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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Marine bacteria in the seawater and seafloor are essential parts of Earth's biodiversity, as they are critical participants of the global energy flow and the material cycles. However, their spatial-temporal variations and interactions among varied biotopes in artificial habitat are poorly understood. In this study, we profiled the variations of bacterial communities among seasons and areas in the water and sediment of artificial reefs using 16S rRNA gene sequencing, and analyzed the interaction patterns of microorganisms based on three co-occurrence networks. Distinct bacterial community structures in the two biotopes were exhibited. The Shannon diversity and the richness of phyla in the sediment were higher, while the differences among the four seasons were more evident in the water. The seasonal variations of bacterial communities in the water were more distinct, while significant variations among four areas were only observed in the sediment. Correlation analysis revealed that nitrite and mud content were the most important factors influencing the abundant OTUs in the water and sediment, respectively. Potential interactions and keystone species were identified based on three co-occurrence networks. Results showed that the connections among bacterial communities in the sediment were less than in the water. In addition, the abundance of the top five abundant species and keystone species had different changing patterns among four seasons and four areas. These results enriched our understanding of the microbial structures, dynamics, and interactions of microbial communities in artificial habitats, which could provide new insights into planning, constructing and managing these special habitats in the future.

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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 interactions among varied biotopes in artificial habitat are poorly

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

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

24 analyzed the interaction patterns of microorganisms based on three co-occurrence networks.

25 Distinct bacterial community structures in the two biotopes were exhibited. The Shannon

26 diversity and the richness of phyla in the sediment were higher, while the differences among the

27 four seasons were more evident in the water. The seasonal variations of bacterial communities in

28 the water were more distinct, while significant variations among four areas were only observed

29 in the sediment. Correlation analysis revealed that nitrite and mud content were the most

30 important factors influencing the abundant OTUs in the water and sediment, respectively.

31 Potential interactions and keystone species were identified based on three co-occurrence

32 networks. Results showed that the connections among bacterial communities in the sediment

33 were less than in the water. In addition, the abundance of the top five abundant species and

34 keystone species had different changing patterns among four seasons and four areas. These

35 results enriched our understanding of the microbial structures, dynamics, and interactions of

36 microbial communities in artificial habitats, which could provide new insights into planning,

37 constructing and managing these special habitats in the future.

38 **Keywords:** Marine biodiversity, Habitat degradation, Artificial reefs, Interaction, Keystone

39 species

40

Introduction

41

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

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

44 2010; Todd et al., 2019). The decline of global biodiversity has resulted in changes in marine

45 ecosystem structures (Verity et al., 2002). Habitat loss and degradation are tricky problems that

46 must be faced to protect biodiversity in coastal waters worldwide (Jackson et al., 2001;

47 Koldewey et al., 2010). To recover marine habitats that provide feeding, spawning grounds, and

48 shelters, artificial habitats are constructed to support the functions of coastal ecosystems (Janiak

49 and Branson, 2021). Artificial reefs (ARs) offer a series of ecological services like improving

50 marine habitats, increasing fishery resources, and manipulating assemblages of ocean organisms

51 (Seaman and Sprague, 1993; Lima et al., 2019), which is an important form of artificial habitats

52 in marine fisheries. ARs facilitate biological production and enhance fishery resources by

53 creating additional habitats for marine organisms (Ng et al., 2017). At present, ARs have been

54 deployed worldwide by creating different structures related to fishing, scuba diving and coastal

55 recreation (Lima et al., 2019). In China, the practice of deploying ARs to create artificial habitats

56 has been proposed by the government since the 1970s (Sun et al., 2017). The annual investment

57 in ARs is about 100 million dollars in recent years. The primary purposes of constructing ARs

58 are conserving marine environment, enhancing fishery resources, and developing recreational

59 fishing (Yang et al., 2019; Xu et al., 2021). With the increasing attention to ARs, most studies

60 have focused on the behaviors of targeted species (Williams-Grove and Szedlmayer, 2017),

61 designs and engineering (Woo et al., 2014), ecological impacts (Shin et al., 2014), fishery

62 management (Lima et al., 2020) and others (Chen et al., 2013). However, our understanding of

63 the bacterial community structure in ARs is still limited.

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

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

66   Recognizing that the functions of microbial communities are essential to master the restoration

67   mechanism of artificial habitats. Scientific attempts have been made to isolate and characterize

68   particular microbiomes to explain the ecological roles of artificial habitats in the 1990s (Zentgraf

69   et al., 1992). Then, the dynamics of bacterial communities associated with coral reefs (natural

70   habitats) and artificial habitats were compared to verify the ecological effects of two habitats

71   using terminal restriction fragment length polymorphisms (Soka et al., 2011). Nowadays, high-

72   throughput sequencing has dramatically facilitated the understanding of the mechanisms of

73   marine microbial ecology (Langille et al., 2013). Despite great advancements, studies focusing

74   on the bacterial communities in the water and sediment of ARs are limited. Wang et al. (2019c)

75   assessed the impact of ARs on bacterial communities in the sediment to reveal the changes of

76   microbial structure and functions. Qin et al. (2019) and Zhu et al. (2020) studied the shifts of

77   protist communities among habitats and their response to 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 km². 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 m³

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

96   For decades, artificial reefs have been deployed worldwide around coasts to recover marine

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

98   benthos, and nekton (Aleksandrov et al., 2002; Scott et al., 2015; Ng et al., 2017; Chen et al.,

99   2019). However, few studies have examined the microbial communities. This study elucidated

100   the bacterial communities in the water and sediment of artificial habitat with two types of ARs

101   using 16S 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 species in the ARs.

106

107   **Materials and Methods**

108   **Study sites and sample collection**

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

110   in January (winter), May (spring), August (summer), and November (autumn), 2020 (Fig. S1).

111   Ninety-six samples from four sampling areas in ARs were studied: rock reefs (RR), transition

112   areas (TA), concrete reefs (CR) and adjacent areas (AA). For the water samples, we used a

113   plexiglass to collect 2 L bottom water at every sampling area. For the sediment samples, grab

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

115   and sediment samples were stored at a cooler filled with ice and immediately transported back to

116   the laboratory in two hours. The methods for treating samples were followed as previously

117   described in Fang et al. (2021). All field experiment was permitted by "Measures for annual

118   evaluation and reexamination of national marine ranching demonstration areas", which was

119   promulgated by 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-

126   L 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

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

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

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

138 deionized 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 we chose was 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-

143 GGACTACHVGGGTWTCTAAT-3′), which amplified 468 bp in V3-V4 hypervariable region

144 of 16S rRNA gene. The procedures of the PCR amplification was based on the manufacturer's

145 instructions and the standard protocols of Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai,

146 China). Raw sequence reads were analyzed using QIIME 1.9 (Caporaso et al., 2010). As for the

147 detailed processes, that were all described in Supp. File 1. The sequencing data have been

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

149 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   Three co-occurrence networks were built using Cytoscape 3.8.2 for the all bacterial

166   communities in the water, sediment and both two habitats (Michael et al., 2011). To improve the

167   visibility and sensitivity of the networks, OTUs observed in more than 50% of all samples and

168   the mean relative abundance higher than 0.5‰ were selected. Then, we calculated the Spearman

169   correlations and significances between OTUs. Significant edges ($P < 0.001$) with high

170   correlations ($|r| > 0.8$) were chose to construct the co-occurrence networks. The network

171   topology parameters were calculated using the Analyze Network plugin to identify the keystone

172   OTUs in the networks (Cheung et al., 2018). Three topology parameters were: (1) degree: the

173   number of edges that a node has; (2) betweenness centrality: the number of shortest paths

174   between any two nodes in the graph passing through a node; (3) closeness centrality: the average

175   distance of a node to any other node. After that, min-max scaling was applied to standardize

176   degree, 1-betweenness centrality, and closeness centrality, respectively. Last, keystoneness was

177   defined as the average of degree, 1-betweenness centrality, and closeness centrality after min-

178   max scaling was calculated (Berry and Widder, 2014); and the OTUs with top 10 highest scores

179   were identified as the keystone species (Cheung et al., 2018). Jaccard index was applied to

180   analyze the similarities of OTUs among networks (Dmitry et al., 2016).

181

182   **Results**

183   **Bacterial alpha diversities and community compositions**

184   A total of 5,427,513 high-quality bacterial 16S rRNA gene sequences and 17207 OTUs with

185   97% similarity levels were identified from 96 water and sediment samples. Sediment samples

186   (15010 OTUs) had higher OTUs than water samples (9423 OTUs), and 7226 OTUs were shared

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

188   current sequencing depth was sufficient for this study. Alpha diversity indices showed significant

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

190   four areas. The Shannon, Chao 1 and Ace of water samples were significantly lower than

191   sediment, while Simpson and Good's coverage of water were higher (Table 1). A seasonal

192   pattern of alpha diversity indices (e.g., Shannon) could be observed in the water samples, which

193   varied from 4.09 to 5.16 (Fig. 1). However, no significant seasonal differences were found in the

194   sediment except for the Simpson (Table 1).

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

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

197   phyla was over 74% for the water samples, while those were about 50% for the sediment

198   samples. For the water samples, the three most abundant phyla (>10%) were Proteobacteria,

199   Cyanobacteria, and Actinobacteria, with mean relative abundances of 42.8%, 18.3% and 13.1%,

200   respectively (Fig. 2). Marked seasonal changes were observed in many bacterial phyla. The

201   phylum Proteobacteria had a relative abundance greater than 56.8% in autumn, while its

202   abundance was 23.6% in summer; the phyla Actinobacteria and Cyanobacteria had the highest

203   abundances in summer (20.9% and 29.4%) and the lowest in winter (6.6% and 13.2%). In the

204   sediment, the bacterial community was dominated (>10%) by the phyla Proteobacteria,

205   Desulfobacterota, and Acidobacteria, which comprised approximately 28.4%, 10.8%, and 10.2%

206   of the relative abundances, respectively (Fig. 2). The relative abundance of the phylum

207   Chloroflexi (mean abundance 8.6%, ranked 4th) had marked seasonal changes, whose abundance

208   in autumn (12.0%) was twice that in winter (6.7%).

209   **Bacterial community structure**

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

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

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

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

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

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

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

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

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

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

220   bacterial communities in the areas RR and TA were different from areas CR and AA in the water

221   samples (Fig. S3a); area CR was separate from other three areas in the sediment (Fig. S3b).

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

223   The relationships among bacterial communities and environmental factors were significant

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

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

226 Temp, Trans, PO_4, DO, SiO_3, and COD were significant correlating factors. In the sediment, MC

227 was the only factor that significantly impacted the bacterial community. The relative abundances

228 of the top abundant OTUs were noticeably related to the environmental factors (Fig. 5). For the

229 water samples, thirty-three OTUs were significantly correlated with Temp, while only two OTUs

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

231 EC, and four OTUs were related to WC and OM (Fig. 5b). What's more, two bacterial groups

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

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

234 sediment samples, correspondingly.

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

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

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

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

239 S5). For the total bacteria network, 185 nodes belonging to 18 phyla and 6832 edges were

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

241 (Fig. 6a). The most abundant OTUs in the network were OTU18562 (Firmicutes), OTU18460

242 (Cyanobacteria), OTU10090 (Proteobacteria), OTU17592 (Proteobacteria), and OTU18751

243 (Actinobacteria). Four OTUs mainly from water samples exhibited seasonal variations in their

244 abundances; OTU10090 was from sediment samples, which had no seasonal change (Fig. 7a).

245 Differences of five abundant OTUs among four areas were not significant except OTU18562

246 (Fig. 8a). The bacterial network for the water samples comprised 189 nodes belonging to 15

247 phyla, while only 1772 edges were observed (Fig. S4). OTU18562 (Firmicutes), OTU14763

248 (Cyanobacteria), OTU17592 (Proteobacteria), OTU18751 (Actinobacteriota), and OTU18053

249   (Proteobacteria) had the highest abundances in the network. Similar seasonal changes of

250   OTU18562 and OTU17592 in abundance were observed, and the other three OTUs showed

251   similar trends (Fig. 7b). OTU18562 showed a most apparent difference among four areas than

252   other four abundant OTUs (Fig. 8b). For the sediment bacterial network, 274 nodes belonging to

253   24 phyla and 1166 edges were observed (Fig. S5). The top five most abundant OTUs in this

254   network were OTU10090 (Proteobacteria), OTU3621 (Desulfobacterota), OTU13814

255   (Myxococcota), OTU13137 (Desulfobacterota), and OTU6550 (Chloroflexi). The seasonal

256   variations of the five OTUs were not evident (Fig. 7c), and OTU10090 showed lower

257   abundances in areas RR and CR than in TA and AA (Fig. 8c).

258   The top keystone OTUs with relative abundances of more than 1‰ were identified as the

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

260   top 10 keystone OTUs were affiliated to five phyla, Myxococcota, Proteobacteria,

261   Gemmatimonadota, Chloroflexi and Actinobacteriota (Table S2a). The capital keystone OTU

262   was OTU13814 (Myxococcota, keystoneness = 0.840), which ranked 15 among all OTUs with a

263   relative abundance of 12.3‰; the abundance OTU12321 (Proteobacteria, keystoneness = 0.836)

264   ranked 150 with a relative abundance of 1.8‰. There was no significant seasonal abundance

265   variation for the top five keystone except OTU13814, and no evident differences among the four

266   areas were found (Fig. S6a, S7a). For the water network, the top 10 keystone OTUs included

267   members of the phyla Bacteroidota, Proteobacteria, Desulfobacterota, Gemmatimonadota and

268   Acidobacteriota (Table S2a). The top two keystone OTUs (OTU525 and OTU7969) had

269   abundances of 2.3‰ (ranked 90) and 1.4‰ (ranked 123), respectively. Similar seasonal trends of

270   the abundance for the top five keystone OTUs were observed, which showed higher abundance

271   in spring and autumn (Fig. S6b). For the sediment network, the top 10 keystone OTUs belonged

272   to three phyla, Proteobacteria, Firmicutes and Chloroflexi (Table S2c). OTU6991 (abundance

273   2.3‰, ranked 114) and OTU3902 (abundance 8.8‰, ranked 24) were the only two keystone

274   OTU with keystoneness greater than 0.7. The changing patterns among the four seasons and four

275   areas for keystone OTU17592, OTU16870, and OTU17616 were consistent (Fig. S6c and Fig.

276   S7c).

277
278   **Discussion**

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

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

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

282   2007). In this study, Proteobacteria, Cyanobacteria, and Actinobacteria were the most abundant

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

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

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

286   water and sediment, and five-sixteenths phyla in the water had higher relative abundances than

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

288   samples may be the main reason. The higher abundance of Proteobacteria (42.8%) in the water,

289   which restricted the population size of other bacterial phyla. Many studies showed similar results

290   (e.g., Sun et al., 2019). Higher diversities of the bacterial communities were observed in the

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

292   (2009) and Abia et al. (2017). Some reasonable assumptions were proposed. Ye et al. (2009)

293   stated that sediments within aquatic environments formed more complex environments, which

294   resulted in more prosperous bacterial communities; Perkins et al. (2014) noted that sediment

295   provided shelters for bacteria, which helped to defend against the impacts of predation and

296   sunlight; Liu et al. (2018) explained that lower concentration of suspended sediment in the water

297   would lead to lower microbial diversity.

298   The heatmap of the abundant OTUs with four areas revealed bacterial abundance in RR was

299   inconsistent with other areas, especially in the sediment (Fig. S3). For one thing, the aquaculture

300   activities of *A. japonicus* in RR might result in these distinctions. The diet preference of *A.*

301   *japonicus* can change the microbial communities in the sediment, which have been investigated

302   thoroughly (e.g., Zhao et al., 2020). For another thing, rock reefs were deployed more intensive

303   than concrete reefs, which might impact the bacterial communities strongly. As for the

304   differences of bacterial abundance among four areas, some inconsistencies were observed. For

305   instance, OTU1283 (Actinobacteriota) showed significantly higher abundance in RR and CR

306   than that in TA and AA, which suggested that it favored ARs. The phylum Actinobacteria plays

307   important roles in mineralizing organic matter in marine sediment (Bell et al., 1998), indicating

308   that the organic matter content in the ARs was higher than that in typical marine sediments.

309   OTU7413 (family Rhodobacteraceae) was regarded as an important food resource for *A.*

310   *japonicus* (Zhao et al., 2020), which showed a lower abundance in RR than other three areas.

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

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

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

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

315   dissimilarities among microbial communities did not significantly correlated with environmental

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

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

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

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

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

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

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

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

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

325   Shannon diversity occurred in spring. Similar seasonal patterns in the Shannon diversity index

326   have been observed in other seawaters (Piwosz et al., 2018; Zhu et al., 2020). Previous studies

327   indicated that sudden decay of microbial plankton communities in winter and new growing

328   season in spring preceding the spring bloom (Espinoza-González et al., 2012; Figueiras et al.,

329   2020) could promote these seasonal patterns. Therefore, higher diversity in spring can be

330   explained by the intrinsically high growth rate of bacterioplankton (Agawin et al., 2000).

331   However, no significant differences of bacterial diversity in the sediment were detected. Marine

332   sediment is characterized as a habitat type with high biodiversity, complicated community

333   structures and spatial heterogeneity (Brandt et al., 2016). The bacterial communities in the

334   sediment had wider niche ranges and sufficient nutrients, which helped maintain and facilitate

335   prosperous diversities (Shu et al., 2020).

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

337   water samples, our results showed that Actinobacteria had the highest abundance in summer,

338   when the salinity was lowest because of higher precipitation level. Shen et al. (2018) confirmed

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

340   Cyanobacteria occurred in summer. Martin et al. (2017) also reported that the autotrophic

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

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

343   with water samples. Phylum Chloroflexi was the most abundant in autumn. Chloroflexi is

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

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

346   probably explain their higher abundances of Chloroflexi.

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

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

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

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

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

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

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

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

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

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

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

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

359   suggested.

360   **Environmental characteristics and bacterial communities**

361   Environmental factors significantly affected bacterial communities, such as nitrite, water

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

363   (Lee et al., 2020). In the present study, the mantel test revealed that nitrite, temperature, and

364   transparency significantly correlated with the communities in the water, while mud content was

365 the only related factor in the sediment (Table 3). Although N and P are limiting factors of

366 eutrophication, they are important nutrients affecting the proliferation of bacteria (Huang et al.,

367 2017). Our results revealed that dissolved inorganic nitrogen (NO_3-N, NH_4-N, NO_2-N) was

368 correlated with many abundant OTUs (Fig. 5a). Water temperature is an important factor that

369 governs microbial growth and activity (Margesin, 2009), and thirty-three abundant OTUs were

370 significantly related to temperature. Varied light intensities may shape different microbial

371 communities (Davies and Evison, 1991), and our studies confirmed that the correlation between

372 transparency and the bacterial communities was significant. Different electrical conductivity

373 levels can influence bacteria (Silverman and Munoz, 1975), which was in accordance with the

374 results that the abundances of thirteen dominant OTUs were affected by EC. Mud content was a

375 vital factor influencing microorganisms, higher content could offer more substrates, which was

376 meaningful to support greater microbial biomass (Wei et al., 2014).

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

378   Although the physical and chemical properties between water and sediment were

379 fundamentally different, the microbial communities are connected through the sedimentation of

380 organic matters (Lucie et al., 2011). To reveal the potential interactions among the bacterial

381 communities in two habitats, co-occurrence networks were used to promote our understanding of

382 the microbiomes in marine ecosystems (Berry and Widder, 2014). Species similarity between the

383 water and sediment networks was low (Jaccard = 11.8%), and the similarity between the water

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

385 total bacterial network (31.5%). These results indicated that habitats significantly affected the

386 compositions of microorganisms in the co-occurrence networks, which was consistent with

387   Zhang et al. (2020a). Our results also suggested that environmental filtering plays a vital role in

388   shaping microbial community compositions (Williams et al., 2014).

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

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

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

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

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

394   resistance (Zhang et al., 2021). Significant seasonality of the top five abundant OTUs for the

395   networks was exhibited, while the dissimilarities of their abundance among the four areas were

396   relatively low (Fig. 7 and Fig. 8). OTU18562 was the most abundant OTU in the total network

397   and water network, which showed evident temporal and spatial differences, concurrently.

398   OTU18562 affiliates to family Planococcaceae, which was correlated with the decomposition of

399   fertilizers (Suzuki et al., 2021). OTU17592 had higher abundance in RR and CR than in TA and

400   AA in the total network and water network, indicating positive relationships between OTU17592

401   and ARs existed. OTU18751 (family Microtrichales), which showed the highest abundance in

402   summer and lowest abundance in spring, played crucial roles in hydrolyzing and utilizing

403   complex organic matters (Li et al., 2021).

404   Keystone species are highly connected taxa in networks that play crucial roles in maintaining

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

406   co-occurrence networks were significantly different. Differences of key microbial groups at ARs

407   and open waters have also been reported (Zhu et al., 2020). The ranks of relative abundance for

408   the top ten keystone OTUs in the three network were low (Table S2). Two explanations could

409   explain this phenomenon. On the one hand, it was suggested that abundant bacteria contribute

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

411   community. Moreover, rare species may be disturbed by the rapid changes of the environment

412   (Shade et al., 2014). On the other hand, rare taxa could considerably increase in abundance to

413   respond the environmental disturbances; and these dynamics may be the reasons for their greater

414   contributions to microbial communities (Caron and Countway, 2009).

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

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

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

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

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

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

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

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

423   S6c). Three keystone OTU (OTU17592, OTU16870 and OTU17616) in the sediment network

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

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

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

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

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

429   keystone OTUs were also crucial in the particular ecological process. For instance, the

430   abundance of OTU7413 (family Rhodobacteraceae) ranked 137th in the water network (Table

431   S2b), which was regarded as the key member of the initial microbial biofilm in coastal seawater

432 (Hila et al., 2013). OTU11572 and OTU8614 belong to family Thermoanaerobaculaceae, may

433 play key roles in nitrogen transformation in water (Wang et al., 2019a).

434

435 **Conclusions**

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

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

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

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

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

441 deployment of ARs impacted the sediment bacteria. The correlations among bacterial

442 communities in the sediment were lowest in the three networks, suggesting less niche overlap

443 existed. The abundant and keystone species in the networks showed discordant variations among

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

445 functions. ARs as an important artificial habitat in marine fishery, our discoveries are crucial to

446 better comprehend the mechanisms of microbial ecology in artificial habitats, to promote

447 restoration efficiency, and to improve biodiversity and recover the marine environment. In

448 particular, marine microbial loops are comprehensive networks containing bacteria, archaea, and

449 eukaryotes, and further studies are needed to reveal the interactions among these microbiomes in

450 artificial habitats.

451

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457

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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.
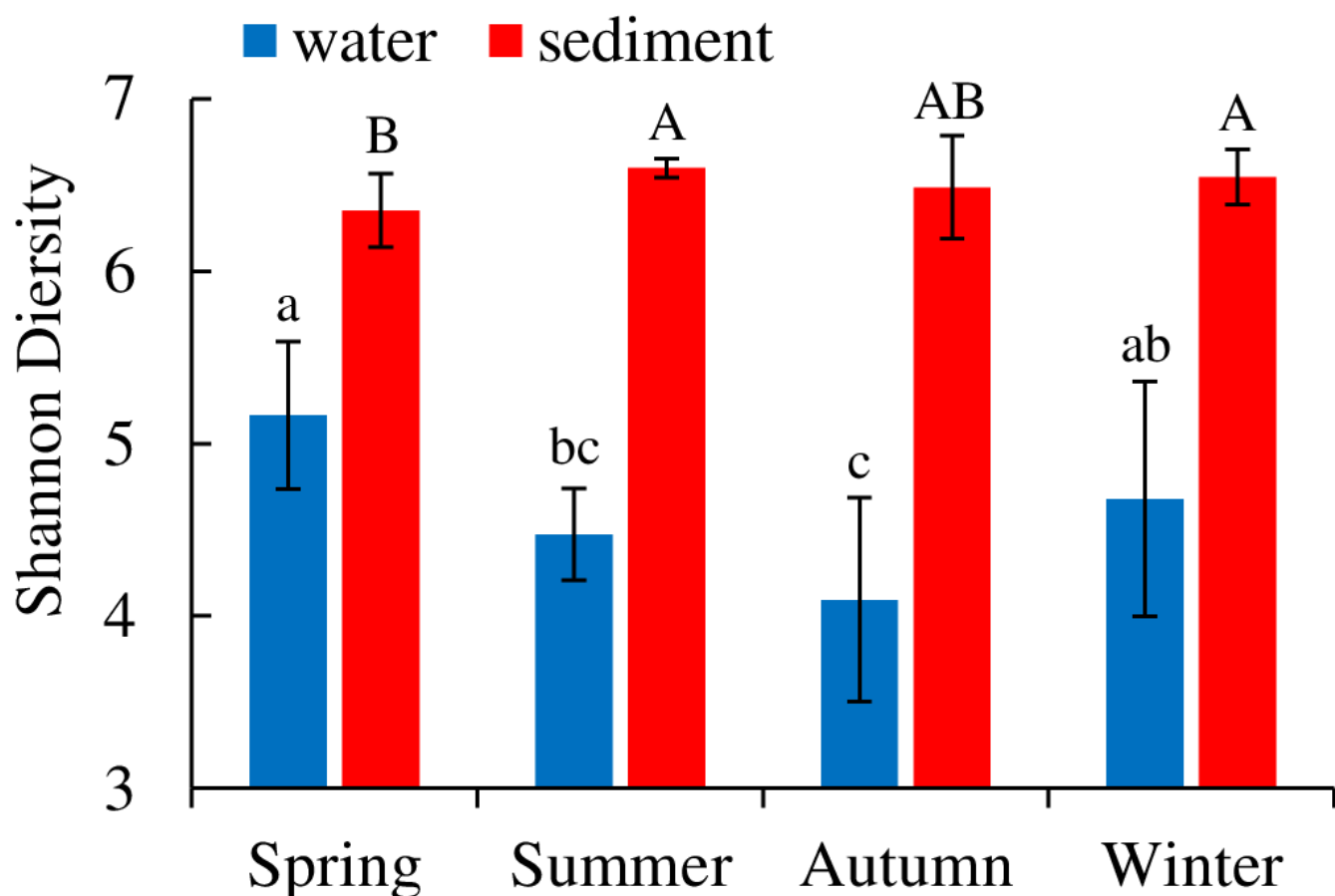
Figure 5

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)

Class

OTU17369 Alphaproteobacteria
OTU16031 Cyanobacteriia
OTU18751 Acidimicrobiia
OTU18022 Acidimicrobiia
OTU18460 Cyanobacteriia
OTU14763 Cyanobacteriia
OTU16379 Gammaproteobacteria
OTU16046 Alphaproteobacteria
OTU18180 Actinobacteria
OTU17767 Gammaproteobacteria
OTU16371 Gammaproteobacteria
OTU15592 Gammaproteobacteria
OTU18053 Gammaproteobacteria
OTU18466 Actinobacteria
OTU14778 Bacilli
OTU18546 Acidimicrobiia
OTU16727 Gammaproteobacteria
OTU17917 Gammaproteobacteria
OTU14911 Acidimicrobiia
OTU16076 Gammaproteobacteria
OTU17592 Gammaproteobacteria
OTU18494 Cyanobacteriia
OTU19453 Alphaproteobacteria
OTU16155 Gammaproteobacteria
OTU17521 Alphaproteobacteria
OTU19164 Gammaproteobacteria
OTU18514 Gammaproteobacteria
OTU17166 Actinobacteria
OTU18450 Alphaproteobacteria
OTU18562 Bacilli
OTU17239 Bacilli
OTU18468 Bacilli
OTU10090 Gammaproteobacteria
OTU17616 Gammaproteobacteria
OTU16870 Gammaproteobacteria
OTU15105 Cyanobacteriia
OTU19096 Cyanobacteriia
OTU3095 Cyanobacteriia
OTU16224 Gammaproteobacteria

Temp Trans COD SiO$_3$ SPM TOC NH$_4$-N Dep Chla DO NO$_2$-N Turb PO$_4$

(b)

Class

OTU3902 Gammaproteobacteria
OTU13814 Polyangia
OTU6550 Anaerolineae
OTU6409 Desulfobulbia
OTU9361 Gammaproteobacteria
OTU14579 Gammaproteobacteria
OTU12291 Gammaproteobacteria
OTU13137 Desulfobulbia
OTU13914 Gammaproteobacteria
OTU11572 Thermoanaerobaculia
OTU956 Desulfobacteria
OTU10090 Gammaproteobacteria
OTU16612 Bacteroidia
OTU13624 Campylobacteria
OTU525 Bacteroidia
OTU17166 Actinobacteria
OTU10279 Thermodesulfovibrionia
OTU9985 PAUC43f_marine_benthic_group
OTU3417 Alphaproteobacteria
OTU3621 Syntrophobacteria
OTU4433 Alphaproteobacteria
OTU4443 Alphaproteobacteria
OTU8614 Thermoanaerobaculia
OTU5350 Alphaproteobacteria
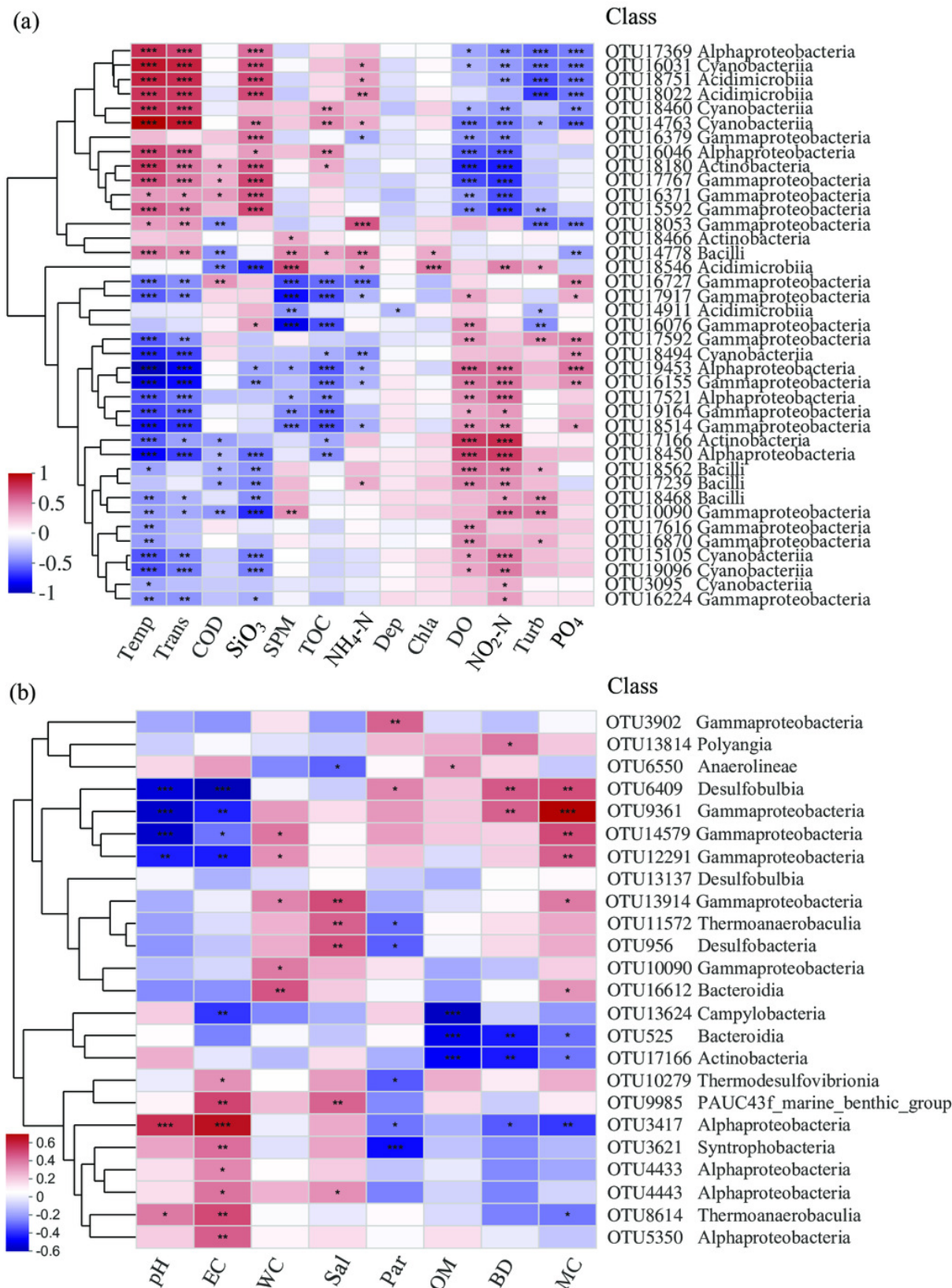
pH EC WC Sal Par OM BD MC

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.
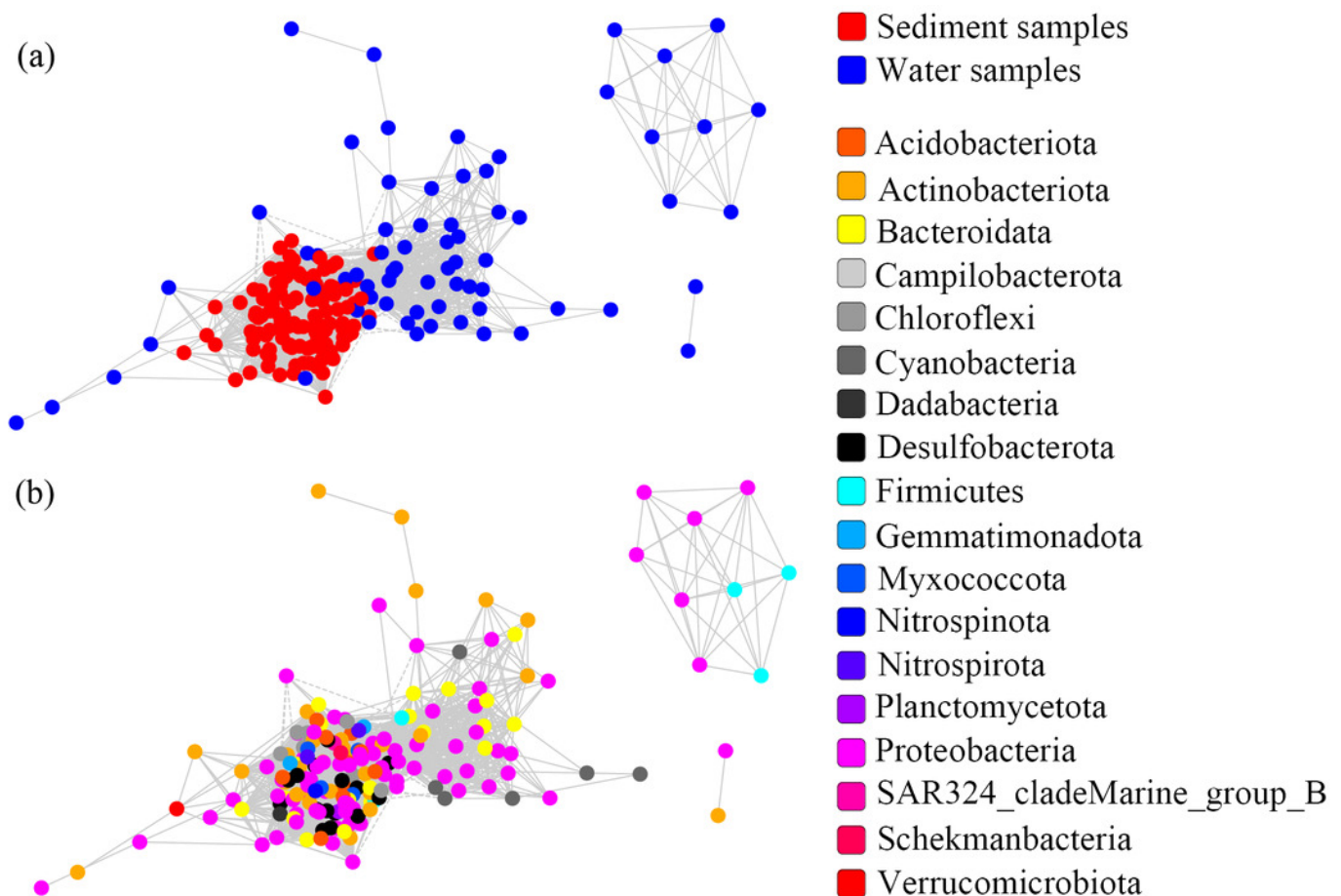
Figure 7

Seasonal changes of the relative abundance for the top 5 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
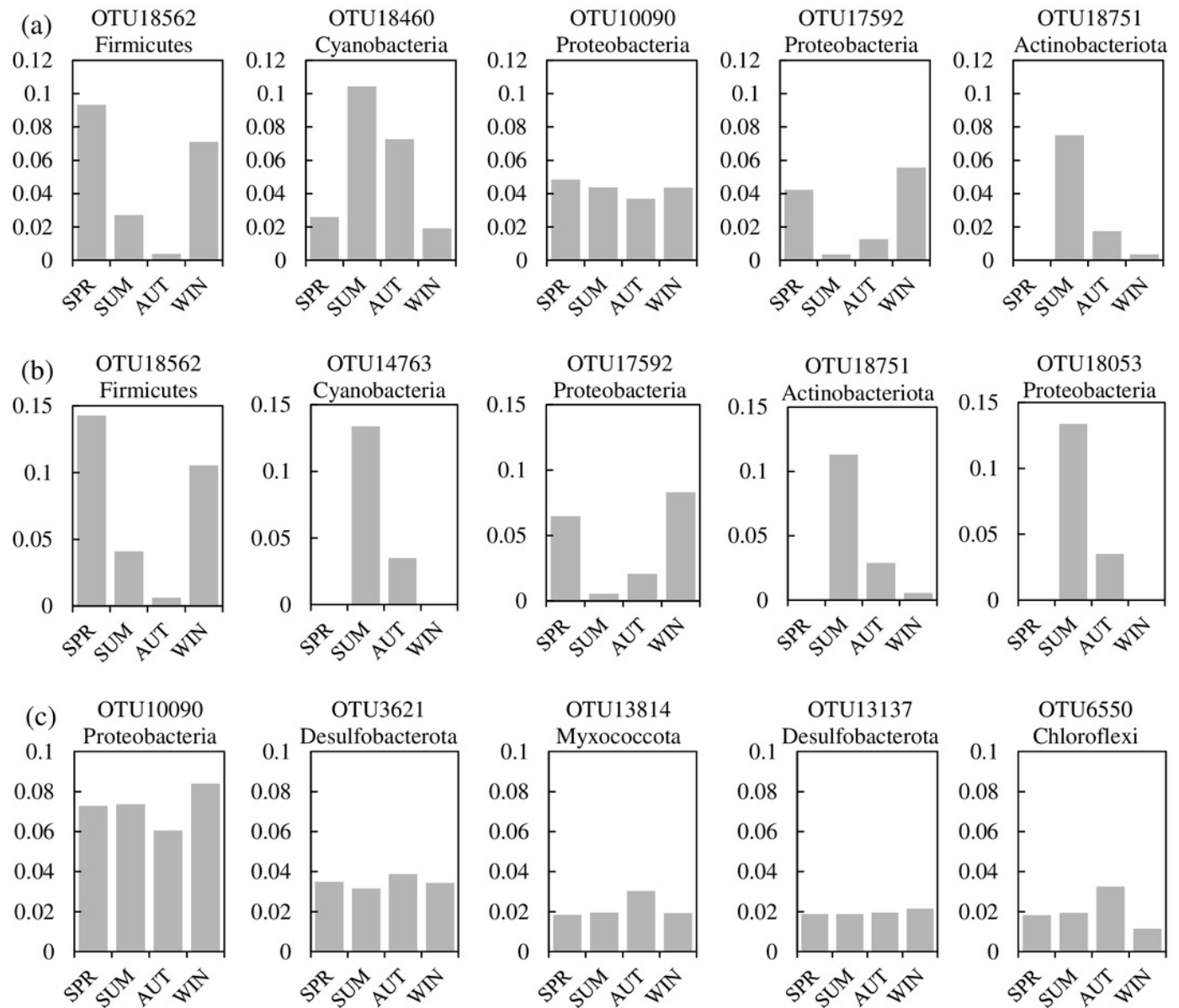Desulfobacterota

OTU6550
Chloroflexi

Figure 8

Variations of the relative abundance for the top 5 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).
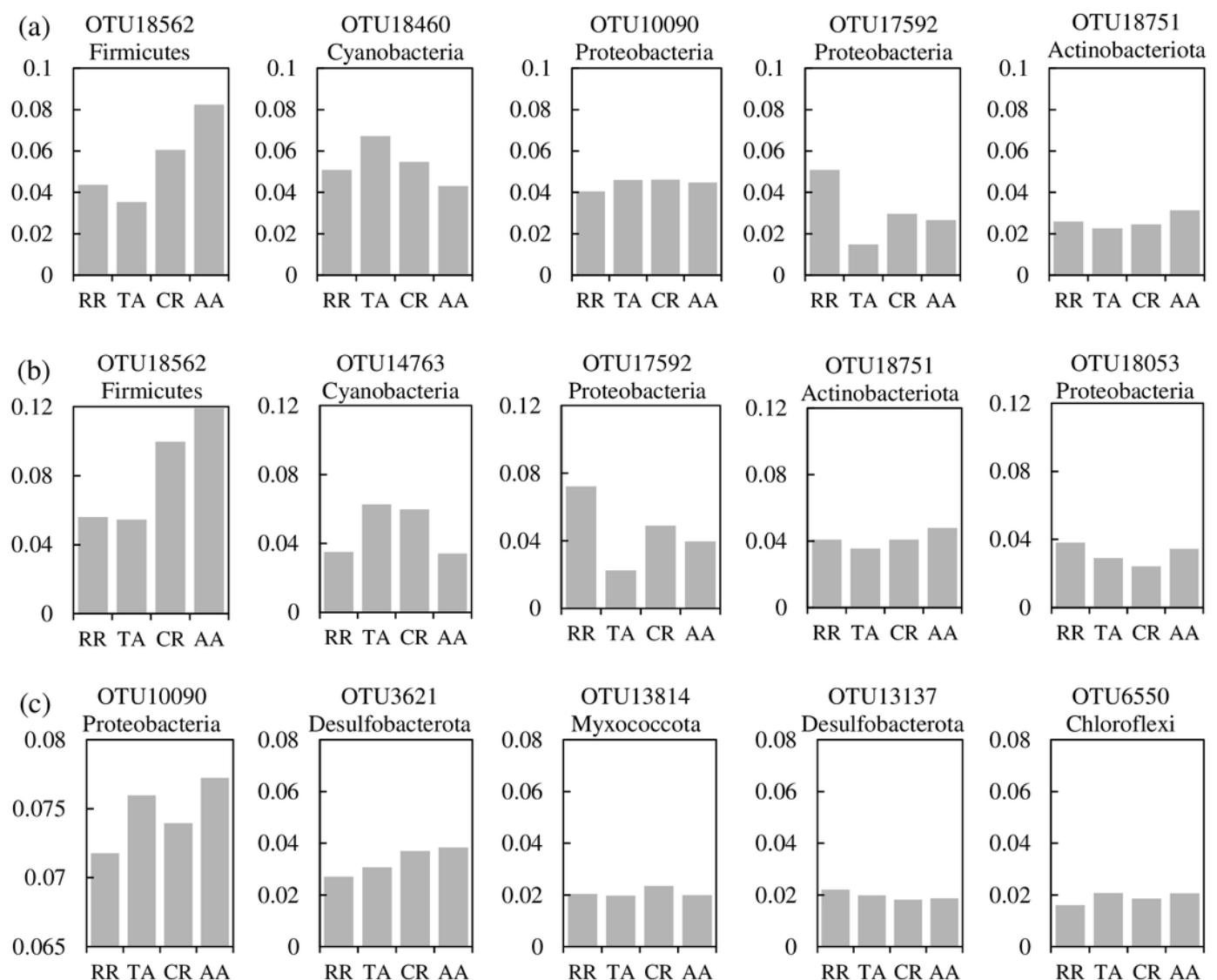
Table 1(on next page)

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 α = 0.05 level.

1

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	

2

3

Table 2(on next page)

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.

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1

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	-	-

2

Table 3(on next page)

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 α = 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).

1

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			

2

3