Characteristics of planktonic and sediment bacterial communities in a heavily polluted urban river

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Urban rivers represent a unique ecosystem in which pollution occurs regularly, altering the biogeochemical characteristics of waterbodies and sediments. However, little is presently known about the spatiotemporal patterns of planktonic and sediment bacterial community diversities and compositions in urban rivers. Herein, Illumina MiSeq high-throughput sequencing was performed to reveal the spatiotemporal dynamics of bacterial populations in Liangtan River, a heavily polluted urban river in Chongqing City (China). The results showed the richness and diversity of sediment bacteria were significantly higher than those of planktonic bacteria, whereas a strong overlap (46.7%) in OTUs was identified between water and sediment samples. Bacterial community composition remarkably differed in waters and sediments. Planktonic bacterial communities were dominated by *Proteobacteria, Bacteroidetes*, *Cyanobacteria* and *Actinobacteria,* while sediment bacterial communities mainly included *Proteobacteria, Actinobacteria, Chloroflexi* and *Bacteroidetes*. Additionally, several taxonomic groups of potential bacterial pathogens showed an increasing trend in water and sediment samples from residential and industrial areas (RI). Variation partition analysis (VPA) indicated that temperature and nutrient were identified as the main drivers determining the planktonic and sediment bacterial assemblages. These results highlight that bacterial communities in the polluted urban river exhibit spatiotemporal variation due to the combined influence of environmental factors associated with sewage discharge and hydropower dams.

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Abstract

Urban rivers represent a unique ecosystem in which pollution occurs regularly, altering the biogeochemical characteristics of waterbodies and sediments. However, little is presently known about the spatiotemporal patterns of planktonic and sediment bacterial community diversities and compositions in urban rivers. Herein, Illumina MiSeq high-throughput sequencing was performed to reveal the spatiotemporal dynamics of bacterial populations in Liangtan River, a heavily polluted urban river in Chongqing City (China). The results showed the richness and diversity of sediment bacteria were significantly higher than those of planktonic bacteria, whereas a strong overlap (46.7%) in OTUs was identified between water and sediment samples. Bacterial community composition remarkably differed in waters and sediments. Planktonic bacterial communities were dominated by *Proteobacteria, Bacteroidetes*, *Cyanobacteria* and *Actinobacteria,* while sediment bacterial communities mainly included *Proteobacteria, Actinobacteria, Chloroflexi* and *Bacteroidetes*. Additionally, several taxonomic groups of potential bacterial pathogens showed an increasing trend in water and sediment samples from residential and industrial areas (RI). Variation partition analysis (VPA) indicated that season and nutrient were identified as the main drivers determining the planktonic and sediment bacterial assemblages. These results highlight that bacterial communities in the polluted urban river exhibit spatiotemporal variation due to the combined influence of environmental factors associated with sewage discharge and hydropower dams.

Keywords: Urban river; Bacterial community structure; Diversity; Illumina MiSeq high-throughput sequencing; Sewage discharge

Introduction

Bacteria are the important components of river ecosystems and play a vital role in biogeochemical processes of nutrients and biotransformation of organic matter (*Madsen et al., 2002; Wang et al., 2017*). Bacteria communities are extremely sensitive to environmental perturbations and serve as potential integrative indicators for measuring the stability of river ecosystems (*Marshall et al., 2008; Zhang et al., 2016*). Therefore, it is particularly critical to unravel the bacterial community diversity and structure to understand the responses of bacteria to elevated environmental pressures in urban rivers (*Crump et al., 2007; Quan et al., 2010*).

Several previous studies have documented the shift in planktonic bacterial community diversity and composition can be influenced by water physicochemical properties including organic matter, nitrogen, phosphorus, pH, and temperature (*Esteves et al., 2015; Fortunato et al., 2013; Liu et al., 2012; Paudel et al., 2019; Yang et al., 2019; Zhang et al., 2019*). Some of these influential factors might collectively regulate planktonic bacterial assemblages in river ecosystems. Moreover, the distribution of sediment bacterial community in river ecosystems has received increasing attention. A number of determined environmental factors have been proposed to play a pivotal role in river sediment bacterial communities including organic matter, nitrogen, phosphorus, pH and heavy metal (*Bouskill et al., 2009; Peng et al., 2019; Su et al., 2019; Xia et al., 2014; Xie et al., 2016*). However, water column and sediments are two different habitats in river ecosystems, characterized by distinctive properties, which result in profound dissimilarity of planktonic and sediment bacterial communities (*Liu et al., 2018; Mao et al., 2019*). Thus, there is a need for comparative analysis of planktonic and sediment bacterial

61   communities in river ecosystems and their driving factors, given the heterogeneity of habitats in

62   space and time.

63

64   Compared with natural aquatic ecosystems, urban rivers represent a heterogeneous ecosystem

65   that is mainly influenced by the surrounding terrestrial environment (*Kaestli et al., 2017*).

66   Sewage from the residential and industrial areas containing excessive pollutants notably alters

67   the basic parameters of water and sediments, such as dissolved oxygen, nitrogen, temperature,

68   and pH, which could further reshape the bacterial community structure and diversity (*Drury et*

69   *al., 2013; Esteves et al., 2015; Mark Ibekwe et al., 2016*). Sewage also introduces

70   microbiological contamination (i.e. bacterial pathogens) to the urban aquatic ecosystems which

71   can impact the composition and function of bacterial community (*Drury et al., 2013; Mark*

72   *Ibekwe et al., 2016*). Additionally, construction of dams considerably modifies water discharge,

73   regulate flow circulation and disturb nutrient transport by retaining suspended materials, thereby

74   altering the hydrodynamic condition of rivers and the distribution of bacterial community (*Dai et*

75   *al., 2013; Yan et al., 2015*). Substantial differences in bacterial community composition have

76   been observed between the upstream and downstream of dams (*Ruiz-Gonzalez et al., 2013; Liu*

77   *et al., 2018*). However, the joint effects of sewage discharge and dam construction on bacterial

78   communities in a certain urban river remain inadequately understood.

79

80   Considering this background, the biogeography of bacterial communities was studied in Liangtan

81   River, a heavily polluted urban river in Chongqing City, China. Specifically, the aim of this

82   current study was to investigate the spatiotemporal dynamics of both planktonic and sediment

83   bacterial populations in the urban river and their responses to dam construction and sewage

84 discharge. Our findings will improve the understanding of multiple environmental gradients on

85 bacterial communities and provide an ecological reference for assessing urban aquatic

86 ecosystems.

87

88 **Materials & Methods**

89 *Sites description and sample collection*

90 Liangtan River (watershed area of 498 km^2 and mainstream length of 88 km), located in

91 Chongqing City (China), is one of major tributaries of Jiangling River. As a typical urban river,

92 Liangtan River is completely surrounded by residential and industrial areas. Excessive

93 anthropogenic input results in the deterioration of this urban river. Based on the Environmental

94 Quality Standards for Surface Water of China, the water quality in all sections of Liangtan River

95 has always been classified as worse than grade 5 (*Ran et al., 2016*). In this study, five different

96 sampling sites were selected from upstream to downstream in Liangtan River (Figure 1). The

97 distance between two adjacent sampling sites is approximately 8-12km. Sites L1 and L2 are

98 located downstream and upstream of Xiema hydropower station (named as HS), respectively.

99 Sites L3, L4, and L5 are surrounded by residential and industrial areas (named as RI), where the

100 increasing anthropogenic activities have had a great impact on the health of the river ecosystem.

101 Additionally, Sites L3 and L4 are adjacent to Xiyong and Tuzhu sewage plants, respectively. The

102 sewage runoff from RI area is mainly composed of domestic pollution such as feces, nitrogen,

103 and phosphorus etc. Triplicate water samples (30 cm depth below water surface, about 10 L) and

104 surface sediments (0-10 cm) were collected from the five sampling sites of Liangtan River in

105 February (winter; the average air temperature is 10-25℃; the average rainfall is 59.8mm) and

106 August (summer; the average air temperature is 20-29℃; the average rainfall is 438.3mm) in

107   2019, using plexiglass water sampler and tube core sampler, respectively. These water samples

108   and surface sediments were placed in sterile containers and immediately transported to the

109   laboratory for subsequent chemical and molecular analysis.

110   The physicochemical properties (pH, temperature, ammonia nitrogen (NH_4^+-N) , nitrate nitrogen

111   (NO_3^--N), total nitrogen (TN), total phosphorus (TP), and total organic carbon (TOC)) of water

112   and sediment samples were measured following the methods described in literature (*China*

113   *Environmental Protection Agency, 2002; Wang, 2012*) and were shown in Tables S1 and S2,

114   respectively.

115

116   *Molecular analyses*

117   In the present study, 0.22-μm pore-size membrane (diameter 50 mm; Millipore) was used to

118   retain water microbial cells. Genomic DNA of water and sediment samples were extracted using

119   E.Z.N.A. Water DNA kit (Omega, USA) and Powersoil DNA extraction kit (Mobio

120   Laboratories, USA), respectively, according to manufacturer instructions. The V3-V4 region of

121   bacterial 16S ribosomal RNA (rRNA) gene was amplified using the primer sets 338F (5'-

122   ACTCCTACGGGAGGCAGCAG-3')/806R (5'- GGACTACHVGGGTWTCTAAT-3'), as

123   previously described (*Xu et al., 2016*). The amplicons from each triplicate sample were pooled in

124   equal amounts and sequenced on an Illumina MiSeq platform at Shanghai Majorbio Bio-pharm

125   Technology Co., Ltd (China). The obtained raw reads were deposited in the NCBI Sequence

126   Read Archive (SRA) database under accession numbers SRP248929 for water and sediment

127   samples. The reads from the original DNA fragments were merged using FLASH and further

128   processed following the protocol by *Caporaso et al. (2010)*. Chimeric reads were discarded

129   using UCHIME (*Edgar et al., 2011*). Sequences were assigned to their corresponding samples

130 according to the barcode sequence and then quality-trimmed with an average Phred quality score

131 cutoff of 20. After quality filtering and the removal of chimeric sequences, data analyses were

132 performed to standardize the sequencing effort across samples. Chimeric-free sequences sharing

133 ≥97 % similarity were grouped into operational taxonomic units (OTUs) and alpha-diversity

134 (Chao1 richness estimator, Shannon diversity index, and Simpson evenness index) was further

135 obtained using the UPARSE pipeline (*Edgar, 2013*). The representative sequences of each OTU

136 were classified with the Ribosomal Database Project (RDP) classifier (*Wang et al., 2007*).

137

138 *Statistical analysis*

139 One-way analysis of variance (ANOVA), followed by Fisher's least significant difference (LSD)

140 test, was used to determine significant differences (P < 0.05) between alpha-diversity indices

141 and the relative abundances of potential bacterial pathogens among sample groups. Potential

142 relationships between physicochemical properties and (i) bacterial community richness; (ii)

143 diversity; and (iii) the proportion of the major bacterial groups were examined with Spearman

144 rank correlation analysis using the software SPSS 20.0.

145 To elucidate the microbial interactions of different environments, molecular ecological networks

146 were constructed in Molecular Ecological Network Analysis pipeline (MENA,

147 http://ieg4.rccc.ou.edu/mena/). Person correlation analysis based on random matrix theory

148 (RMT) was used to construct networks. Each network analysis used bacterial OTUs data from

149 ten samples in every ecosystem. OTUs detected in more than half of the samples were kept for

150 the network construction as default settings in the pipeline to ensure the accuracy of OTUs

151 interrelationships. Global network properties, individual nodes' centrality, and module separation

152 and modularity were calculated. Among these properties, each node is an estimable molecular

153 marker (e.g. OTU) and each edge (e.g. interaction) represents the strength of the correlation

154 between them. Logarithm transitivity was used to infer the stability of the community. The

155 constructed network graphs were visualized by Cytoscape (version 3.7.2) and Gephi software

156 (version 0.9.2).

157 To examine the difference in the overall community composition between each pair of samples

158 at the OTU level, Beta-diversity using Bray-Curtis dissimilarities (*Bray and Curtis,1957*) was

159 used to examine the difference in the overall community composition between samples and

160 visualized on a PCoA plot. Permutational multivariate analysis of variance (PerMANOVA) was

161 performed to test for significant differences between sample groups based on Bray-Curtis

162 distance matrices. Multivariate analysis was used to determine which physicochemical properties

163 had the biggest impact on planktonic and sediment bacterial communities. Detrended

164 correspondence analysis (DCA) was first applied to determine the suitable ordination analysis

165 method for exploring the correlations between overall bacterial community composition (OTU

166 level) and the environmental factors. The longest DCA axis had a gradient length less than 3

167 standard deviation units, so redundancy analysis (RDA) was performed (*Lepš and Šmilauer,*

168 *2003*; *Li et al., 2019*). Variance inflation factor (VIF) was used for multicollinearity among

169 environmental variables; variables with VIF > 10 were eliminated from RDA analysis. Variation

170 partition analysis (VPA) was applied for assessing the individual and shared effects of season

171 (temperature and rainfall), nutrient (NH_4^+-N, NO_3^--N, TN, TP, and TOC) and geographic

172 distance (latitude and longitude) on planktonic and sediment bacterial community structures.

173 Species data were log transformed and environmental data were z-score standardized prior to

174 beta-diversity calculations. R (version 4.0.2) was used for statistical testing unless otherwise

175 indicated. PCoA, PerMANOVA, DCA, RDA, VIF and VPA were performed using the "vegan"

176 package.

177

178 **Results**

179 *Bacterial community richness and diversity*

180 In this study, the obtained high-quality bacterial reads from each sample ranged between 28,133

181 and 63,295, normalized to 28,133 for the comparison of bacterial community richness and

182 diversity. High Good's coverage indicated that the OTUs of each bacterial library had been well

183 captured, with the values ranging from 95.5 % to 98.4%. Water bacterial libraries were

184 composed of 1,706–2,783 OTUs, while sediment bacterial libraries comprised 3,488–4,731

185 OTUs (Table S1). The Chao1 richness estimators of planktonic and sediment bacterial

186 communities were 2,865–3,810 and 4,083–6,015, respectively. At a given sampling site,

187 sediment sample was generally found to have more OTUs and higher Chao1 richness estimator

188 than the corresponding water one. Additionally, a significant increase of planktonic bacterial

189 richness was observed in summer ($P < 0.05$) (Figure 2), yet the trend of seasonal variation of

190 sediment bacterial richness was not clear. Shannon and Simpson indices of planktonic bacterial

191 communities were 4.49–5.20 and 0.0208–0.0449, while those of sediment bacterial communities

192 were 6.40–7.09 and 0.002–0.009. In summer, Shannon and Simpson indices of both planktonic

193 and sediment bacterial communities in HS area were significantly different from those in RI area

194 ($P<0.05$). However, a significant seasonal difference of sediment bacterial diversity (Shannon

195 and Simpson indices) was only observed in RI area ($P<0.05$).

196

197   In order to explore the interactions between water and sediment bacterial communities, the OTUs

198   were used to construct Microbial Ecological Networks (MENs) (Figure S1 and S2). 46.7% of

199   OTUs were shared between water and sediment samples. Water samples contained 1273 unique

200   OTUs that accounted for 14.0% of the total OTUs. The sediment network contained 2801 edges

201   among 1746 nodes, which were fewer than the networks of water (9927 edges among 795

202   nodes), reflecting a smaller number of co-occurrences in sediment (edge/node ratio=1.60). The

203   decreases in average connectivity and average clustering coefficient further demonstrated a

204   reduction in network complexity from water to sediment (Table S2). Additionally, 51.3%

205   positive edges and 48.7% negative edges were presented in sediment while 47.5% positive edges

206   and 52.5% negative edges showed in water. These results show that the topological features

207   between water and sediment were distinguishable, and the interactions among the bacterial

208   communities in water were much complex than that in sediment. The results of PCoA analysis

209   revealed a marked discrepancy in the structure of planktonic and sediment bacterial communities

210   (Figure 3). PerMANOVA confirmed that the bacterial communities in the water were

211   significantly different from those in the sediment ($R^2 = 0.561$, P= 0.001) (Table 1). Planktonic

212   bacterial communities for summer samples significantly differed from winter samples ($R^2 =$

213   0.731, P= 0.011), yet the trend for seasonal change of sediment bacterial community structure

214   was not apparent. An obvious and significant difference of sediment bacterial communities was

215   found between HS and RI areas ($R^2 = 0.345$, P= 0.006). Additionally, the interaction effects of

216   seasons (winter or summer) and areas (HS or RI) on planktonic and sediment bacterial

217   communities were statistically significant ($R^2 = 0.886$, P= 0.002; $R^2 = 0.886$, P= 0.002,

218   respectively).

219

220   *Bacterial community composition*

221   In the present study, a total of 13 major bacterial phyla (relative abundance $>$ 1% in each

222   sample) were identified, including *Proteobacteria*, *Bacteroidetes*, *Actinobacteria*, *Chloroflexi*,

223   *Cyanobacteria*, *Firmicutes*, *Acidobacteria*, *Epsilonbacteraeota*, *Patescibacteria*,

224   *Gemmatimonadetes*, *Spirochaetes*, *Nitrospirae*, and *Latescibacteria* (Figure 4). *Proteobacteria*

225   showed the dominance in the water samples (39.9–54.8%) and sediment samples (28.0–48.4%),

226   mainly consisting of three classes (*Alphaproteobacteria*, *Gammaproteobacteria*, and

227   *Deltaproteobacteria*) (Figure 5). *Gammaproteobacteria* predominated proteobacterial classes

228   across all samples, and was relatively higher in water samples. Moreover, winter water samples

229   generally had higher *Gammaproteobacteria* proportion than summer samples, yet the seasonal

230   variation of *Gammaproteobacteria* organisms in sediments was not clear. *Alphaproteobacteria*

231   was the second largest proteobacterial class in all samples, but the abundance of planktonic

232   *Alphaproteobacteria* organisms revealed an evident decrease in winter. *Deltaproteobacteria* was

233   also an important proteobacterial member in sediment, but showed lower proportion in water.

234   The second largest bacterial phylum in water samples was *Bacteroidetes* (10.7–26.5%), but its

235   proportion was relatively low in sediment samples (6.1–13.3%). However, *Actinobacteria* and

236   *Firmicutes* were more prevalent in sediment samples (*Actinobacteria* 7.1–19.5%, *Firmicutes*

237   4.1–8.7%) than those in water samples (*Actinobacteria* 3.9–15.4%, *Firmicutes* 1.5–5.2%).

238   Additionally, *Chloroflexi* and *Acidobacteria* showed relatively high proportion in sediment

239   samples, but became the minor planktonic bacterial groups. In contrast, *Cyanobacteria* and

240   *Epsilonbacteraeota*, as two major components of planktonic bacterial communities, were rarely

241   detected in sediments.

242

243   To determine the heterogeneity of potential bacterial pathogens at the genus level among the

244   sample groups, a total of 12 major bacterial genus (relative abundance $> 0.1\%$ in each sample)

245   were detected, including *Pseudomonas*, *Arcobacter*, *Aeromonas*, *Escherichia-Shigella*,

246   *Legionella*, *Acinetobacter*, *Enterobacter*, *Bacteroides*, *Flavobacterium*, *Mycobacterium*,

247   *Bacillus,* and *Clostridium* (Table S3). Water samples were generally found to have a higher

248   proportion of bacterial pathogens as mentioned above than sediment samples. In either summer

249   or winter, the average relative abundance of potential bacterial pathogens in water and sediment

250   samples from RI area was significantly higher than those from HS areas ($P < 0.05$) (Table 2).

251

252   *Influential factors on bacterial communities*

253   Spearman rank correlation analysis indicated that planktonic bacterial community richness (OTU

254   number and Chao1 estimator) and the taxa (*Proteobacteria* and *Alphaproteobacteria*) displayed

255   positive correlations with the water temperature ($P < 0.05$ or $P < 0.01$), but were negatively

256   correlated to TP ($P < 0.05$ or $P < 0.01$) (Table 3). Planktonic bacterial community Shannon

257   diversity showed a significant negative correlation with water NH_4^+–N and TOC concentrations

258   ($P < 0.05$). Moreover, *Bacteroidetes* displayed a negative correlation with TN and TP ($P < 0.05$),

259   while *Cyanobacteria* was positively correlated to NH_4^+–N, TN, and TOC ($P < 0.05$). In addition,

260   water temperature and TP were identified as key drivers determining the abundance of

261   *Actinobacteria* ($P < 0.05$). Water temperature also positively correlated to *Patescibacteria* ($P <$

262   0.05). The results of RDA analysis revealed that the water environmental factors in the first two

263   RDA axes respectively accounted for 59.1% and 24.3% of the total variance for planktonic

264   bacterial OTU composition (Figure 6). Rainfall (F=7.31, P=0.012, 499 Monte Carlo

265   permutations), water temperature (F=6.93, P=0.006, 499 Monte Carlo permutations), TP (F=4.05

266   P=0.028, 499 Monte Carlo permutations), and TN (F=4.00 P=0.022, 499 Monte Carlo

267   permutations) significantly contributed to the planktonic bacterial population–environment

268   relationship. The VPA indicated that season, nutrients, and geographic distance explained 78.7%

269   of the variation in the planktonic bacterial community (Figure 7a). Season, nutrients, and

270   geographic distance explained 42.5%, 18.1%, and 13.7% of the total variation, respectively.

271   Therefore, the variation in planktonic bacterial community was mainly determined by season and

272   nutrient condition.

273

274   Sediment bacterial Shannon diversity exhibited a positive correlation with sediment NH_4^+–N,

275   NO_3^-–N, and TN concentrations (P < 0.05) (Table 4). *Proteobacteria* was negatively correlated

276   to NH_4^+–N and NO_3^-–N (P < 0.05), while *Deltaproteobacteria* displayed a positive correlation

277   with sediment pH (P < 0.01). Moreover, *Alphaproteobacteria* were positively correlated to the

278   water temperature (P < 0.05), but negatively correlated to TP and TOC (P < 0.01).

279   *Actinobacteria* was positively correlated to NH_4^+–N, NO_3^-–N, and TN (P < 0.05), while

280   *Acidobacteria* showed a negatively correlation with NH_4^+–N, NO_3^-–N, and TN (P < 0.05).

281   *Chloroflexi* was found to be related to sediment pH, TP, and TOC (P < 0.05 or P < 0.01).

282   Additionally, *Gemmatimonadetes* and *Nitrospirae* were positively correlated to NH_4^+–N and

283   NO_3^-–N (P < 0.05 or P < 0.01), while *Bacteroidetes* showed a positive correlation with sediment

284   TP and TOC concentrations (P < 0.05 or P < 0.01). RDA analysis for sediment communities

285   showed that sediment physicochemical factors explained 44.5% and 31.4% of the total variation

286   (Figure 8), respectively. Sediment TP (F=3.89, P=0.006, 499 Monte Carlo permutations), rainfall

287   (F=3.38, P=0.016, 499 Monte Carlo permutations) and temperature (F=3.38, P=0.012, 499

288   Monte Carlo permutations) passed the Monte Carlo significance test (P < 0.05), suggesting a

289   significant contribution to the sediment bacterial community composition–environment

290   relationship. The VPA showed the season, nutrients, and geographic distance contributed 69.1%

291   of the variation in the sediment bacterial community (Figure 7b). Season and nutrients explained

292   54.4% of the variation, indicating a major contribution in shaping the sediment bacterial

293   community.

294

295   **Discussion**

296   *Bacterial community richness and diversity in Liangtan river*

297   Numerous previous studies have shown the spatiotemporal variation of bacterial community

298   richness and diversity in aquatic ecosystems (*Adhikari et al., 2019; Dai et al., 2016; Mai et al.,*

299   *2018; Mao et al., 2019; Yang et al., 2019; Sheng et al., 2016; Zhang et al., 2014; Zhang et al.,*

300   *2019; Zhu et al., 2019*). However, little is known about the shift of bacterial richness and

301   diversity in given urban rivers. In this study, Illumina Miseq high-throughput sequencing was

302   used to reveal the richness and diversity of planktonic and sediment bacterial communities in

303   Liangtan river. The Chao1 richness estimators and Shannon diversity index of planktonic

304   bacterial communities were 2,865–3,810 and 4.49–5.20, while those of sediment communities

305   were 4,083–6,015 and 6.40–7.09, respectively. However, lower Chao1 richness estimator but

306   higher Shannon diversity index of both planktonic and sediment bacterial communities were

307   detected in Jiangling river, the mainstream of Liangtan river (*Wang et al., 2018*). Additionally,

308   the Chao1 richness and Shannon diversity of sediment in Liangtan river were markedly higher

309   than those of their corresponding water column, which were in agreement with previous studies

310   in river systems (*Dai et al., 2016; Mao et al., 2019; Staley et al., 2015*). In this study, a

311   remarkable seasonal fluctuation of planktonic bacterial community richness occurred in Liangtan

312   river, but the trend of seasonal variation of sediment community diversity was not clear.

313   Moreover, significant differences in Shannon diversity of both planktonic and sediment bacterial

314   communities were observed between HS and RI areas in summer (P<0.05), suggesting that the

315   spatial distribution of bacterial communities. The spatial change of bacterial community diversity

316   was dependent on sampling date. *Dai et al. (2016)* indicated a remarkable spatial variation of

317   bacterial Shannon diversity in summer compared to spring, which further supported the results.

318

319   Previous literatures suggest that planktonic bacterial community richness or/and diversity in river

320   ecosystems might be influenced by water temperature, pH, and nutrients (*Mao et al., 2019;*

321   *Staley et al., 2015; Wang et al., 2016*). The results of Spearman rank correlation analysis

322   revealed that water temperature and TP were the dominant driving forces for planktonic bacterial

323   community richness, while NH_4^+–N and TOC were identified as key drivers determining

324   planktonic bacterial community Shannon diversity. Numerous influential factors on sediment

325   bacterial communities in river systems have been documented previously (*Mao et al., 2019;*

326   *Zhang et al., 2019*), where bacterial diversity was correlated with nutrients and heavy metals.

327   *Sheng et al. (2016)* indicated that increases in TN and TP may lead to high bacterial richness and

328   diversity. Our study identified the sediment bacterial Shannon diversity was positively correlated

329   to the level of sediment NH_4^+–N, NO_3^-–N, and TN, yet no determined indicators could well

330   explain the sediment bacterial richness.

331

332   *Bacterial community composition in Liangtan river*

333   There is a large degree of variation in bacterial communities in terms of taxonomic composition

334   in aquatic ecosystems (*Adhikari et al., 2019; Chen et al., 2018; Dai et al., 2016; Mai et al.,*

335 *2018; Sun et al., 2019; Xia et al., 2014; Yang et al., 2019; Zhang et al., 2014; Zhu et al., 2019*),

336 whereas the characteristics of bacterial communities in given urban rivers remains poorly

337 understood. In this study, the results of PCoA and PerMANOVA revealed strong heterogeneity

338 in the structure of planktonic and sediment bacterial communities, which was supported by

339 previous studies in river systems (*Ibekwe et al., 2016; Liu et al., 2018; Staley et al., 2015; Wang*

340 *et al., 2018*). However, a strong overlap (46.7%) in OTUs was identified between water and

341 sediment samples from Liangtan river. The coexisting bacteria accounted for 76.9% of the whole

342 communities in water column, indicating that planktonic bacterial communities was mostly

343 seeded from sediment bacterial communities. As proposed by *Staley et al. (2015)*, sediment can

344 contribute up to 50% of the community composition in the water column explaining the big

345 overlap. The positive and negative correlation in water and sediment accounted for nearly 50%,

346 indicating a similar co-occurrence patterns that complex ecological relationships (e.g. symbiosis,

347 competition, or predation) in both water and sediment. However, a higher co-occurrence were

348 found in water, which might be attributed to environmental filtering and niche differentiation.

349 The most abundant phylum in water and sediment of Liangtan river was *Proteobacteria,* which

350 was in line with previous studies in river ecosystems (*Liu et al., 2012; Liu et al., 2018; Mao et*

351 *al., 2019; Staley et al.,2015; Tao et al.,2019; Wang et al., 2018; Zhang et al., 2019*). Noticeably,

352 the genera *Pseudomonas*, *Arcobacter*, *Acinetobacter* belonging to phylum *Proteobacteria* were

353 detected in high abundance in water and/or sediment samples. Additionally, taxa displayed

354 higher abundances in the RI area compared to the HS area (Table S3) that may be attributed to

355 the high nutrient sources from industrial and residential sewage. *Newton et al*. (*2013*) identified

356 three bacterial genera (*Acinetobacter, Arcobacter, and Trichococcus*) as sewer signatures in

357 urban wastewater. *Yang et al*. (*2019*) revealed that members of genus *Acinetobacter* comprise of

358   potentially pathogenic as well as sewage indicator bacteria. *Bacteroidetes* was the second largest

359   phylum in the water samples from Liangtan river. Specific species within this phylum are

360   associated with the gut microbiota of many mammals (*Shanks et al., 2011*) and humans (*Candela*

361   *et al., 2010*). Consequently, *Bacteroidetes* might be proposed as effective alternative fecal

362   indicators (*Wéry et al., 2008*). In our study, water samples from RI area generally had higher

363   abundance of the potential pathogenic bacteria *Bacteroides* (phylum *Bacteroidetes*) than those

364   from HS area (Table S3). Another indicator of fecal contamination is *Firmicutes*, which is one of

365   the markers for identifying human feces (*Zheng et al., 2009*). The relative abundance of

366   *Firmicutes* was higher in sediment samples than that in water samples. Interestingly, the genera

367   *Bacillus* and *Clostridium* (phylum *Firmicutes*) were found mainly in sediment samples from RI

368   area associated with the presence of untreated fecal sewage.

369

370   *Cyanobacteria* was another predominant group of planktonic bacterial communities in Liangtan

371   river. A remarkable increase of *Cyanobacteria* abundance in site L2 compared to that in site L1

372   was detected which might be associated with hydropower station. Similar observations were

373   documented for the Guadiana river (*Domingues et al., 2014*) and Jiangling river (*Wang et al.,*

374   *2018*). The impact of dam construction was predicted to increase eutrophication conditions and

375   possibly promote *Cyanobacteria* blooms (*Wang et al., 2018*). Additionally, the abundance of

376   *Cyanobacteria* in our study was positively correlated to water NH_4^+–N, TN, and TOC

377   concentrations, suggesting its potential to serve as an important indicator of river eutrophication.

378   These results suggest environmental dominators drive the potentially responses of bacterial

379   community structure to the domestic sewage-polluted conditions and construction of hydropower

380   station.

381

382   The VPA revealed a major contribution of season and nutrients in shaping planktonic and

383   sediment bacterial communities, yet the effect of geographical distance on bacterial community

384   structure was negligent. Environmental factors are more likely to contribute to variation in

385   bacterial composition than geographical distance at small or intermediate spatial scales (less than

386   3000 km; *Martiny et al. 2006*). Hence, environmental factors (season and nutrients) were more

387   important than geographical distance in shaping bacterial community structure in Liangtan River.

388   Specifically, the results of RDA suggested that rainfall, water temperature, TP, and TN between

389   seasons mainly accounted for the shift of the planktonic bacterial community structure, while TP,

390   rainfall and temperature were identified as key drivers determining sediment bacterial

391   community composition. The fluctuations of rainfall and temperature between seasons may

392   mainly account for the seasonal variations in bacterial community structure. As proposed by

393   previous studies (Haque *et al., 2018; Staley et al., 2015)*, seasonal hydrological regime (i.e.

394   rainfall) directly causes a "wash-out effect" of nutrients and bacterial assemblages. Additionally,

395   nutrients were found to be another key driver explaining bacterial community structure. *Ibekwe et*

396   *al. (2016)* highlighted that phosphorus and nitrogen are the major factors significantly related to

397   the changes in planktonic bacterial community. *Zhang et al. (2019)* also stressed the significant

398   roles of TP and temperature on the variation of sediment bacterial community.

399

400   *Implications of the research*

401   Sediments within aquatic ecosystems represent a complex matrix, usually

402   providing microorganisms with numerous advantages not found in the water column.

403 *Perkins et al. (2014)* reported that sediments contain more nutrients than the water column, and

404 sediments can shield bacteria from the effect of sunlight and protect bacteria against predation.

405 In the current study, the sediment samples clustered distinctively from water samples and

406 displayed a higher bacterial diversity than the water samples, strengthening the fact that

407 sediments may serve as reservoirs of diverse microbial populations and highlighting the need to

408 include sediments in river monitoring. Additionally, the fecal bacteria and candidate pathogens

409 accounted for a higher proportion in RI area, suggesting their potential to serve as important

410 indicators for the health and status of river ecosystems. Nevertheless, more extensive researches

411 should be conducted in our future work, including (i) a long-term investigation (i.e. samples will

412 be collected seasonally during two years) of planktonic and sediment bacterial communities in

413 urban rivers polluted by continuous domestic sewage is of paramount importance; (ii) the role of

414 functional microorganisms, especially high abundance of bacterial pathogens, is furtherly

415 elucidated to provide insight into the monitoring and control of contamination in urban rivers.

416

Conclusions

418 Our study revealed a comprehensive understanding of the integrated biogeography of planktonic

419 and sediment bacterial communities in Liangtan river. Water and sediment habitats differed

420 greatly in bacterial community richness, diversity and structure. Additionally, the relative

421 abundance of potential bacterial pathogens in water and sediment samples from RI area was

422 significantly higher than those from HS area. Under the joint influence of sewage discharge and

423 hydropower dams, rainfall, temperature and nutrient were identified as the deterministic forces

424 shaping the bacterial communities in the heavily polluted urban river. Our study offers further

425 reference for the environmental management of Liangtan River. It also provides typical data for

426 the comparison with other urban rivers, and thus contributes to the increasing knowledge of

427 microbial ecology in contaminated urban rivers.

428

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604

Figure 1

Location of sampling sites in Liangtan River.

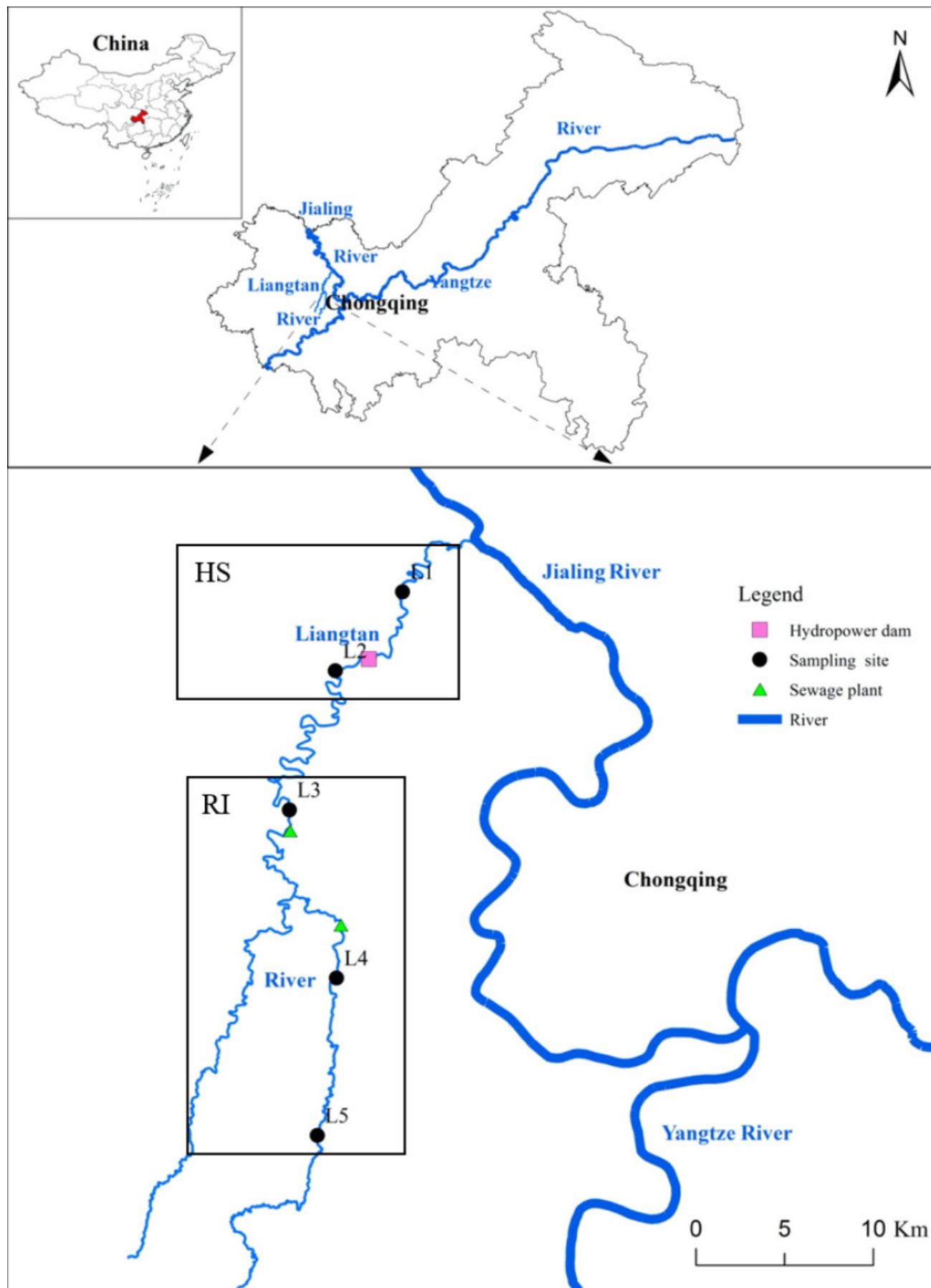



Figure 2

Comparisons of Chao1 richness estimator , Shannon diversity index, and Simpson evenness index of planktonic and sediment bacterial communities in HS and RI areas. Different lowercase letters indicate significant differences (P < 0.05) in mean values.

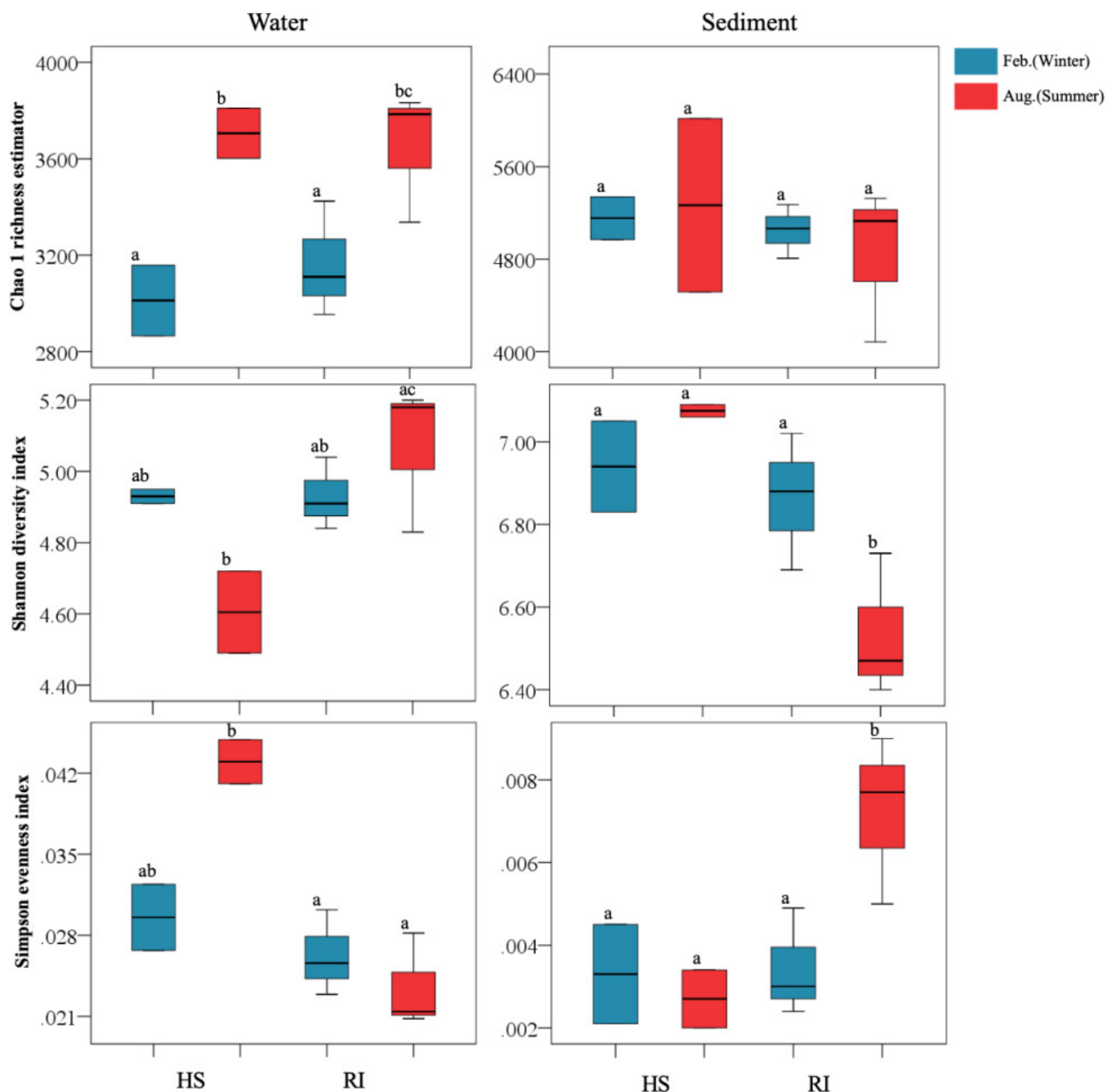



Figure 3

Principal coordinates analysis (PCoA) for spatiotemporal variations of planktonic and sediment bacterial communities according to Bray-Curtis distance matrices.Green circle symbols represent the February water samples from sites L1–L5. Green triangle symb

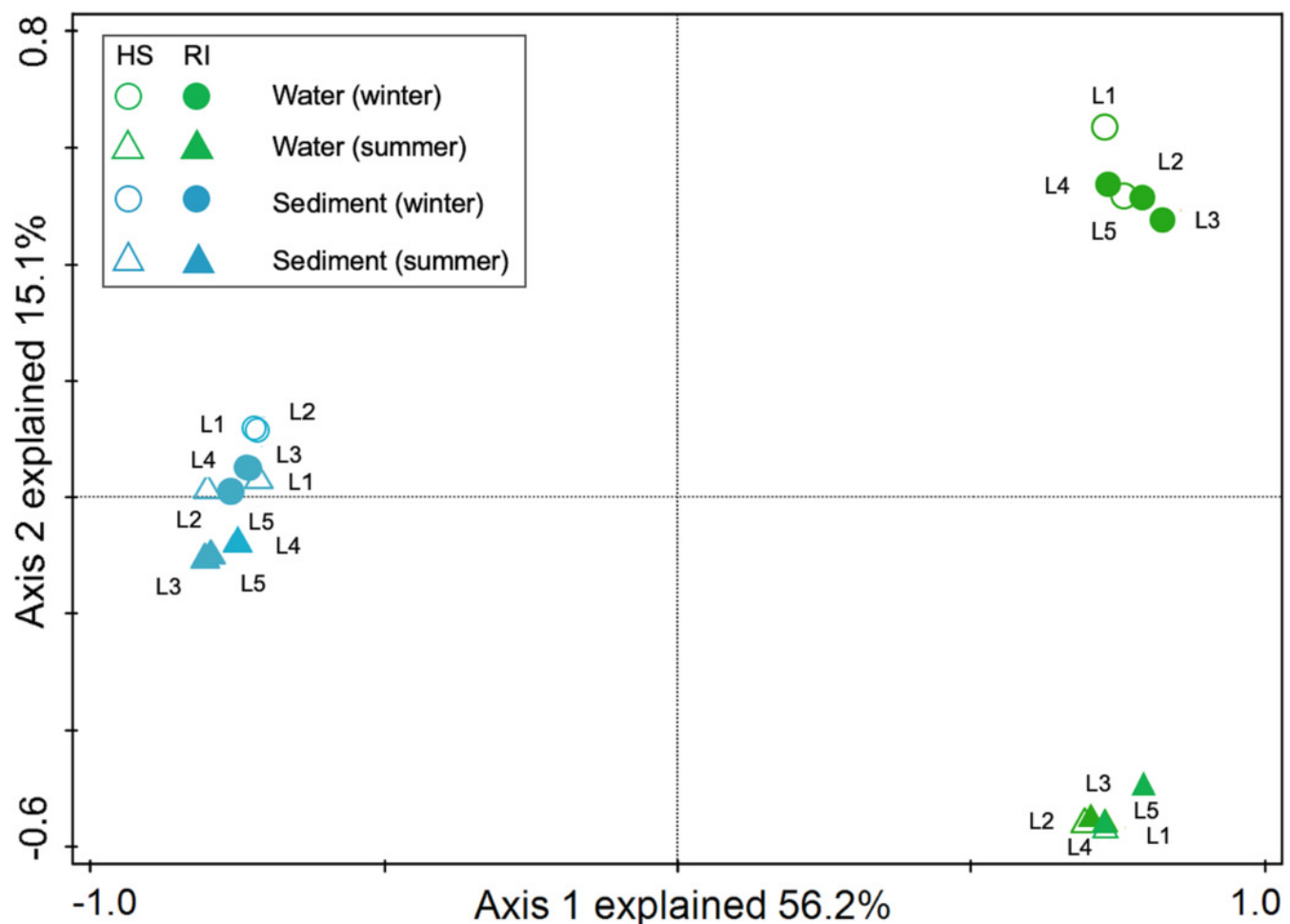



Figure 4

Relative abundance of dominant bacterial phyla in waters and sediments derived from the sampling sites. Bacterial phyla with relative abundance less than 1% of in each sample are classified as others.

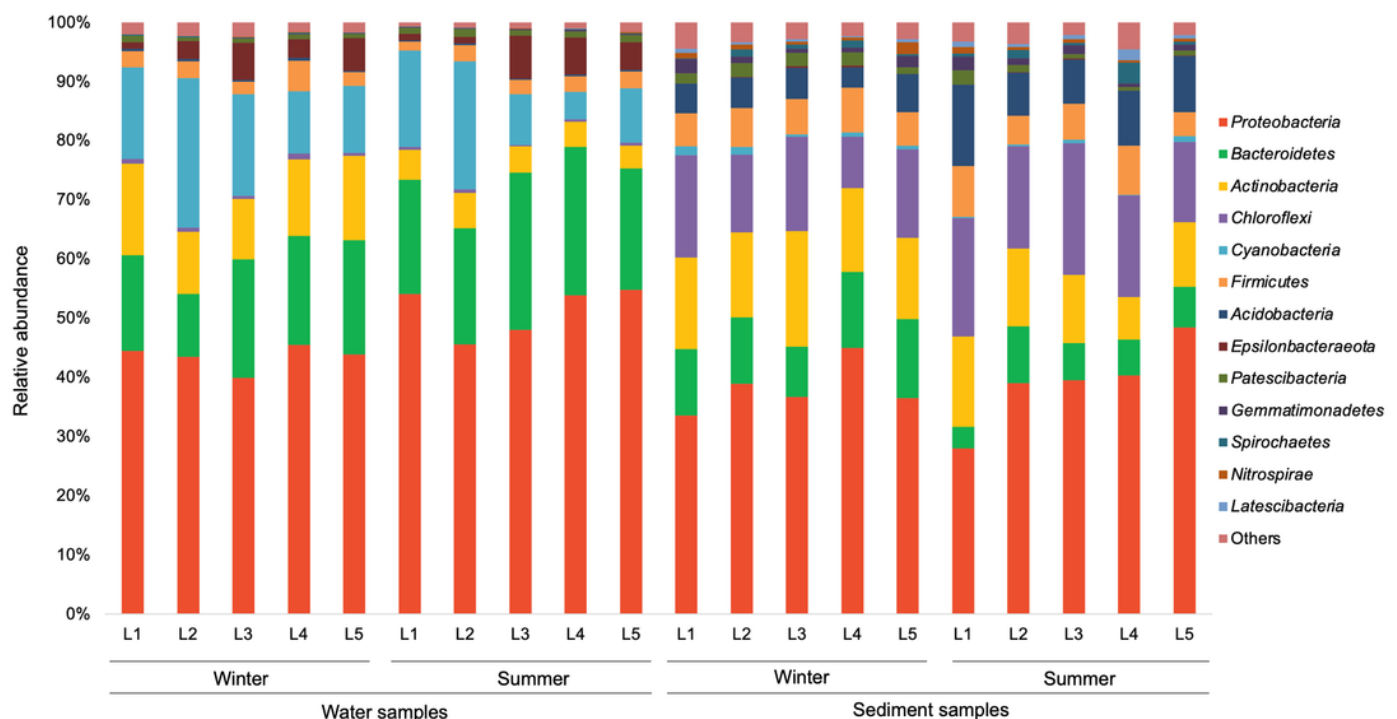



Figure 5

Relative abundance of proteobacterial classes in waters and sediments derived from the sampling sites. Unclassified Proteobacteria are classified as others.

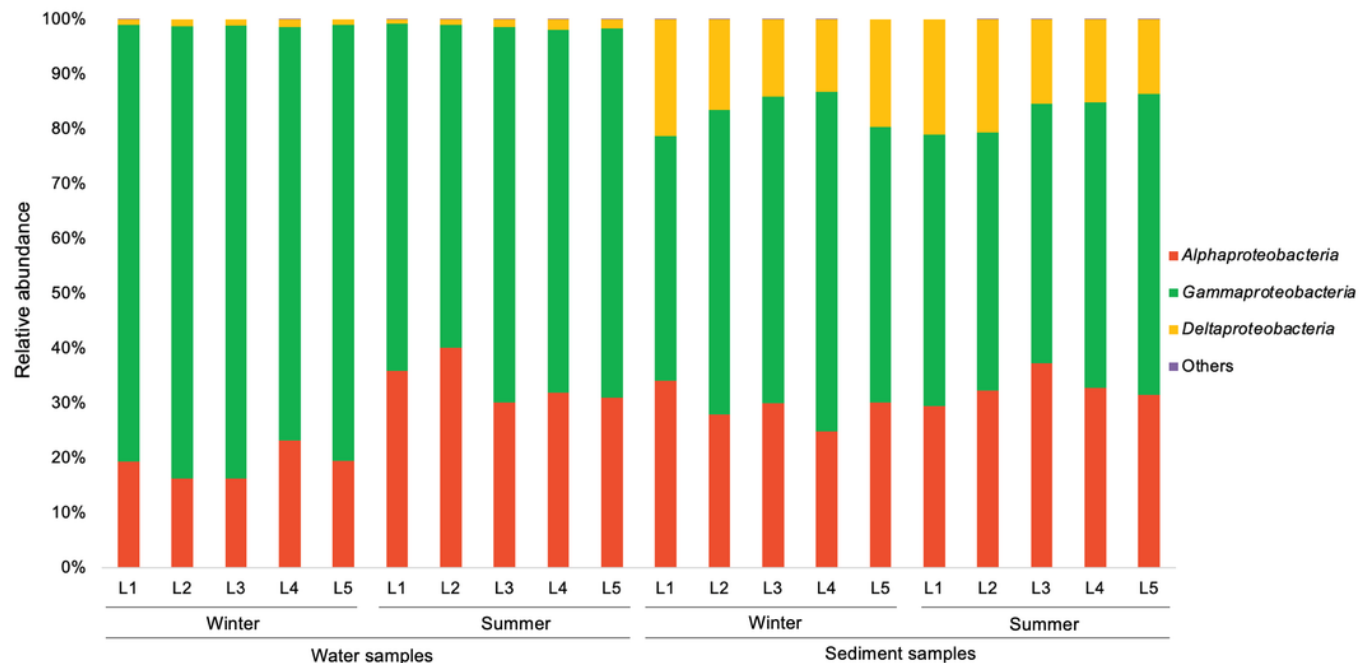



Figure 6

Redundancy analysis (RDA) for the first two principal dimensions of the relationships between planktonic bacterial OTU composition and water properties. Green triangle symbols represent the August water samples from sites L1–L5. Blue circle symbols repres

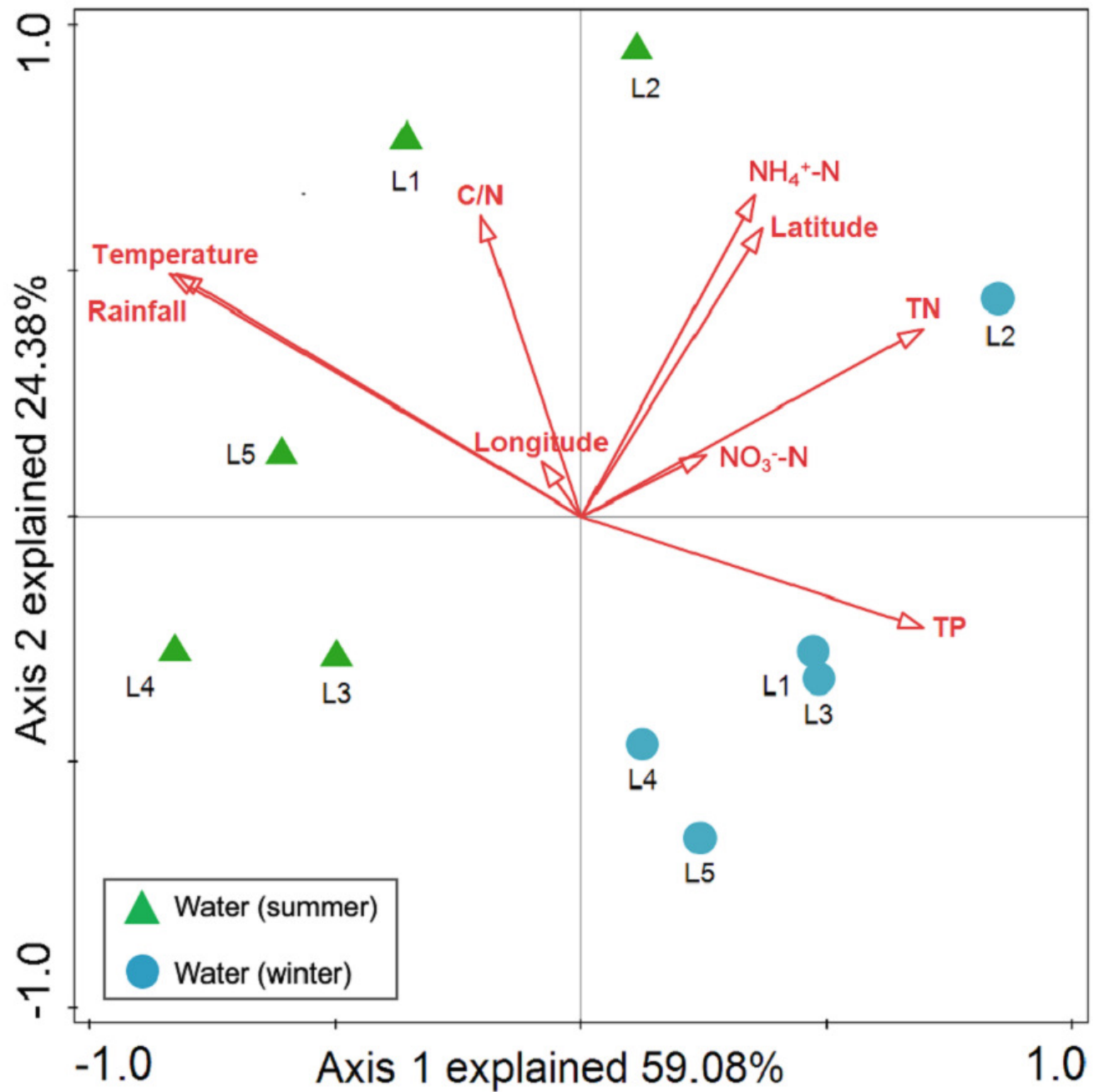



Figure 7

Variation partitioning analysis (VPA) of the effects of season, nutrients, and geographic distance on planktonic (a) and sediment (b) bacterial communities.

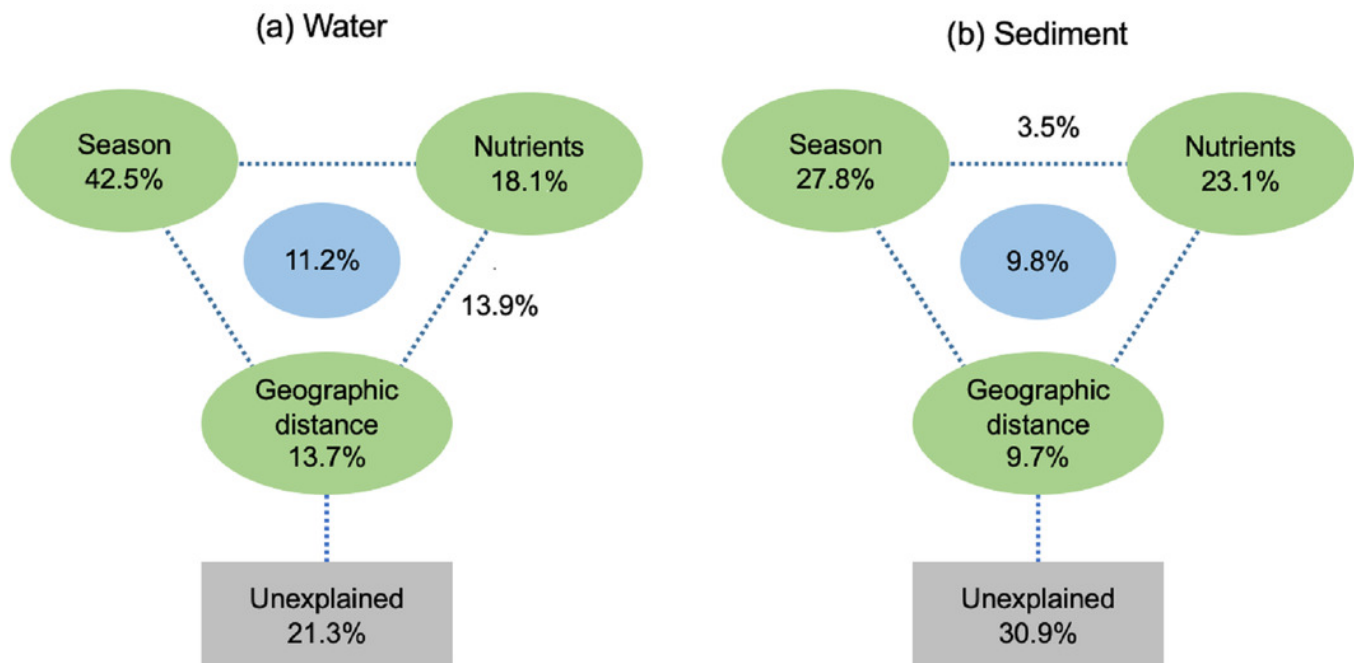
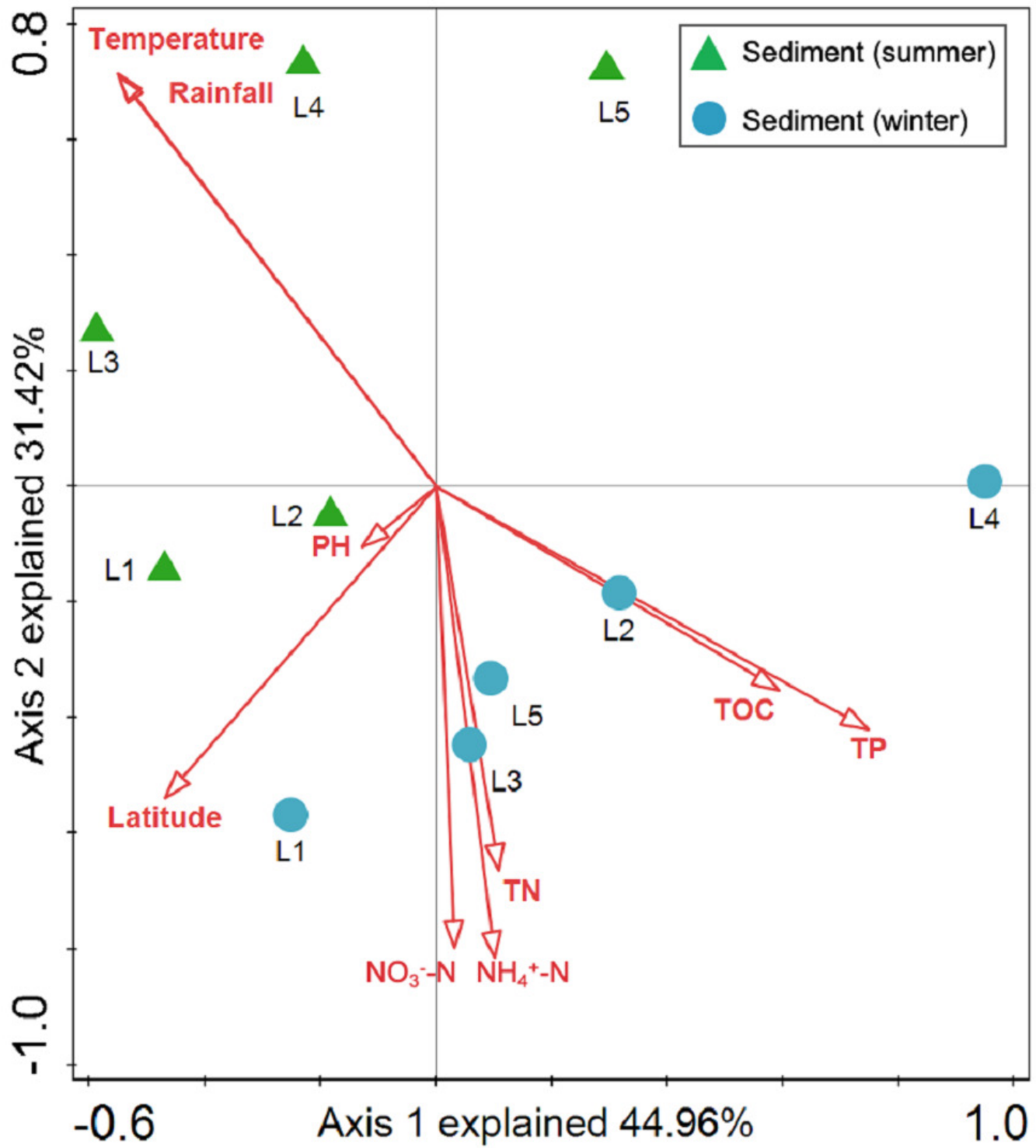



Figure 8

Redundancy analysis (RDA) for the first two principal dimensions of the relationships between sediment bacterial OTU composition and sediment properties. Green triangle symbols represent the August sediment samples from sites L1–L5. Blue circle symbols re

Quantitative effects of seasons (winter or summer) and areas (HS or RI) on planktonic and sediment bacterial communities using permutational multivariate analysis of variance (PerMANOVA).

1 **Table 1** Quantitative effects of seasons (winter or summer) and areas (HS or RI) on planktonic and sediment bacterial communities

2 using permutational multivariate analysis of variance (PerMANOVA). R^2 value represents the proportion of the community variation

3 constrained by each variable and their interaction. P value less than 0.05 means significant.

Groups	Water (Water vs. sediment: $R^2 = 0.561$, $P=$ **0.001**)		Sediment	
	R^2	P	R^2	P
Season (winter vs. summer)	0.731	**0.011**	0.197	0.063
Area (HS vs. RI)	0.120	0.496	0.345	**0.006**
Season×area	0.886	**0.002**	0.611	**0.001**

Comparisons of potential bacterial pathogen sequences at the genus level in waters and sediments derived from HS and RI areas. The data in bold indicate significant differences (P < 0.05) in mean values.

1 **Table 2** Comparisons of potential bacterial pathogen sequences at the genus level in waters and sediments derived from HS and RI

2 areas. The data in bold indicate significant differences (P < 0.05) in mean values.

Season	Area	Water/sediment	Groups				Water/sediment	Area	Season
			Winter		Summer				
			HS	RI	HS	RI			
Winter	HS	Water	—	**0.024**	0.089	0.123	Sediment	HS	Winter
	RI	Water	**0.019**	—	**0.002**	0.228	Sediment	RI	
Summer	HS	Water	0.148	0.217	—	**0.007**	Sediment	HS	Summer
	RI	Water	0.000	**0.003**	**0.001**	—	Sediment	RI	

Spearman rank correlation analysis of water environmental factors with the richness and diversity of planktonic bacterial community or the proportion of the major planktonic bacterial groups. The data in bold indicate significant differences.

1 **Table 3** Spearman rank correlation analysis of water environmental factors with the richness and diversity of bacterioplankton

2 community or the proportion of the major planktonic bacterial groups. The data in bold indicate significant differences.

Parameters	pH	Temperature	NH_4^+-N	NO_3^--N	TN	TP	TOC
OTUs	0.012	**0.669[a]**	-0.103	-0.067	-0.382	**-0.770[b]**	-0.139
Chao1 estimator	0.012	**0.693[a]**	-0.236	0.067	-0.236	**-0.770[b]**	-0.103
Shannon index	0.255	-0.264	**-0.745[a]**	0.358	0.115	0.345	**-0.697[a]**
Proteobacteria	-0.134	**0.865[b]**	-0.188	-0.127	-0.370	**-0.673[a]**	-0.115
Alphaproteobacteria	-0.158	**0.853[b]**	0.079	-0.018	-0.236	**-0.794[b]**	0.224
Gammaproteobacteria	0.255	-0.239	-0.467	0.333	0.139	0.491	-0.576
Bacteroidetes	-0.146	0.497	-0.139	-0.321	**-0.673[a]**	**-0.709[a]**	-0.430
Actinobacteria	0.182	**-0.632 [a]**	-0.006	0.200	0.382	**0.648[a]**	0.176
Cyanobacteria	0.292	-0.485	**0.721[a]**	0.030	**0.661[a]**	0.394	**0.636[a]**
Epsilonbacteraeota	-0.219	-0.080	-0.273	-0.200	-0.564	-0.176	-0.515
Firmicutes	0.316	-0.178	-0.248	0.600	0.600	0.236	-0.030
Patescibacteria	0.365	**0.669[a]**	-0.006	-0.006	0.042	-0.576	-0.055

3 [a] Correlation is significant at the 0.05 level

4 [b] Correlation is significant at the 0.01 level

Spearman rank correlation analysis of sediment environmental factors with the richness and diversity of sediment bacterial community or the proportion of the major sediment bacterial groups. The data in bold indicate significant differences.

1 **Table 4** Spearman rank correlation analysis of sediment environmental factors with the richness and diversity of sediment bacterial

2 community or the proportion of the major sediment bacterial groups. The data in bold indicate significant differences.

Parameters	pH	Temperature	NH$_4^+$-N	NO$_3^-$-N	TN	TP	TOC
OTUs	-0.103	0.299	0.030	0.176	-0.018	-0.309	-0.297
Chao1 estimator	-0.212	0.079	0.055	0.321	0.006	-0.030	-0.006
Shannon index	0.430	0.177	**0.709[a]**	**0.745[a]**	**0.758[a]**	-0.079	-0.103
Proteobacteria	-0.479	0.128	**-0.770[a]**	**-0.661[a]**	-0.600	-0.030	-0.030
Alphaproteobacteria	-0.042	**0.695[a]**	-0.624	-0.527	-0.358	**-0.733[a]**	**-0.709[a]**
Gammaproteobacteria	-0.503	-0.28	-0.467	-0.491	-0.394	0.248	0.236
Deltaproteobacteria	**0.770[b]**	0.488	0.455	0.515	0.539	-0.236	-0.164
Bacteroidetes	0.103	-0.622	0.539	0.467	0.309	**0.842[b]**	**0.794[b]**
Actinobacteria	-0.055	-0.482	**0.673[a]**	**0.636[a]**	**0.648[a]**	0.358	0.261
Chloroflexi	0.164	**0.701[a]**	-0.030	0.042	0.079	**-0.782[b]**	**-0.758[a]**
Firmicutes	-0.079	0.037	-0.006	-0.127	-0.055	0.067	0.103
Acidobacteria	-0.055	0.305	**-0.733[a]**	**-0.636[a]**	**-0.758[a]**	-0.515	-0.430
Gemmatimonadetes	0.552	0.171	**0.782[b]**	**0.673[a]**	0.612	-0.127	-0.103
Nitrospirae	0.527	-0.055	**0.879[b]**	**0.709[a]**	0.600	0.115	0.127

3 [a] Correlation is significant at the 0.05 level

4 [b] Correlation is significant at the 0.01 level