: a
518   simulation study and an application to the 1000 Genomes Project. *Bioinformatics* 32(9): 1366-
519   1372. DOI 10.1093/bioinformatics/btv752
520 **Eleftheriou A.** 2013. Methods for the study of marine benthos. John Wiley & Sons Ltd. Sussex,
521   UK. pp 82-84.

522 **Espinoza-González O, Figueiras FG, Crespo BG, Teixeira IG, Castro CG.** 2012. Autotrophic
523   and heterotrophic microbial plankton biomass in the NW Iberian upwelling: seasonal
524   assessment of metabolic balance. *Aquatic Microbial Ecology* 67(1): 77-89. DOI
525   10.3354/ame01584
526 **Eswaran R, Khandeparker L.** 2019. Seasonal variation in beta-glucosidase-producing
527   culturable bacterial diversity in a monsoon-influenced tropical estuary. *Environmental
528   Monitoring and Assessment* 191: 662. DOI 10.1007/s10661-019-7818-0
529 **Fadeev E, Salter I, Schourup-Kristensen V, Nthig EM, Bienhold C.** 2018. Microbial
530   communities in the east and west Fram Strait during sea ice melting season. *Frontiers in
531   Marine Science* 5: 429. DOI 10.3389/fmars.2018.00429
532 **Fang GJ, Yu HL, Sheng HX, Tang YL, Liang ZL.** 2021. Comparative analysis of microbial
533   communities between water and sediment in Laoshan Bay marine ranching with varied
534   aquaculture activities. *Marine Pollution Bulletin* 173: 112990. DOI:
535   10.1016/j.marpolbul.2021.112990
536 **Faust K, Raes J.** 2012. Microbial interactions: from networks to models. *Nature Reviews
537   Microbiology* 10(8): 538-550. DOI 10.1038/nrmicro2832
538 **Feng BW, Li XR, Wang JH, Hu ZY, Meng H, Xiang LY.** 2009. Bacterial diversity of water
539   and sediment in the Changjiang estuary and coastal area of the East China Sea. *FEMS
540   Microbiology Ecology* 70(2): 80-92. DOI 10.1111/j.1574-6941.2009.00772.x
541 **Figueiras FG, Teixeira IG, Froján M, Zúiga D, Castro CG.** 2020. Seasonal variability in the
542   microbial plankton community in a semienclosed bay affected by upwelling: the role of a
543   nutrient trap. *Frontiers in Marine Science* 7: 578042. DOI 10.3389/fmars.2020.578042
544 **Fournier B, Samaritani E, Frey B, Seppey C, Mitchell E.** 2020. Higher spatial than seasonal
545   variation in floodplain soil eukaryotic microbial communities. *Soil Biology and Biochemistry*
546   147: 107842. DOI 10.1016/j.soilbio.2020.107842
547 **Genitsaris S, Monchy S, Viscogliosi E, Sime-Ngando T, Ferreira S, Christaki U.** 2015.
548   Seasonal variations of marine protist community structure based on taxon-specific traits using
549   the Eastern English Channel as a model coastal system. *FEMS Microbiology Ecology* 91(5):
550   fiv034. DOI 10.1093/femsec/fiv034
551 **Hatosy SM, Martiny J, Sachdeva R, Steele J, Fuhrman JA, Martiny AC.** 2013. Beta
552   diversity of marine bacteria depends on temporal scale. *Ecology* 94(9): 1898-1904. DOI
553   10.1890/12-2125.1
554 **Hila E, Gilad H, Meir A, Yehuda C, Dror M.** Rhodobacteraceae are the key members of the
555   microbial community of the initial biofilm formed in eastern Mediterranean coastal seawater.
556   *FEMS Microbiology Ecology* 85(2): 348-357. DOI 10.1111/1574-6941.12122
557 **Huang W, Chen X, Jiang X, Zheng B.** 2017. Characterization of sediment bacterial
558   communities in plain lakes with different trophic statuses. *Microbiologyopen* 6: e503. DOI
559   10.1002/mbo3.503
560 **Jackson J, Kirby MX, Berger WH, Bjorndal KA, Botsford LW, Bourque BJ.** 2001.
561   Historical overfishing and the recent collapse of coastal ecosystems. *Science* 293: 629-638.
562   DOI 10.1126/science.1059199
563 **Janiak DS, Branson D.** 2021. Impacts of habitat and predation on epifaunal communities from
564   seagrass beds and artificial structures. *Marine Environmental Research* 163: 105225. DOI
565   10.1016/j.marenvres.2020.105225
566 **Koldewey HJ, Curnick D, Harding S, Harrison LR, Gollock M.** 2010. Potential benefits to
567   fisheries and biodiversity of the Chagos Archipelago/British Indian Ocean Territory as a no-
568   take marine reserve. *Marine Pollution Bulletin* 60(11): 1906-1915. DOI
569   10.1016/j.marpolbul.2010.10.002

Langille MG, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA, Clemente JC, Burkepile DE, Thurber RL, Knight R, Beiko RG, Huttenhower C. 2013. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nature Biotechnology* 31(9): 814-821. DOI 10.1038/nbt.2676

Lee SY, Eom YB. 2016. Analysis of microbial composition associated with freshwater and seawater. *Biomedical Science Letter* 22: 150-159. DOI 10.15616/BSL.2016.22.4.150

Lee H, Heo Y, Kwon SL, Yoo Y, Kim D, Lee J, Kwon BO, Khim JS, Kim JJ. 2020. Environmental drivers affecting the bacterial community of intertidal sediments in the Yellow Sea. *Science of The Total Environment* 755: 142726. DOI 10.1016/j.scitotenv.2020.142726

Li J, Zheng L, Ye CB, Ni BS, Wang XZ, Liu H. 2021. Evaluation of an intermittent-aeration constructed wetland for removing residual organics and nutrients from secondary effluent: performance and microbial analysis. *Bioresource Technology* 329: 124897. DOI 10.1016/j.biortech.2021.124897

Lima JS, Zalmon IR, Love M. 2019. Overview and trends of ecological and socioeconomic research on artificial reefs. *Marine Environmental Research* 145: 81-96. DOI 10.1016/j.marenvres.2019.01.010

Lima JS, Atalah J, Sanchezjerez P, Zalmon IR. 2020. Evaluating the performance and management of artificial reefs using artificial reef multimetric index (ARMI). *Ocean & Coastal Management* 198, 105350. DOI 10.1016/j.ocecoaman.2020.105350

Liu X, Hu HW, Liu YR, Xiao KQ, Cheng FS, Ji L. 2015. Bacterial composition and spatiotemporal variation in sediments of Jiaozhou Bay, China. *Journal of Soils and Sediments* 15(3): 732-744. DOI 10.1007/s11368-014-1045-7

Liu T, Zhang AN, Wang J, Liu S, Jiang X, Dang C. 2018. Integrated biogeography of planktonic and sedimentary bacterial communities in the Yangtze River. *Microbiome* 6(1): 16. DOI 10.1186/s40168-017-0388-x

Lucie Z, Amaral-Zettler LA, Fuhrman JA, Claire H, Huse SM, Mark W. 2011. Global patterns of bacterial beta-diversity in seafloor and seawater ecosystems. *PloS One* 6(9): e24570. DOI 10.1371/journal.pone.0024570

Mangot JF, Domaizon I, Taib N, Marouni N, Duffaud E, Bronner G. 2013. Short-term dynamics of diversity patterns: evidence of continual reassembly within lacustrine small eukaryotes. *Environmental Microbiology* 15(6): 1745-1758. DOI 10.1111/1462-2920.12065

Margesin R. 2009. Effect of temperature on growth parameters of psychrophilic bacteria and yeasts. *Extremophiles* 13(2): 257. DOI 10.1007/s00792-008-0213-3

Marquardt M, Vader A, Stübner EI, Reigstad M, Gabrielsen TM. 2016. Strong seasonality of marine microbial eukaryotes in a high-Arctic fjord (Isfjorden, West Spitsbergen). *Applied and Environmental Microbiology* 82(6): 1868-1880. DOI 10.1128/AEM.03208-15

Martin A, Thomas P, Rhena S. 2017. Identification of Cyanobacteria in a eutrophic coastal lagoon on the Southern Baltic Coast. *Frontiers in Microbiology* 8: 923. DOI 10.3389/fmicb.2017.00923

Mikhailov IS, Zakharova YR, Bukin YS, Galachyants YP, Petrova DP, Sakirko MV. 2019. Co-occurrence networks among bacteria and microbial eukaryotes of lake Baikal during a spring phytoplankton bloom. *Microbial Ecology* 77(1): 96-109. DOI 10.1007/s00248-018-1212-2

Ming HX, Fan JF, Liu JW, Su J, Wan ZY, Wang YT, Li DW, Li MF, Shi TT, Jin Y, Huang HL, Song JX. 2021. Full-length 16S rRNA gene sequencing reveals spatiotemporal dynamics of bacterial community in a heavily polluted estuary, China. *Environmental Pollution* 275(2): 116567. DOI: 10.1016/j.envpol.2021.116567

617 **Mohapatra M, Behera P, Ji YK, Rastogi G.** 2020. Seasonal and spatial dynamics of
618   bacterioplankton communities in a brackish water coastal lagoon. *Science of the Total*
619   *Environment* 705: 134729. DOI 10.1016/j.scitotenv.2019.134729
620 **Mußmann M, Pjevac P, Krüger K.** 2017. Genomic repertoire of the Woeseiaceae/JTB255,
621   cosmopolitan and abundant core members of microbial communities in marine sediments. *The*
622   *ISME Journal* 11: 1276-1281. DOI 10.1038/ismej.2016.185
623 **Ng C, Toh TC, Chou LM.** 2017. Artificial reefs as a reef restoration strategy in sediment-
624   affected environments: insights from long-term monitoring. *Aquatic Conservation: Marine*
625   *and Freshwater Ecosystems* 2017: 1-10. DOI 10.1002/aqc.2755
626 **Palit K, Das S.** 2020. Community structure, taxonomic diversity and spatio-temporal variation of
627   sediment and water bacteria in bhitarkanika mangrove ecosystem, India. *International Journal*
628   *of Environmental Science and Technology* 18: 1147-1166. DOI 10.1007/s13762-020-02851-5
629 **Pavlovska M, Prekrasna I, Dykyi E, Zotov A, Stoica E.** 2021. Niche partitioning of bacterial
630   communities along the stratified water column in the Black Sea. *MicrobiologyOpen* 10: e1195.
631   DOI 10.1002/mbo3.1195
632 **Pereira HM, Leadley PW, Proenca V, Alkemade R, Scharlemann J, Fernandez-Manjarres**
633   **JF.** 2010. Scenarios for global biodiversity in the 21st century. *Science* 330(6010): 1496-1501.
634   DOI 10.1126/science.1196624
635 **Perkins TL, Clements K, Baas JH, Jago CF, Jones DL, Malham SK, McDonald JE.** 2014.
636   Sediment composition influences spatial variation in the abundance of human pathogen
637   indicator bacteria within an estuarine environment. *PLoS One* 9: e112951. DOI
638   10.1371/journal.pone.0112951
639 **Pinhassi J, Gómez-Consarnau L, Alonso-Sáez L, Sala M, Vidal M, Pedrós-Alió C.** 2006.
640   Seasonal changes in bacterioplankton nutrient limitation and their effects on bacterial
641   community composition in the NW Mediterranean Sea. *Aquatic Microbial Ecology* 44(3): 241-
642   252. DOI 10.3354/ame044241
643 **Piwosz K., Calkiewicz J, Golebiewski M, Creer S.** 2018. Diversity and community
644   composition of pico- and nanoplanktonic protists in the Vistula river estuary (Gulf of Gdansk,
645   Baltic Sea). *Estuarine Coastal and Shelf Science* 207: 242-249. DOI
646   10.1016/j.ecss.2018.04.013
647 **Pommier T, Canbck B, Riemann L, Bostrm KH, Simu K, Lundberg P.** 2007. Global patterns
648   of diversity and community structure in marine bacterioplankton. *Molecular Ecology* 16(4):
649   867-880. DOI 10.1111/j.1365-294X.2006.03189.x
650 **Qin C, Cn QA, Zhu W, Ma H, Pan W.** 2019. Are habitat changes driving protist community
651   shifts? a case study in Daya Bay, China. *Estuarine Coastal and Shelf Science* 227: 106356.
652   DOI 10.1016/j.ecss.2019.106356
653 **Scott ME, Smith JA, Lowry MB, Taylor MD, Suthers IM.** 2015. The influence of an offshore
654   artificial reef on the abundance of fish in the surrounding pelagic environment. *Marine &*
655   *Freshwater Research* 66: 429-437. DOI 10.1071/MF14064
656 **Seaman WJ, Sprague LM.** 1993. Artificial habitats for marine and freshwater fisheries.
657   *Fisheries Research* 16(1): 89-92. DOI 10.1007/BF00043299
658 **Shade A, Jones SE, Caporaso JG, Handelsman J, Knight R, Fierer N.** 2014. Conditionally
659   rare taxa disproportionately contribute to temporal changes in microbial diversity. *mBio* 5(4):
660   e01371-14. DOI 10.1128/mBio.01371-14
661 **Shen D, Jürgens K, Beier S.** 2018. Experimental insights into the importance of ecologically
662   dissimilar bacteria to community assembly along a salinity gradient. *Environmental*
663   *Microbiology* 20(3): 1170-1184. DOI 10.1111/1462-2920.14059

664   **Sheng H, Tang Y, Wang X.** 2018. Relationship between environmental factors and benthic
665   macroalgae communities of artificial reefs in Laoshan Bay. *Indian Journal of Geo Marine*
666   *Sciences* 47(11): 2248-2254.
667   **Shin PK, Cheung SG, Tsang TY, Wai HY.** 2014. Ecology of artificial reefs in the subtropics.
668   *Advances in Marine Biology* 68: 1-63. DOI 10.1016/B978-0-12-800169-1.00001-X
669   **Shu W, Wang P, Zhang H, Ding M, Wu B.** 2020. Seasonal and spatial distribution and
670   assembly processes of bacterioplankton communities in a subtropical urban river. *FEMS*
671   *Microbiology Ecology* 96: fiaa154. DOI 10.1093/femsec/fiaa154
672   **Silverman MP, Munoz EF.** 1975. Microbial metabolism and dynamic changes in the electrical
673   conductivity of soil solutions: a method for detecting extraterrestrial life. *Applied microbiology*
674   28(6): 960-967. DOI 10.1128/AEM.28.6.960-967.1974
675   **Smouse PE, Long JC, Sokal RR**. 1986. Multiple regression and correlation extensions of the
676   mantel test of matrix correspondence. *Systematic Zoology* 35(4), 627-632. DOI:
677   10.2307/2413122
678   **Soka S, Hutagalung RA, Yogiara AC.** 2011. Population dynamic of *Dendronephthya* sp.-
679   associated bacteria in natural and artificial habitats. *HAYATI Journal of Biosciences* 18(2): 57-
680   60. DOI 10.4308/hjb.18.2.57
681   **Sun P, Liu X, Tang Y, Cheng W, Sun R, Wang X.** 2017. The bio-economic effects of artificial
682   reefs: mixed evidence from Shandong, China. *ICES Journal of Marine Science* 74(8): 2239-
683   2248. DOI 10.1093/icesjms/fsx058
684   **Sun Y, Li X, Liu J, Yao Q, Jin J, Liu X.** 2019. Comparative analysis of bacterial community
685   compositions between sediment and water in different types of wetlands of Northeast China.
686   *Journal of Soil & Sediments* 19(7): 3083-3097. DOI 10.1007/s11368-019-02301-x
687   **Suzuki K, Naoya K, Kota N, Rasit A, Naoki H.** 2021. Impacts of application of calcium
688   cyanamide and the consequent increase in soil pH on N_2O emissions and soil bacterial
689   community compositions. *Biology and Fertility of Soils* 57(2): 269-279. DOI 10.1007/s00374-
690   020-01523-3
691   **State Bureau of Quality and Technical Supervision of China**. 2007. GB/T 12763-2007
692   Specifications for oceanographic survey. China Standards Press, Beijing (in Chinese).
693   **Steele JA, Countway PD, Xia L, Vigil PD, Beman JM, Kim DY, Chow CET, Sachdeva R,**
694   **Jones AC, Schwalbach MS.** 2011. Marine bacterial, archaeal and protistan association
695   networks reveal ecological linkages. *The ISME Journal* 5(9): 1414-25. DOI
696   10.1038/ismej.2011.24
697   **Sunagawa S, Coelho LP, Chaffron S, Kultima JR, Labadie K, Salazar G, Djahanschiri B,**
698   **Zeller G, Mende DR, Alberti A, Cornejo-Castillo FM, Costea PI, Cruaud C, d'Ovidio F,**
699   **Engelen S, Ferrera I, Gasol JM, Guidi L, Hildebrand F, Kokoszka F, Lepoivre C, Lima-**
700   **Mendez G, Poulain J, Poulos BT, Royo-Llonch M, Sarmento H, Vieira-Silva S, Dimier C,**
701   **Picheral M, Searson S, Kandels-Lewis S; Tara Oceans coordinators, Bowler C, de Vargas**
702   **C, Gorsky G, Grimsley N, Hingamp P, Iudicone D, Jaillon O, Not F, Ogata H, Pesant S,**
703   **Speich S, Stemmann L, Sullivan MB, Weissenbach J, Wincker P, Karsenti E, Raes J,**
704   **Acinas SG, Bork P.** 2015. Ocean plankton. Structure and function of the global ocean
705   microbiome. *Science* 348(6237): 1261359. DOI 10.1126/science.1261359.
706   **Tang YL, Wei SW, Yang MD, Wang XX, Zhao FF**. 2022. Experimental investigation of local
707   scour around artificial reefs in steady currents. *Journal of Ocean University of China* 21(2): 1-
708   12. DOI 10.1007/s11802-022-4883-8
709   **Timothy EE, Anne HB, John W.** 2006. Fishing through marine food webs, *Proceedings of the*
710   *National Academy of Sciences* 103(9): 3171-3175. DOI 10.1073/pnas.0510964103

711 **Todd PA, Heery EC, Loke L, Thurstan RH, Kotze DJ, Swan C.** 2019. Towards an urban
712   marine ecology: characterizing the drivers, patterns and processes of marine ecosystems in
713   coastal cities. *Oikos* 128(9): 1215-1242. DOI 10.1111/oik.05946
714 **Verity PG, Smetacek V, Smetacek V, Smayda TJ, Smayda TJ.** 2002. Status, trends and future
715   of the marine pelagic ecosystem. *Environmental Conservation* 29(2): 207-237. DOI
716   10.1017/S0376892902000139
717 **Wang M, Xiong WG, Zou Y, Lin MX, Zhou Q, Xie XY, Sun YX.** 2019a. Evaluating the net
718   effect of sulfadimidine on nitrogen removal in an aquatic microcosm environment.
719   *Environmental Pollution* 248: 1010-1019. DOI 10.1016/j.envpol.2019.02.048
720 **Wang X, Liu L, Zhao L, Xu H, Zhang X.** 2019b. Assessment of dissolved heavy metals in the
721   Laoshan Bay, China. *Marine Pollution Bulletin* 149: 110608. DOI
722   10.1016/j.marpolbul.2019.110608
723 **Wang Y, Sun J, Fang E, Guo B, Dai Y, Gao Y, Wang H, Zhang X, Xu X, Yu Y, Liu K.**
724   2019c. Impact of artificial reefs on sediment bacterial structure and function in Bohai Bay.
725   *Canadian Journal of Microbiology* 65(3): 191-200. DOI 10.1139/cjm-2018-0157.
726 **Wei H, Guenet B, Vicca S, Nunan N, Asard H, AbdElgawad H, Shen WJ, Janssens IA.**
727   2014. High clay content accelerates the decomposition of fresh organic matter in artificial
728   soils. *Soil Biology & Biochemistry* 77(7): 100-108. DOI 10.1016/j.soilbio.2014.06.006
729 **Williams-Grove LJ, Szedlmayer ST.** 2017. Depth preferences and three-dimensional
730   movements of red snapper, *Lutjanus campechanus*, on an artificial reef in the Northern Gulf of
731   Mexico. *Fisheries Research* 190: 61-70. DOI 10.1016/j.fishres.2017.01.003
732 **Wilms R, Kopke B, Sass H, Chang TS, Cypionka H, Engelen B.** 2006. Deep biosphere-related
733   bacteria within the subsurface of tidal flat sediments. *Environmental Microbiology* 8 (4): 709-
734   719. DOI 10.1111/j.1462-2920.2005.00949.x.
735 **Woo J, Kim D, Yoon HS, Na WB.** 2014. Characterizing Korean general artificial reefs by drag
736   coefficients. *Ocean Engineering* 82: 105-114. DOI 10.1016/j.oceaneng.2014.02.025
737 **Xu M, Yang XY, Song XJ, Xu KD, Yang LL.** 2021. Seasonal analysis of artificial oyster reef
738   ecosystems: implications for sustainable fisheries management. *Aquaculture International*
739   29(1): 167-192. DOI 10.1007/s10499-020-00617-x
740 **Yang X, Lin C, Song X, Xu M, Yang H.** 2019. Effects of artificial reefs on the meiofaunal
741   community and benthic environment - a case study in Bohai Sea, China. *Marine Pollution*
742   *Bulletin* 140: 179-187. DOI 10.1016/j.marpolbul.2018.12.031
743 **Ye W, Liu X, Lin S, Tan J, Pan J, Li D, Yang H. 2009.** The vertical distribution of bacterial
744   and archaeal communities in the water and sediment of Lake Taihu. *FEMS Microbiology*
745   *Ecology* 70(2): 107-120. DOI: 10.1111/j.1574-6941.2009.00761.x
746 **Ye Q, Liu J, Du J, Jing Z.** 2016. Bacterial diversity in submarine groundwater along the coasts
747   of the Yellow Sea. *Frontiers in Microbiology* 6: 1519. DOI 10.3389/fmicb.2015.01519
748 **Zentgraf B, Hedlich R, Gwenner C, Uyen NV, Thao NN.** 1992. Isolation and characterization
749   of thermophilic bacteria from natural and artificial habitats. *Acta Biotechnologica* 12(5): 397-
750   403. DOI 10.1002/abio.370120507
751 **Zhang C, Liu Q, Li X, Wang MA, Yang J, Xu J, Jiang Y.** 2020a. Spatial patterns and co-
752   occurrence networks of microbial communities related to environmental heterogeneity in deep-
753   sea surface sediments around Yap Trench, Western Pacific Ocean. *Science of The Total*
754   *Environment* 759: 143799. DOI 10.1016/j.scitotenv.2020.143799.

755 **Zhang X, Zhao C, Yu S, Jiang Z, Huang X.** 2020b. Rhizosphere microbial community
756   structure is selected by habitat but not plant species in two tropical seagrass beds. *Frontiers in*
757   *Microbiology* 11: 161. DOI 10.3389/fmicb.2020.00161
758 **Zhang N, Juneau, P, Huang R, He ZL, Sun B, Zhou JZ, Liang YT.** 2021. Coexistence
759   between antibiotic resistance genes and metal resistance genes in manure-fertilized soils.
760   *Geoderma* 382: 114760. DOI 10.1016/j.geoderma.2020.114760
761 **Zhao ZL, Jiang JW, Pan YJ, Dong Y, Zhou C, Zhang GH, Gao S, Sun HJ, Guan XY, Wang**
762   **B, Xiao Y, Zhou ZC.** 2020. Temporal dynamics of bacterial communities in the water and
763   sediments of sea cucumber (*Apostichopus japonicus*) culture ponds. *Aquaculture 528*: 735498.
764   DOI 10.1016/j.aquaculture.2020.735498
765 **Zhu W, Qin C, Ma H, Xi S, Li C.** 2020. Response of protist community dynamics and co-
766   occurrence patterns to the construction of artificial reefs: A case study in Daya Bay, China.
767   *Science of The Total Environment* 742: 140575. DOI 10.1016/j.scitotenv.2020.140575.
768 **Zorz J, Willis C, Comeau AM, Langille M, Johnson CL, Li WKW.** 2019. Drivers of regional
769   bacterial community structure and diversity in the Northwest Atlantic Ocean. *Frontiers in*
770   *Microbiology* 10: 281. DOI 10.3389/fmicb.2019.00281

Figure 1

Seasonal change of Shannon diversity (mean ± SD) of bacterial communities in the two habitats of artificial reefs.

Means with different letter (lowercase letters for water samples, capital letters for sediment samples) are significantly different with P value < 0.05.

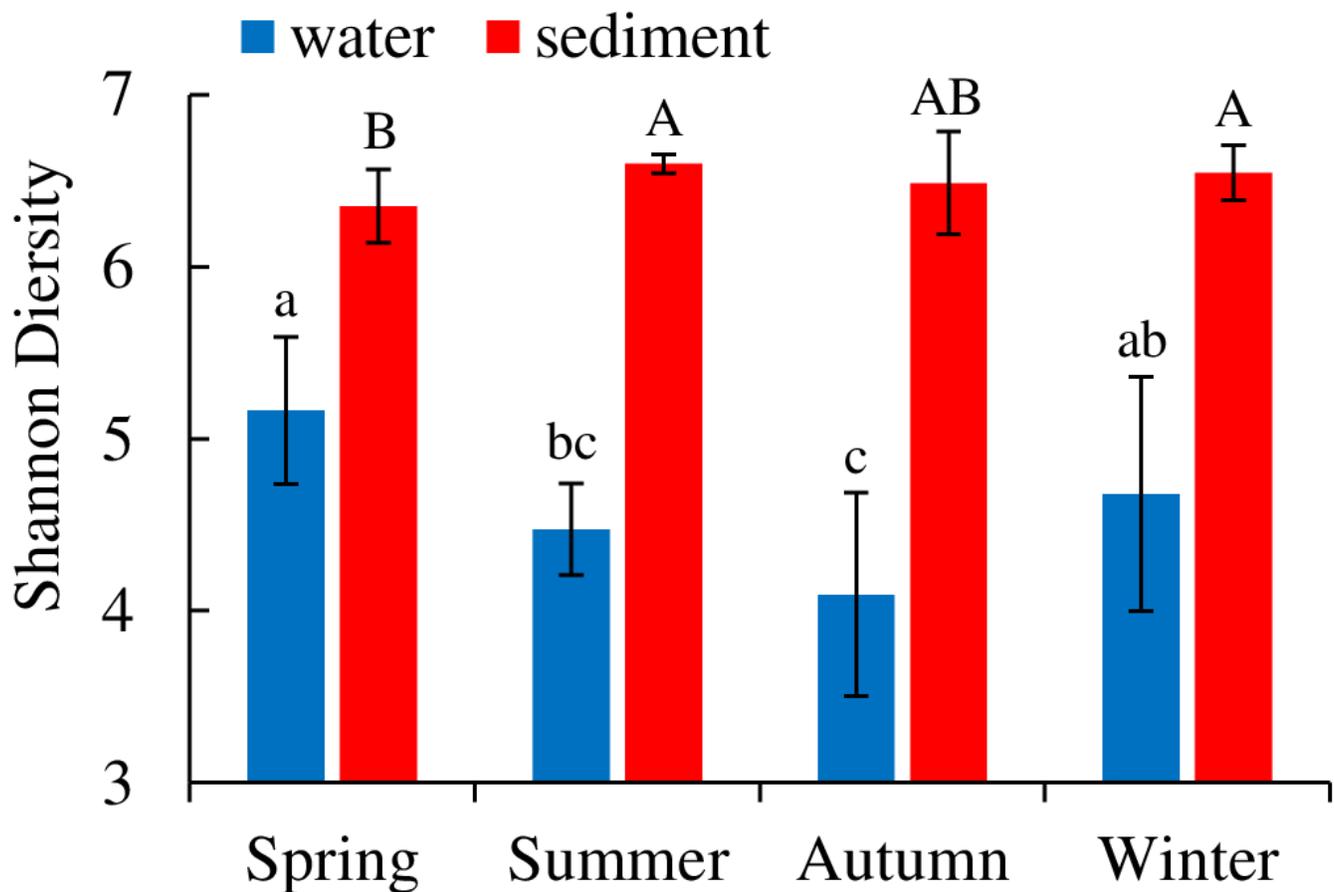



Figure 2

Relative abundance of bacterial communities at phylum levels in the two habitats and four seasons of artificial reefs.

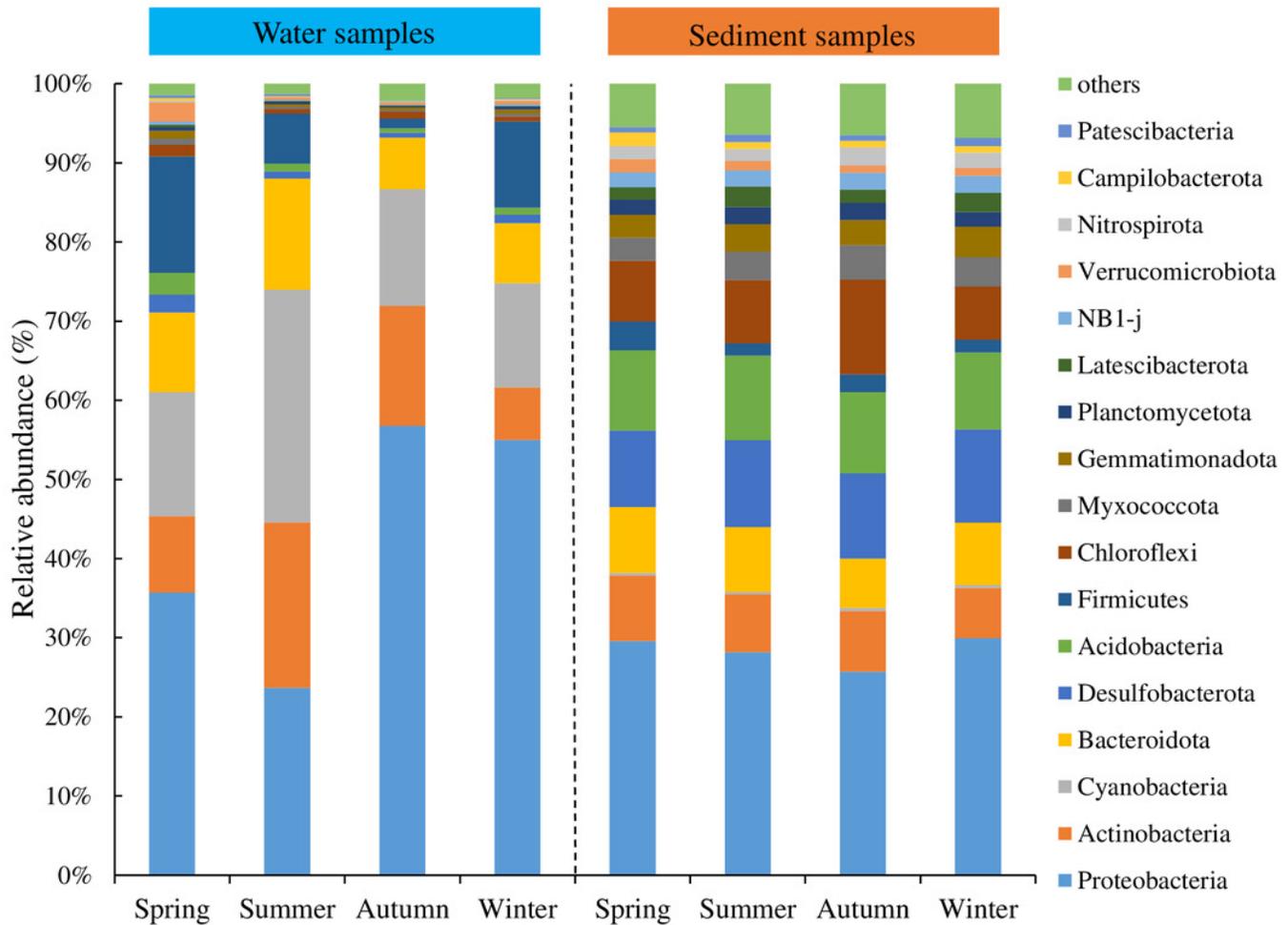



Figure 3

Principal coordinates analysis (PCoA) plots of bacterial communities in the water and sediment of artificial reefs.

R: the test statistical significance of analysis of similarities (ANOSIM); P: statistical significance value at α = 0.05 level.

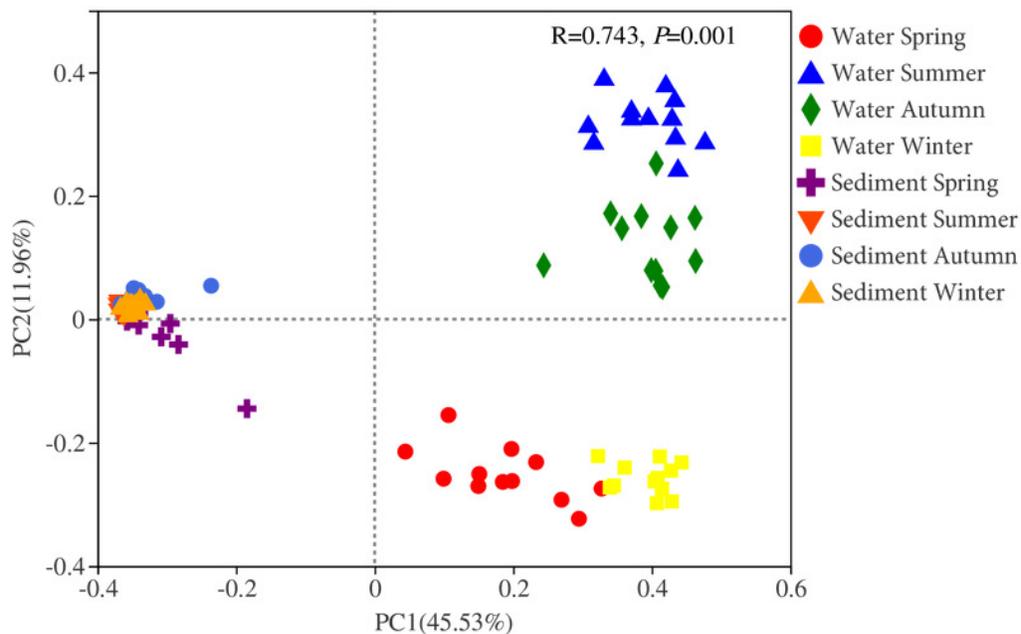



Figure 4

Seasonal variations of bacterial communities among samples in the water and sediment using Bray-Curtis dissimilarity.

Means with different letter (lowercase letters for water samples, capital letters for sediment samples) are significantly different with *P* value < 0.05.

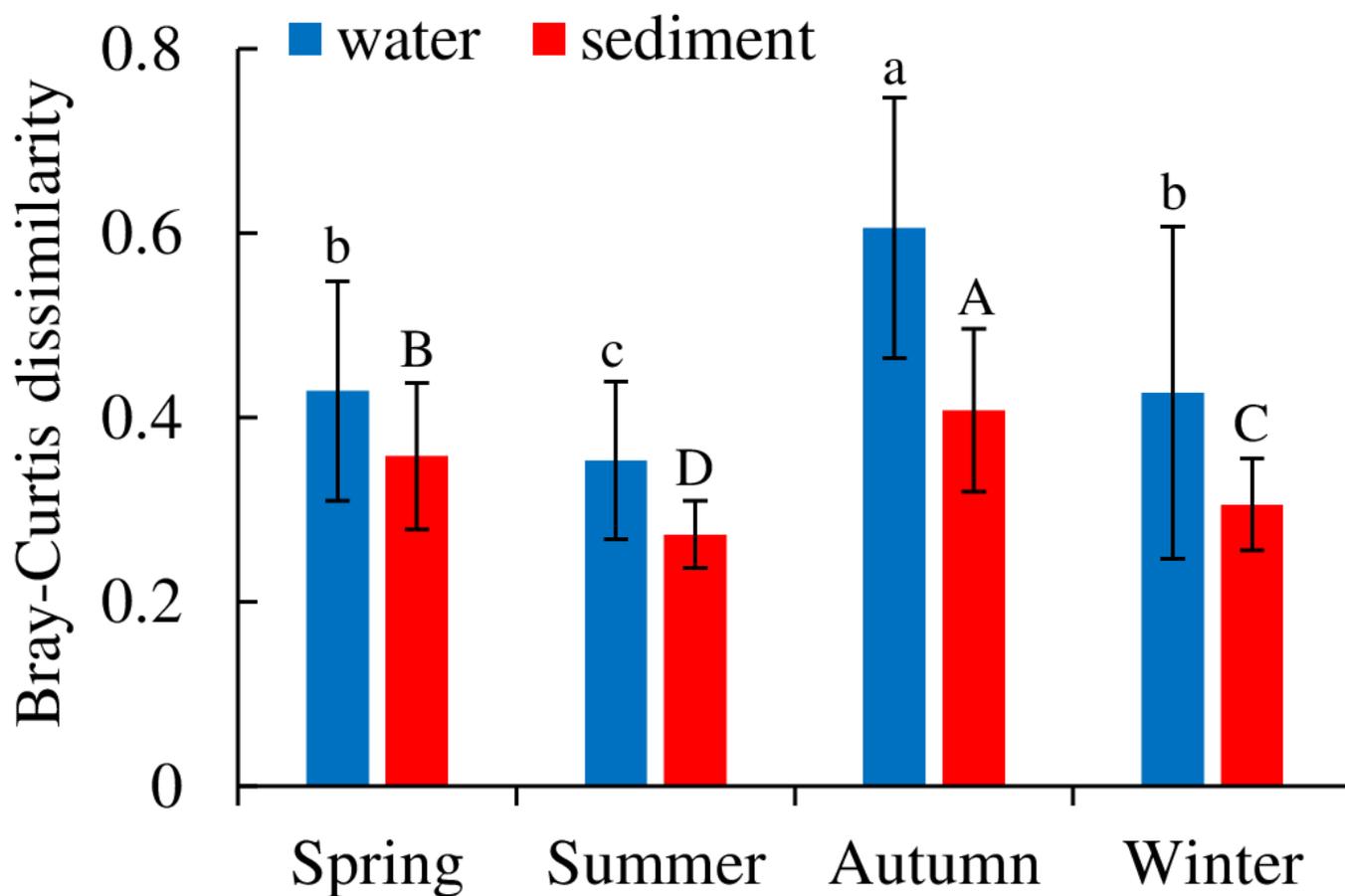



Figure 5

Heatmap of top abundant OTUs of bacterial communities with environmental factors in the (a) water and (b) sediment of artificial reefs.

The cluster trees were analyzed to show the similarity of OTUs using Bray-Curtis distance. Asterisks represent significant correlations between bacterial OTUs and environmental factors at the following α levels: * = 0.05, ** = 0.01, *** = 0.001. Environmental factors: temperature (Temp); transparency (Trans); chemical oxygen demand (COD); active silicate (SiO$_3$); suspended particulate materials (SPM); total organic carbon (TOC); ammonium (NH$_4$-N); depth (Dep); chlorophyll-a (Chla); dissolved oxygen (DO); nitrite (NO$_2$-N); turbidity (Turb); active phosphate (PO$_4$); electrical conductivity (EC); water content (WC); salinity (Sal); mean particle sizes (Par); organic matter content (OM); sediment bulk density (BD); mud content (MC).

(a)

(b)

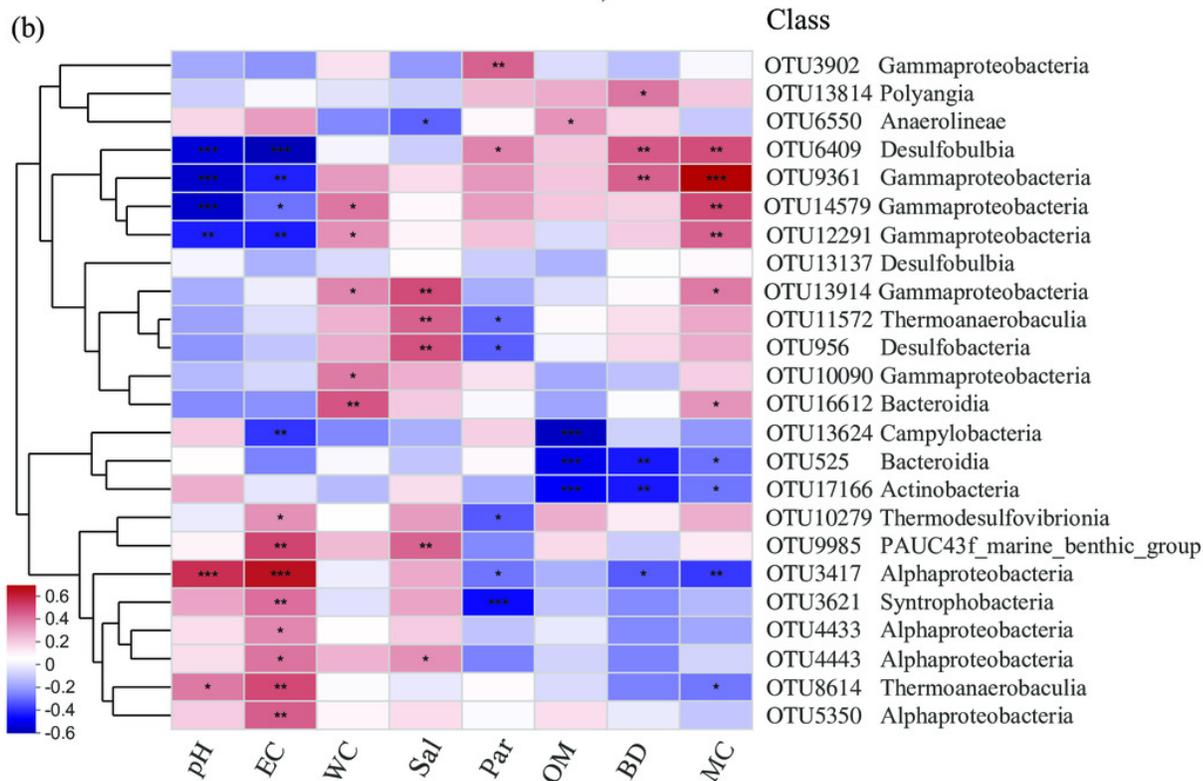
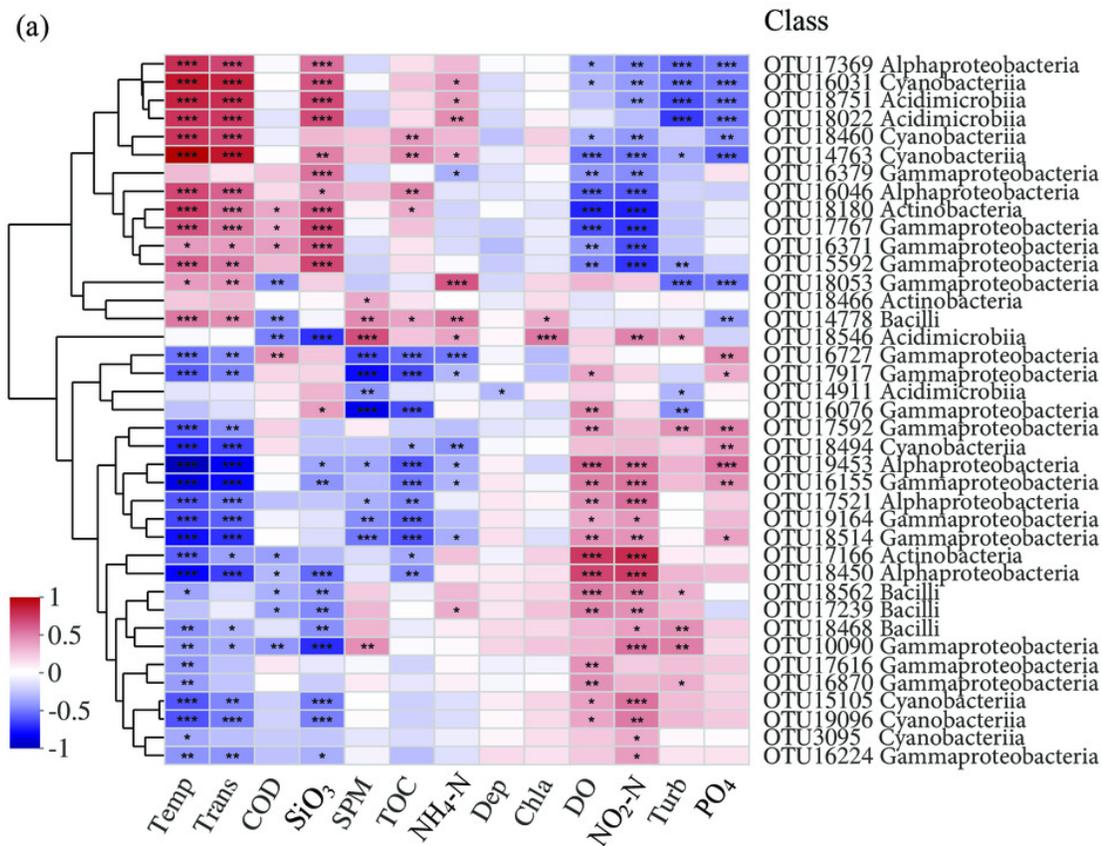



Figure 6

Co-occurrence networks built from abundant bacterial OTUs in the water and sediment of artificial reefs.

Nodes are colored at (a) habitats and (b) phylum levels. Edges with |r| ≥ 0.8 and P ≤ 0.001 are shown in the networks. Positive and negative lines are represented by solid lines and dotted lines, respectively.

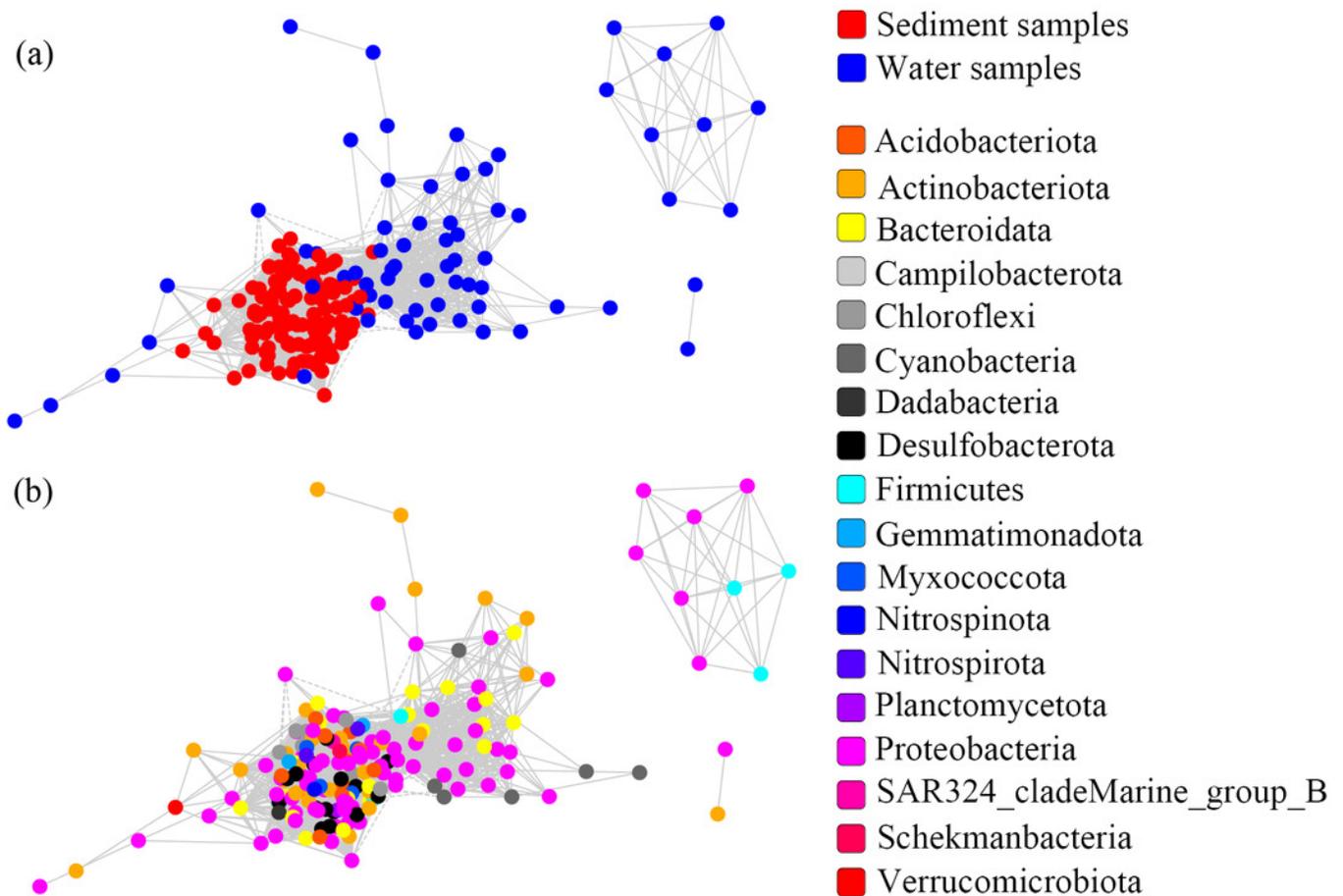



Figure 7

Seasonal changes of the relative abundance for the top five abundant OTUs of the co-occurrence networks in the (a) water and sediment, (b) water and (c) sediment of artificial reefs.

Four seasons: spring (SPR); summer (SUM); autumn (AUT); winter (WIN).

(a)

OTU18562
Firmicutes

OTU18460
Cyanobacteria

OTU10090
Proteobacteria

OTU17592
Proteobacteria

OTU18751
Actinobacteriota

(b)

OTU18562
Firmicutes

OTU14763
Cyanobacteria

OTU17592
Proteobacteria

OTU18751
Actinobacteriota

OTU18053
Proteobacteria

(c)

OTU10090
Proteobacteria

OTU3621
Desulfobacterota

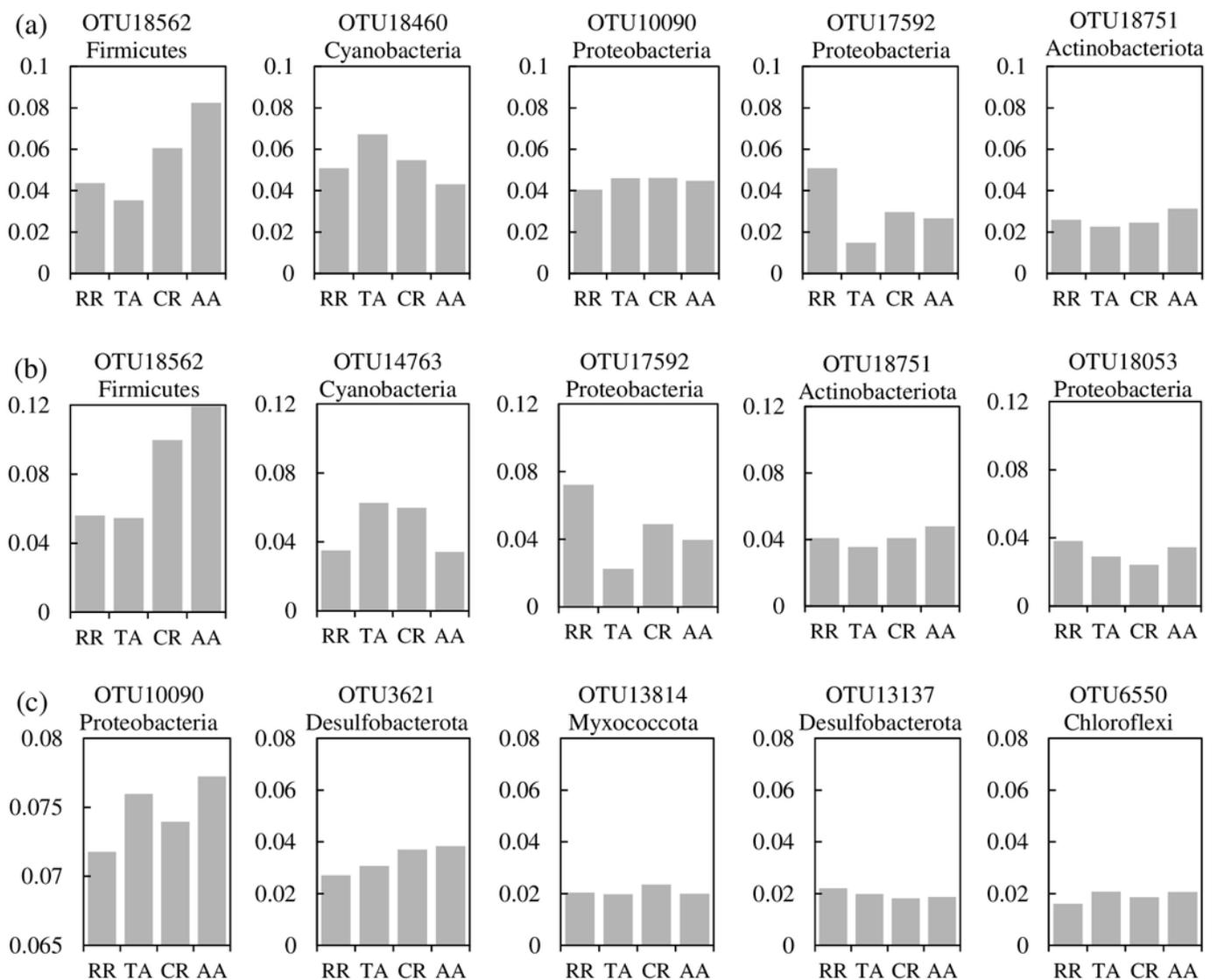
OTU13814
Myxococcota

OTU13137
Desulfobacterota

OTU6550
Chloroflexi

Figure 8

Variations of the relative abundance for the top five abundant OTUs among four sampling areas of the co-occurrence networks in the (a) water and sediment, (b) water and (c) sediment of artificial reefs (ARs).

Four sampling areas in ARs: rock reefs (RR), transition areas (TA), concrete reefs (CR) and adjacent areas (AA).

Number of OTUs and alpha diversity indices in the water and sediment of artificial reefs for four seasons.

Seasonality *P*: statistical difference among four seasons; Water-Sediment *P*: statistical difference between water and sediment samples. Significant difference was at $\alpha = 0.05$ level.

Season	OTUs		Shannon		Simpson		Ace		Chao 1		Coverage	
	Water	Sediment	Water	Sediment	Water	Sediment	Water	Sediment	Water	Sediment	Water	Sediment
Spring	1726	2823	5.16	6.35	0.030	0.008	4742	6875	3349	5111	0.95	0.93
Summer	1227	3056	4.47	6.60	0.040	0.005	3148	7210	2309	5408	0.97	0.92
Autumn	984	3030	4.09	6.49	0.075	0.006	2665	7373	1936	5439	0.97	0.92
Winter	1273	3103	4.68	6.55	0.045	0.006	3765	7490	2609	5584	0.97	0.92
Seasonality P	0.001	0.492	0.001	0.322	0.019	0.030	0.001	0.055	0.001	0.124	0.001	0.136
Water-Sediment P	0.004		0.005		0.025		0.007		0.005		0.011	

The effects of habitat, season and site on bacterial communities (OTU level) based on PERMANOVA and ANOSIM. Interaction effects were only calculated by PERMANOVA.

F: the test statistical significance of Permutational multivariate analysis of variance (PERMANOVA); R: the test statistical significance of analysis of similarities (ANOSIM); P: statistical significance value at $\alpha = 0.05$ level.

Habitat	Effect	PERMANOVA		ANOSIM	
		F	P	R	P
Total	Habitat	73.951	0.001	0.89	0.001
	Season	4.880	0.001	0.12	0.001
	Area	0.463	0.973	0.02	0.862
	Habitat × Season	28.950	0.001	-	-
	Habitat × Area	11.096	0.001	-	-
	Season × Area	1.218	0.139	-	-
	Habitat × Season × Area	8.146	0.001	-	-
Water	Season	17.364	0.001	0.820	0.001
	Area	0.438	0.999	0.060	0.994
	Season × Area	4.172	0.001	-	-
Sediment	Season	2.905	0.001	0.227	0.001
	Area	2.163	0.001	0.133	0.001
	Season × Area	2.689	0.001	-	-

Relationships between bacterial community and environmental factors based on the Mantel test and partial Mantel test.

r: the test statistical significance of Mantel test and partial Mantel test; P: statistical significance value at $\alpha = 0.05$ level. Environmental factors: nitrite (NO_2-N); temperature (Temp); transparency (Trans); active phosphate (PO_4); dissolved oxygen (DO); active silicate (SiO_3); chemical oxygen demand (COD); mud content (MC).

Water			Sediment		
Factor	**r**	***P***	**Factor**	**r**	***P***
Total	0.294	0.001	Total	0.145	0.018
NO$_2$-N	0.414	0.001	MC	0.125	0.050
Temp	0.346	0.001			
Trans	0.287	0.001			
PO$_4$	0.215	0.003			
DO	0.181	0.004			
SiO$_3$	0.115	0.023			
COD	0.141	0.039			