Differentiation of cognate bacterial communities in thermokarst landscapes: implications for ecological consequences of permafrost degradation

Running title: Bacterial communities in thermokarst landscape

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Abstract

Thermokarst processes likely result in new habitats harboring novel bacterial communities in degraded permafrost soil (PB~~Cs~~), thermokarst lake sediments (SB~~Cs~~), and lake water (WB~~Cs~~). Our study aimed to investigate the paired PB~~Cs~~, SB~~Cs~~, and WB~~Cs~~ across the Qinghai-Tibet Plateau (QTP) by assessing the spatial pattern of diversity as well as assembly mechanisms of these bacterial communities. Each habitat had distinct bacterial assemblages, with lower alpha diversity and higher beta diversity in WB~~Cs~~ than in SB~~Cs~~ and PB~~Cs~~. However, up to 41% of the OTUs were shared by PB~~Cs~~, SB~~Cs~~, and WB~~Cs~~, suggesting that many taxa originate from the same sources via dispersal. SB~~Cs~~ and WB~~Cs~~ had reciprocal dispersal effects and both were correlated with PB~~Cs~~. Dispersal limitation was the most dominant assembly process shaping PB~~Cs~~ and SB~~Cs~~ while homogeneous selection was the most dominant for WB~~Cs~~. Bacterial communities of the three habitats correlated differently with environmental variables, but latitude, mean annual precipitation, and pH were the common factors associated with their beta diversity, while total phosphorus was the common factor associated with their assembly processes. Our results imply that thermokarst processes result in diverse habitats that have distinct bacterial communities that differ in diversity, assembly mechanisms, and environmental drivers.

Keywords: thermokarst; permafrost; bacteria, community assembly, Qinghai-Tibet Plateau

1 Introduction

Permafrost is an important landscape in high latitude and altitude regions, covering 15% of the land area of the Northern Hemisphere (Obu, 2021) and 40% of the Qinghai-Tibet Plateau (QTP) (Zou et al., 2017; Gao et al., 2021), and containing twice as much carbon as

is currently present in the atmosphere (Schuur et al., 2009; Hugelius et al., 2014; Mishra et al., 2021). Permafrost is highly sensitive to climate warming (Wu et al., 2007; Jorgenson et al., 2010; Biskaborn et al., 2019), which is expected to reduce 50-90% of permafrost cover by 2100 (Lawrence et al., 2012; Chadburn et al., 2017). As a result of ice-rich permafrost thaw, thermokarst lakes and ponds are formed (Kokelj and Jorgenson, 2013; Farquharson et al., 2016) and extensively distributed across the Arctic and sub-Arctic regions (de Jong et al., 2018) as well as the QTP (Niu et al., 2011; Luo et al., 2020). The initial sediment and water in thermokarst lakes originate from the melting of permafrost, and they are continuously replenished through the collapse of permafrost and precipitation (West and Plug, 2008; de Jong et al., 2018). Thus, thermokarst lake sediments and water, as well as the surrounding degraded permafrost soil, represent three distinct habitats derived from the original permafrost during the process of thermokarst formation (Figure 1Figure 1). It is well known that thermokarst processes substantially influence regional hydrological, ecological, and biogeochemical processes (Chin et al., 2016; In'T Zandt et al., 2020; Manasypov et al., 2021) and initiate a strong positive climate feedback to global warming (Walter et al., 2006; Schuur et al., 2008; Schaefer et al., 2011; Anthony et al., 2018). However, the microbial differences and relationships among these distinct habitats in thermokarst landscapes are largely unknown.

Understanding microbes in thermokarst landscapes, and elsewhere, is important because microbial communities play pivotal roles in driving biogeochemical and ecological processes. To understand thermokarst microbial communities, we need to understand the assembly mechanisms structuring them, a central research topic in microbial ecology (Stegen et al., 2012; Nemergut et al., 2013; Zhou et al., 2014; Zhou and Ning, 2017). In

62 the assembly of microbial communities, both deterministic and stochastic processes occur

63 simultaneously but with contributions that can vary (Chase, 2010; Zhou et al., 2013;

64 Vellend et al., 2014; Makoto et al., 2019). Typically, deterministic processes place a strong

65 emphasis on niche-based mechanisms, including ecological selection, environmental

66 filtering, and biotic interactions (Zhou and Ning, 2017). Conversely, stochastic processes

67 involve neutral mechanisms like random birth and death, unforeseen disturbance,

68 probability-based dispersal, and ecological drift (Chave, 2004; Chase, 2010; Zhou et al.,

69 2014). In various ecosystems or habitats, the significance of deterministic and stochastic

70 processes can differ greatly and be shaped by a multitude of environmental factors (Tripathi

71 et al., 2018; Aguilar and Sommaruga, 2020; Jiao and Lu, 2020; She et al., 2021). During

72 thermokarst formation, vast areas of permafrost have been transformed to thermokarst

73 lakes, leading to major changes in physicochemical environments as well as in biological

74 communities of these regions. Thus, it is also expected that the microbial communities

75 experience major changes in occupying degraded permafrost soil, thermokarst lake

76 sediments, and lake water, and in doing so, display different assembly mechanisms (Figure

77 1Figure 1).

78 Better understanding community assembly in these systems is important because thawing

79 permafrost and thermokarst lakes are greenhouse gas emission hotspots (In'T Zandt et al.,

80 2020; Mu et al., 2020; Elder et al., 2021). Close relationships between biogeochemical

81 processes and microbial community assembly have been generally demonstrated (Bier et

82 al., 2015; Graham et al., 2016; Le Moigne et al., 2020; Ren et al., 2022a). Assembly

83 processes inevitably influence biogeochemical functions by shaping community diversity

84 and composition (Graham et al., 2016; Leibold et al., 2017; Mori et al., 2018). For example,

85   dispersal (a stochastic process) can suppress biogeochemical functioning by increasing the

86   proportion of maladapted taxa (Strickland et al., 2009; Nemergut et al., 2013; Graham and

87   Stegen, 2017), while selection (a deterministic process) may have positive influence on

88   biogeochemical function by facilitating locally adapted taxa (Graham et al., 2016). In

89   particular, stochastic dispersal has been suggested to suppress the mineralization of organic

90   carbon in soil and water (Le Moigne et al., 2020; Luan et al., 2020). Therefore, it is

91   hypothesized that the relative influence of deterministic and stochastic processes on

92   community assembly could impact the biogeochemical functions of microbial

93   communities (Strickland et al., 2009; Nemergut et al., 2013; Pholchan et al., 2013; Graham

94   and Stegen, 2017). Given the importance to understand how microbial community

95   variations affect the biogeochemical cycles in permafrost and thermokarst landscapes, it is

96   necessary to have a deeper understanding of the assembly mechanisms in shaping

97   microbial communities that form following permafrost degradation.

98   In this paper we evaluated these ideas on the Qinghai-Tibet Plateau (QTP), which is known

99   as the "Third Pole" of the Earth and is therefore uniquely positioned as an indicator of

100   global change (Yao et al., 2012). Pronounced environmental changes in response to climate

101   warming on the QTP have been observed and documented, especially in the past half

102   century (Piao et al., 2012; Zhang et al., 2018; Ren et al., 2019a). Major changes are

103   predicted to continue on the QTP and permafrost thawing is among the most prominent but

104   little is known about the microbial communities in these rapidly emerging ecosystems. To

105   fill this gap, we investigated water and sediment in thermokarst lakes across the QTP as

106   well as permafrost soil around the lakes (Figure 1Figure 1). Our aims were to (1) assess

107   the spatial pattern of alpha and beta diversity of bacterial communities, and (2) evaluate

the community assembly mechanisms and environmental responses of the bacterial communities in degraded permafrost soil, as well as in the sediment and water of thermokarst lakes.

2 Methods

2.1 Study area, field sampling, and chemical analysis

This work was conducted across the QTP in July 2021 (Figure S1~~Figure S1~~). In total, 44 sites were investigated by collecting paired samples of lake water, lake sediment, and surrounding permafrost soil (Figure 1~~Figure 1~~a) (Ren et al., 2022a). The sampling strategy and chemical analysis methods were described in detail in our previous publications (Ren et al., 2022a, b). For water sampling of each lake, surface water samples were collected at a depth of 0.3 to 0.5 m with three replicates. For microbial analysis, 200 mL of water was filtered using a 0.2-μm polycarbonate membrane filter (Whatman, UK) for DNA extraction. The remaining water samples were transported to the lab for other physicochemical measurements. For sediment sampling, the top 15 cm of sediment was collected from 3 points. Sediment samples for microbial analysis were collected in a 45-mL sterile centrifuge tube, and the remaining samples were air-dried for analyzing physicochemical properties. For permafrost sampling, five topsoil cores were collected along three 25-m transects with increasing distances to the lake shore, respectively. The soils from one transects were homogenized. Soil samples for microbial analysis were stored in 45-mL sterile centrifuge tubes and the remaining soils were used for analyzing physicochemical properties. For each sampling site, pH, conductivity (Cond), organic carbon (DOC in water and SOC in sediment and soil), total nitrogen (TN), and total phosphorus (TP) were measured according to our previous publications (Ren et al., 2022a, b). Moreover, the QTP

climate dataset (Zhou, 2018) was obtained from the National Tibetan Plateau Data Center

(https://data.tpdc.ac.cn/en/), and was utilized to extract the mean annual temperature (MAT)

and mean annual precipitation (MAP) for each of the study sites.
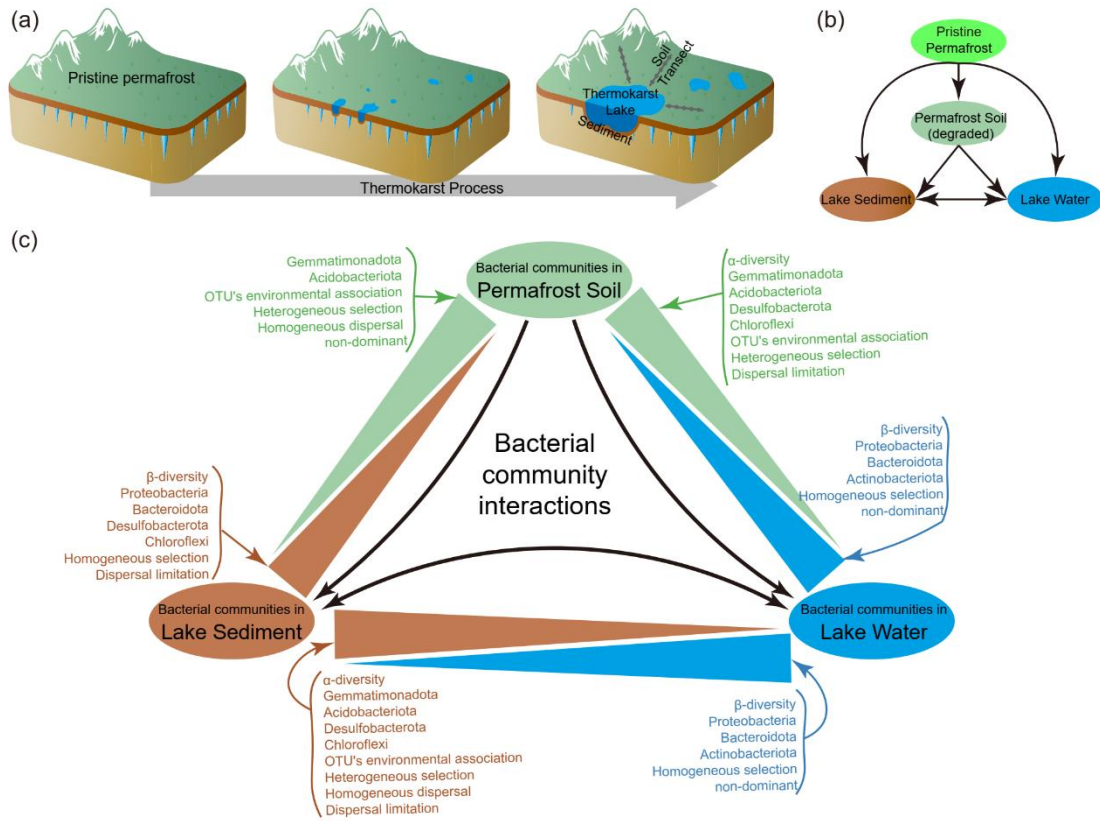
Figure 1 (a) The process of thermokarst lake formation in ice-rich permafrost (modified

from Ren et al, 2022a). (b) A schematic view of the relationships between permafrost soil,

lake sediment, and lake water. (c) Summary of the differences between distinct habitats of

the bacterial communities in permafrost soil, lake sediment, and lake water.

2.2 DNA extraction, PCR, and sequencing

The methods of DNA extraction, PCR, and sequencing were described in detail in our

previous publication (Ren et al., 2022a). In brief, tThe Magen Hipure Soil DNA Kit

(Magen, China) was used to extract DNA from soil (0.5 g frozen soil), sediment (0.5 g

143   frozen sediment), and water (membrane filter) samples according to the manufacturer's

144   protocols. Extraction blanks were routinely performed in parallel. The prokaryotic 16S

145   rRNA gene's V3-V4 hypervariable regions were amplified using universal primers 343F-

146   TACGGRAGGCAGCAG and 798R-AGGGTATCTAATCCT (Nossa et al., 2010). PCRs

147   were conducted in 25 µl reaction mixture containing 2.5 µl of TransStart buffer, 2 µl of

148   dNTPs, 1 µl of each primer, 0.5 µl of TransStart Taq DNA polymerase, and 20 ng template

149   DNA. The PCR reactions were conducted on a thermal cycler (ABI GeneAmp® 9700,

150   USA) using the followed procedure: initial denaturation at 94 °C for 5 min, 24 cycles of

151   denaturation at 94 °C for 30 s followed by annealing at 56 °C for 30 s and extension at

152   72 °C for 20 s, and a final extension at 72 °C for 5 min. To reduce amplification bias, three

153   individual PCR amplifications were performed for each sample and the triplicate PCR

154   products were combined and, purified. DNA libraries were verified on 2% agarose gels

155   and quantified using a Qubit 4 Fluorometer (Thermo Fisher Scientific, Waltham, USA).,

156   and quantified. Next generation sequencing of the amplicon products was conducted on an

157   Illumina Miseq Platform (Illumina, San Diego, CA, USA). Automated cluster generation

158   and 250/300 paired-end sequencing with dual reads were performed following the

159   manufacturer's instructions. Sequencing of the amplicon products was done on the

160   Illumina MiSeq platform (Illumina, San Diego, CA, USA) following the manufacturer's

161   instructions. The forward and reverse reads were joined and assigned to samples based on

162   barcode and truncated by cutting off the barcode and primer sequence. Raw sequences were

163   trimmed of ambiguous bases and low-quality sequences (quality score lower than 20).

164   After trimming, and the paired-end reads were joined and de-noised using QIIME1.9.1

165   (Caporaso et al., 2010). The sequences were subjected to the following denoising criteria:

166 sequences with ambiguous or homologous regions, as well as those below 200 bp in length,

167 were excluded; sequences with at least 75% of bases having a quality score above Q20

168 were retained; and chimeric sequences were identified and eliminated. All sequences from

169 extraction blanks were removed. The effective sequences were grouped into Operational

170 Taxonomic Units (OTUs) using a 97% sequence similarity threshold and annotated the

171 taxonomic classifications against the SILVA 138 database (released on 02-Nov-2020)

172 (Quast et al., 2013). The singletons were removed, and the sequences were rarefied to the

173 lowest number of sequences per sample were normalized to (24,251 sequences) per sample

174 to eliminate the bias from the sampling effort.

175 *2.3 Analyses*

176 Three α-diversity indices, including observed number of OTUs (OTU richness), Shannon

177 diversity, and phylogenetic diversity (PD), were calculated using QIIME 1.9.1 (Caporaso

178 et al., 2010). The "ses.mntd" function in the picante 1.8.2 package was used to calculate

179 the standardized effect size measure of the mean nearest taxon distance (SES.MNTD) for

180 assessing the phylogenetic clustering of bacterial communities (Kembel et al., 2010). The

181 β-diversity was calculated as the Bray-Curtis distance based on the relative abundance of

182 OTUs. In order to determine the habitat niche occupied by each taxon, we utilized the

183 "spaa" package (Zhang, 2016) in R to calculate the Levin's niche width (Levins, 1968). The

184 formula of niche breadth is $B_i = 1/\sum_1^n p_i^2$, where B_i represents the niche breadth of OTU_i

185 across the communities, n is the total number of communities, and p_i is the proportion of

186 OTU_i in each community. Differences in α-diversity and β-diversity among bacterial

187 communities in different habitats, including permafrost soil bacterial communities (PBCs),

188 lake sediment bacterial communities (SBCs), and lake water bacterial communities

9

189 (WB~~Cs~~), were assessed using Wilcoxon rank-sum test. The relationships between

190 taxonomic and environmental variables were assessed using Spearman correlation, and the

191 P-values were corrected using the FDR method (Benjamini and Hochberg, 1995). Mantel

192 tests were performed to examine the correlation between environmental variables and β-

193 diversity. A Non-metric Multidimensional Scaling (NMDS) analysis was conducted to

194 examine the distribution of PB~~Cs~~, SB~~Cs~~, and WB~~Cs~~ using the "metaMDS" function in the

195 vegan 2.5-7 package based on the Bray-Curtis distance using the relative abundance of

196 OTUs. (Oksanen et al., 2020). The distinctiveness of these communities was confirmed

197 through a non-parametric statistical test (ANOSIM) using the "anosim" function in the

198 vegan package. The habitat niche occupied by each species was estimated by calculating

199 Levin's niche breadth (Levins, 1968) with the use of the spa 0.2.2 package (Zhang, 2016).

200 Species with a broader niche breadth were distributed more evenly across a wider range of

201 habitats, compared to those with a narrower niche breadth.

202 Structural equation modeling (SEM) was conducted to assess the relationships among

203 location (including latitude, longitude, and elevation), climate (including mean annual

204 temperature and mean annual precipitation), and physicochemical ~~parameters~~ variables

205 (including pH, conductivity, nutrients concentrations and stoichiometric ratios) of each

206 habitat (permafrost soil, lake sediment, and lake water), as well as their bacterial

207 communities (PB, SB, and WB). In model building, the SEM incorporated prior

208 knowledges: (a) location and climate factors potentially influence all the studied bacterial

209 communities, (b) physicochemical factor of each habitat potentially influences the

210 corresponding bacterial communities, and (c) permafrost soil potentially influences

211 thermokarst lake sediment and water, while lake sediment and water interact with each

212   other. In the SEM, location, climate, and physicochemical environments were reduced in

213   dimensions by principal component analysis (PCA), respectively, using the "prcomp"

214   function of the vegan package, and the first axis (PCA1) was used in SEM. For community

215   structure, the first axis of NMDS was used. SEM was constructed using the lavaan package

216   (Rosseel, 2012). The fit of SEM was assessed using standard indices, including chi-square

217   (χ^2), goodness-of-fit index (GFI), comparative fit index (CFI), root mean square residual

218   (RMR), and root mean squared error of approximation (RMSEA) (Hu and Bentler, 1999;

219   Barrett, 2007).

220   Phylogenetic trees of bacteria were constructed in the R package ggtree 3.2.1 (Yu et al.,

221   2017) using the top 1000 abundant OTUs in PBCs, SBCs, and WBCs, respectively. For

222   each phylogenetic tree, a heatmap was built in the inner ring represents Spearman's

223   correlation between OTUs and environmental variables. The middle ring was built to

224   represent the frequency of the OTUs in our studied sites. The outer ring was built to

225   represent the relative abundance of the OTUs.

226   A null model analysis was performed to investigate the processes shaping the assembly of

227   bacterial communities in permafrost soil, lake sediment, and lake water using the R

228   package picante 1.8.2 (Kembel et al., 2010). This analysis based on the calculation of the

229   beta nearest taxon index (βNTI) to measure the extent of deterministic processes in shaping

230   the phylogenetic composition of the communities, as well as a Bray–Curtis-based Raup-

231   Crick matrix (RC_{Bray}) to assess the relative influences of stochastic processes (Stegen et al.,

232   2013; Zhou and Ning, 2017). Because homogeneous selection results in communities that

233   share greater phylogenetic similarity, the proportion of homogeneous selection was

234   calculated as the fraction of pairwise comparisons with βNTI < −2. On the other hand,

235 heterogeneous selection, leading to communities with lesser phylogenetic similarity, was

236 measured as the fraction of pairwise comparisons with $\beta NTI > +2$. Because homogeneous

237 dispersal results in communities exhibiting greater taxonomic resemblance, the extent of

238 its impact was measured as the proportion of pairwise comparisons with $-2 < \beta NTI < 2$

239 and $RC_{Bray} < -0.95$. Conversely, communities constrained by dispersal limitation display

240 lesser taxonomic similarity, and the measure of dispersal limitation was derived from the

241 fraction of pairwise comparisons with $-2 < \beta NTI < 2$ and $RC_{Bray} > 0.95$. Finally, the

242 fraction of the pairwise comparisons with $-2 < \beta NTI < 2$ and $-0.95 < RC_{Bray} < 0.95$ was

243 identified as "undominated". Mantel tests were conducted to test the relationships between

244 environmental variables and βNTI.

245 All the statistical analyses were carried out in R 4.1.2 (R Core Team, 2020).

246 **3 Results**

247 *3.1 General distribution patterns of α-diversity*

248 After quality filtering, 3,201,132 high quality sequences were obtained and clustered into

249 9,361 OTUs, of which, 3870 OTUs were core OTUs shared by bacterial communities in

250 permafrost soil, lake sediment, and lake water (Figure S2Figure S2). Moreover, a large

251 number of OTUs were shared by PBCs and SBCs (n=7053), of which, 16.4% were enriched

252 in lake sediment and 19.3% were enriched in permafrost soil (Figure S2Figure S2).

253 However, a relatively small number of OTUs were shared by PBCs and WBCs (n=4007)

254 and by SBCs and WBCs (n=4431), and only a very small proportion of OTUs were

255 enriched in lake water (Figure S2Figure S2). Bacterial communities had a significantly

256 lower α-diversity in lake water than in lake sediment and permafrost soil (Figure 2Figure

257 2a). α-diversity was not significantly different between PBCs and SBCs (Figure 2Figure

258 2a). Correlation analyses showed that phylogenetic diversity of PBCs was positively

259 correlated with SOC, TN, and C:N:P ratios (Figure 2Figure 2b). For SBCs, α-diversity

260 indices were positively correlated with MAT, MAP, SOC, TN, C:P and N:P, while

261 negatively correlated with latitude and pH (Figure 2Figure 2b). For WBCs, α-diversity

262 indices were negatively correlated with pH, and Shannon diversity was negatively

263 correlated with DOC, TN, and C:P (Figure 2Figure 2b).

264 PBCs and SBCs had a significantly greater phylogenetic diversity than WBCs (Figure

265 2Figure 2a and Figure 3Figure 3). The OTUs in PBCs had significantly higher frequency

266 than that of SBCs and WBCs (Figure 3Figure 3). The top 1000 abundant OTUs in PBCs

267 were highly correlated with environmental variables, particularly with latitude, MAP, SOC,

268 TN, TP, and C:N:P ratios (Figure 3Figure 3a). The top 1000 abundant OTUs in SBCs were

269 more commonly positively correlated with MAP, SOC, TN, and C:N:P ratios, but more

270 commonly negatively correlated with latitude and pH (Figure 3Figure 3b). The top 1000

271 abundant OTUs in WBCs had relatively fewer significant relationships with environmental

272 variables in general, but were negatively correlated with latitude, conductivity, pH, DOC,

273 TN, and C:N:P ratios, while more positively correlated with MAP (Figure 3Figure 3c). In

274 addition, WBCs had significantly higher SES.MNTD than PBCs and SBCs (Figure 3Figure

275 3d), suggesting higher phylogenetic clustering of bacterial taxa in WBCs.
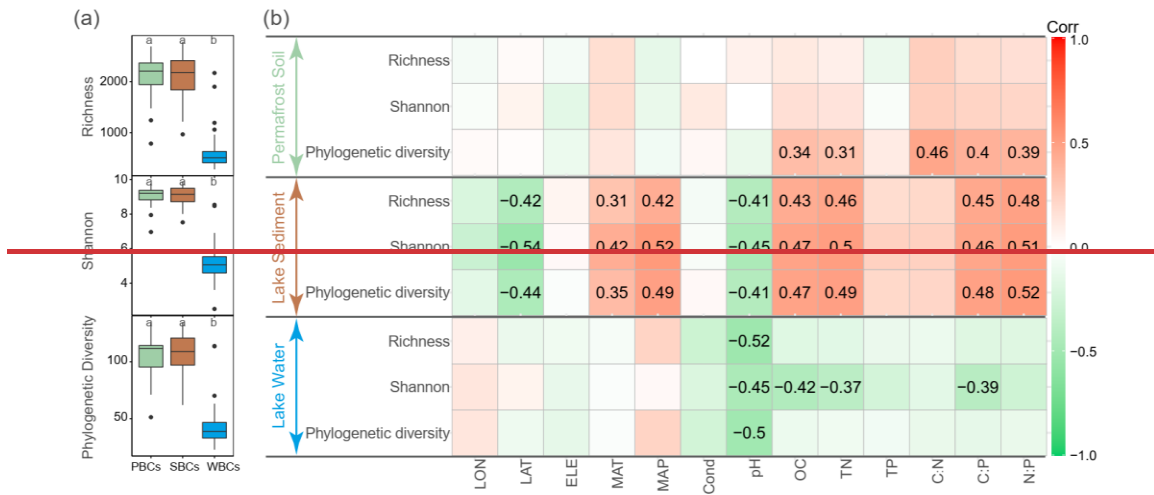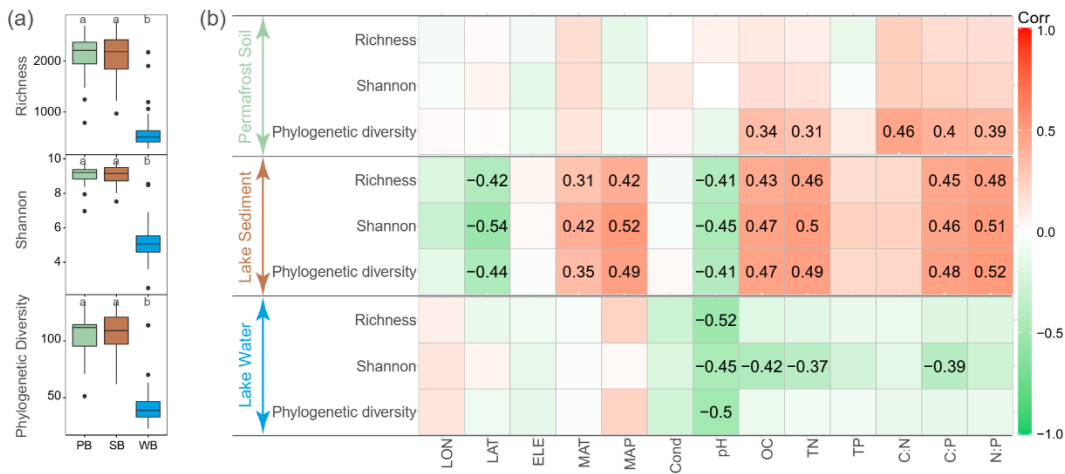
Figure 2 (a) Alpha diversity of bacterial communities in permafrost soil (PBC), lake sediment (SBC), and lake water (WBC). The different low-case letters represent significant differences assessed using Wilcoxon rank-sum test. (b) Spearman correlations show the relationships between alpha diversity and environmental factors. The color represents the correlation coefficient, which is shown in number when the result is statistically significant ($p < 0.05$).

284
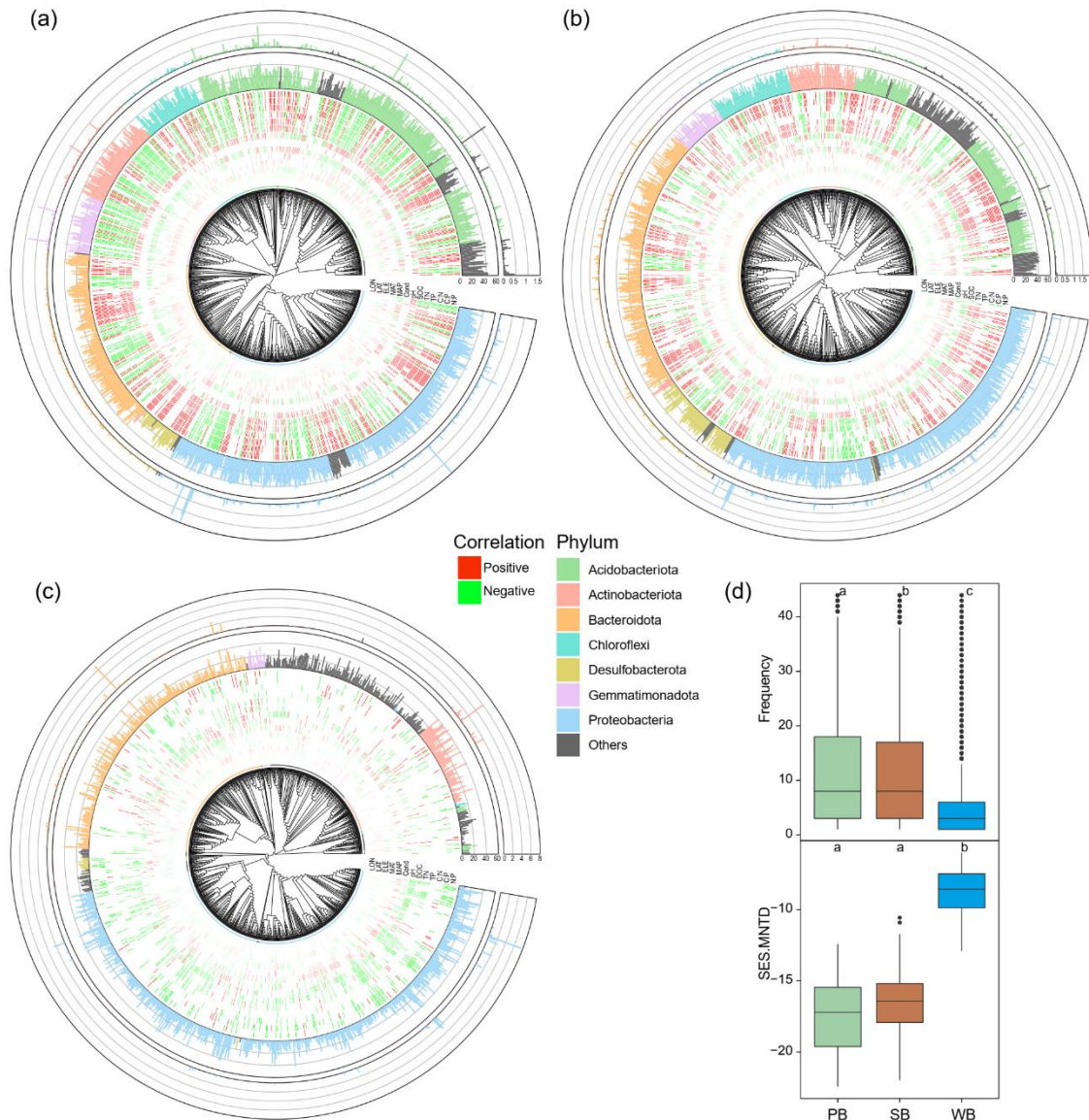
Figure 3 Phylogenetic tree of the top 1000 OTUs in <u>bacterial communities in </u>(a) permafrost soil ~~(PBC)~~, (b) lake sediment ~~(SBC)~~, and (c) lake water ~~(WBC)~~. Tree tips are colored by major phylum. The inner ring of the heatmap represents spearman's correlation between OTUs and environmental variables. Only significant (p<0.05) results are shown. The middle ring represents the frequency of the OTUs in our studied sites. The outer ring represents the relative abundance of the OTUs. (d) Boxplots showing differences of OTU's

291   frequency and SES.MNTD values among bacterial communities in permafrost soil (PBCs),

292   lake sediment (SBCs), and lake water (WBCs). The different lower-case letters represent

293   significant differences assessed using Wilcoxon rank-sum test.

294   **_3.2 Community composition and β-diversity patterns_**

295   PBCs were dominated by Proteobacteria (30.4%), Acidobacteriota (25.3%), Bacteroidota

296   (11.4%), Actinobacteriota (6.8%), Chloroflexi (5.2%), and Gemmatimonadota (5.2%)

297   (Figure 4Figure 4a). SBCs were dominated by Proteobacteria (35.2%), Bacteroidota

298   (20.0%), Acidobacteriota (11.3%), Desulfobacterota (6.4%), Chloroflexi (6.3%), and

299   Actinobacteriota (5.8%) (Figure 4Figure 4a). WBCs were dominated by Proteobacteria

300   (46.9%), Bacteroidota (29.2%), and Actinobacteriota (17.4%) (Figure 4Figure 4a). While

301   Proteobacteria were predominant in all three habitat types, these dominant phyla had

302   significantly different relative abundances among these habitats. Proteobacteria and

303   Bacteroidota had a significantly higher relative abundance in WBCs than in SBCs and

304   PBCs (Figure 4Figure 4a). The relative abundance of Actinobacteriota was the highest in

305   WBCs but was not significantly different between PBCs and SBCs (Figure 4Figure 4a).

306   Gemmatimonadota and Acidobacteriota were significantly enriched in PBCs than in SBCs

307   and WBCs. Desulfobacterota and Chloroflexi were significantly enriched in SBCs than in

308   PBCs and WBCs (Figure 4Figure 4a).

309   These phyla responded differently to environmental variables (Figure 4Figure 4b). For

310   example, Actinobacteriota and Gemmatimonadota in PBCs and Actinobacteriota and

311   Desulfobacterota in SBCs were negatively correlated with nutrient concentrations and

312   ratios, while Desulfobacterota in PBCs and Acidobacteriota in SBCs were positively

313   correlated with nutrient concentrations and ratios (Figure 4Figure 4b). pH was a frequently

16

314 correlated with for taxa in various taxonomic groups across all three habitats (Figure

315 4bFigure 5b)

316 Nonmetric multidimensional scaling (NMDS) analysis along with non-parametric

317 statistical tests showed that bacteria in different habitats formed distinct communities

318 (Figure 5Figure 5a). The extent of difference was larger for WBCs vs PBCs (β=0.98;

319 R_{ANOSIM} = 0.989, P<0.001) than the differences for WBCs vs SBCs (β=0.96; R_{ANOSIM} =

320 0.967, P<0.001). There was the least dissimilarity between PBCs and SBCs (β=0.81;

321 R_{ANOSIM} = 0.384, P<0.001). The fitted SEM model showed that PBCs had direct effects on

322 SBCs and WBCs, and the latter two had reciprocal effects on each other (Figure 5Figure

323 5b). In addition, location, climate, and permafrost soil physicochemical environments had

324 direct effects on PBCs. Climate had direct effects on SBCs while lake water

325 physicochemical environments had direct effects on WBCs (Figure 5Figure 5b).

326 WBCs had a higher β-diversity than SBCs and PBCs, suggesting that bacterial

327 communities were more spatially heterogeneous in lake water than in lake sediment and

328 permafrost soil (Figure 5cFigure 6a). Taxa in PBCs had higher habitat niche breadths than

329 taxa in SBCs and WBCs (Figure 5dFigure 6b). We estimated the distance decay

330 relationship of bacterial community similarity. Significant distance-decay relationships

331 were observed for all communities but the fitness values were relatively low (Figure

332 S3Figure S3), indicating weak decay of community similarity with geographic distance in

333 thermokarst landscape. We also explored the main environmental variables that influence

334 the variations of the bacterial communities (Figure 6Figure 7). β-diversities of PBCs, SBCs,

335 and WBCs were all significantly correlated with latitude, MAP, and pH (Figure 6Figure 7).

336 In addition, β-diversity of PBCs was also significantly correlated with all the other

337 environmental variables except MAT and conductivity. β-diversity of SB~~Cs~~ was also

338 significantly correlated with conductivity and C:N (Figure 6~~Figure 7~~). β-diversity of WB~~Cs~~

339 was also significantly correlated with elevation, MAT, conductivity, DOC, TN, and TP

340 (Figure 6~~Figure 7~~). The results suggested that the compositional variation among PB~~Cs~~,

341 SB~~Cs~~, and WB~~Cs~~ was differentially structured by spatial, climatic, and physicochemical

342 variables.
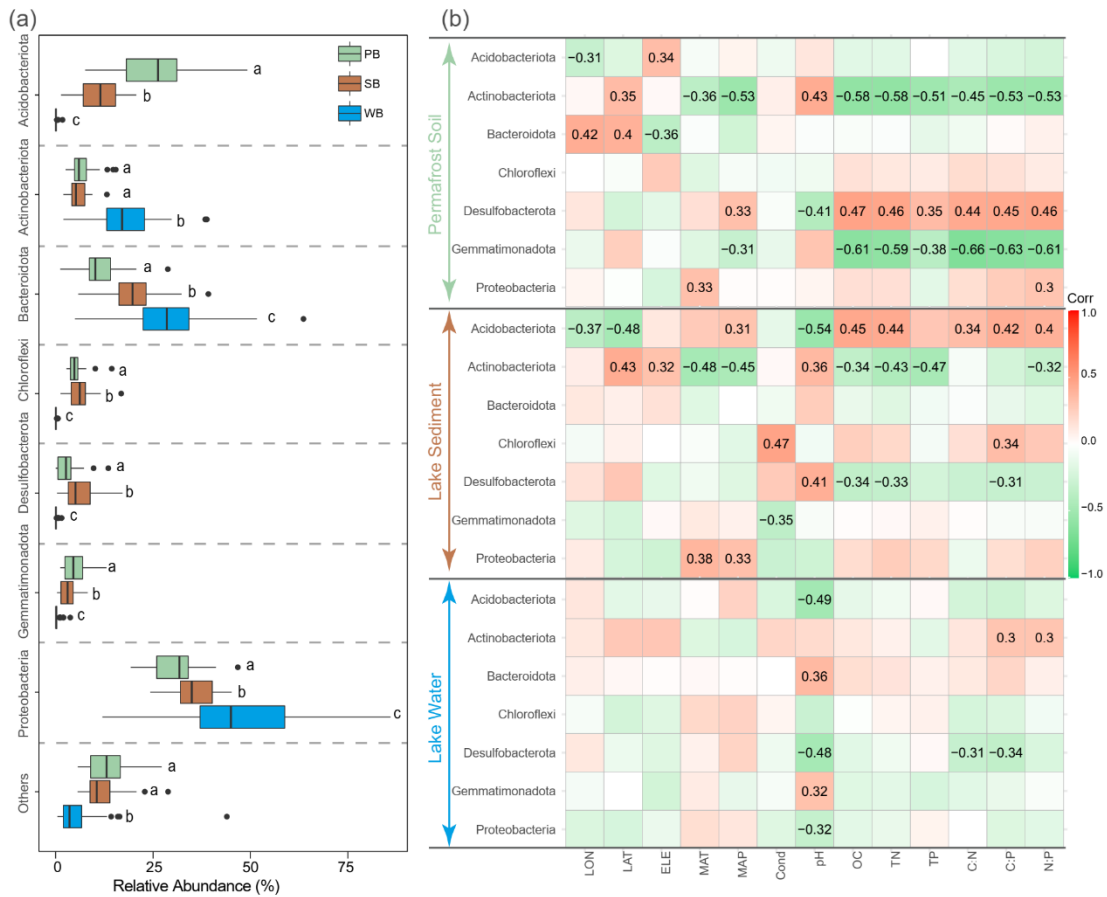
344 Figure 4 (a) Relative abundances of major phyla in bacterial communities in permafrost

345 soil (PB~~Cs~~), lake sediment (SB~~Cs~~), and lake water (WB~~Cs~~). The different low-case letters

346 represent significant differences assessed using Wilcoxon rank-sum test. (b) Spearman

347 correlations show the relationships between the relative abundance of major phyla and

348   environmental factors. The color represents the correlation coefficient, which shown in

349   number when the result is significant (p<0.05).

350
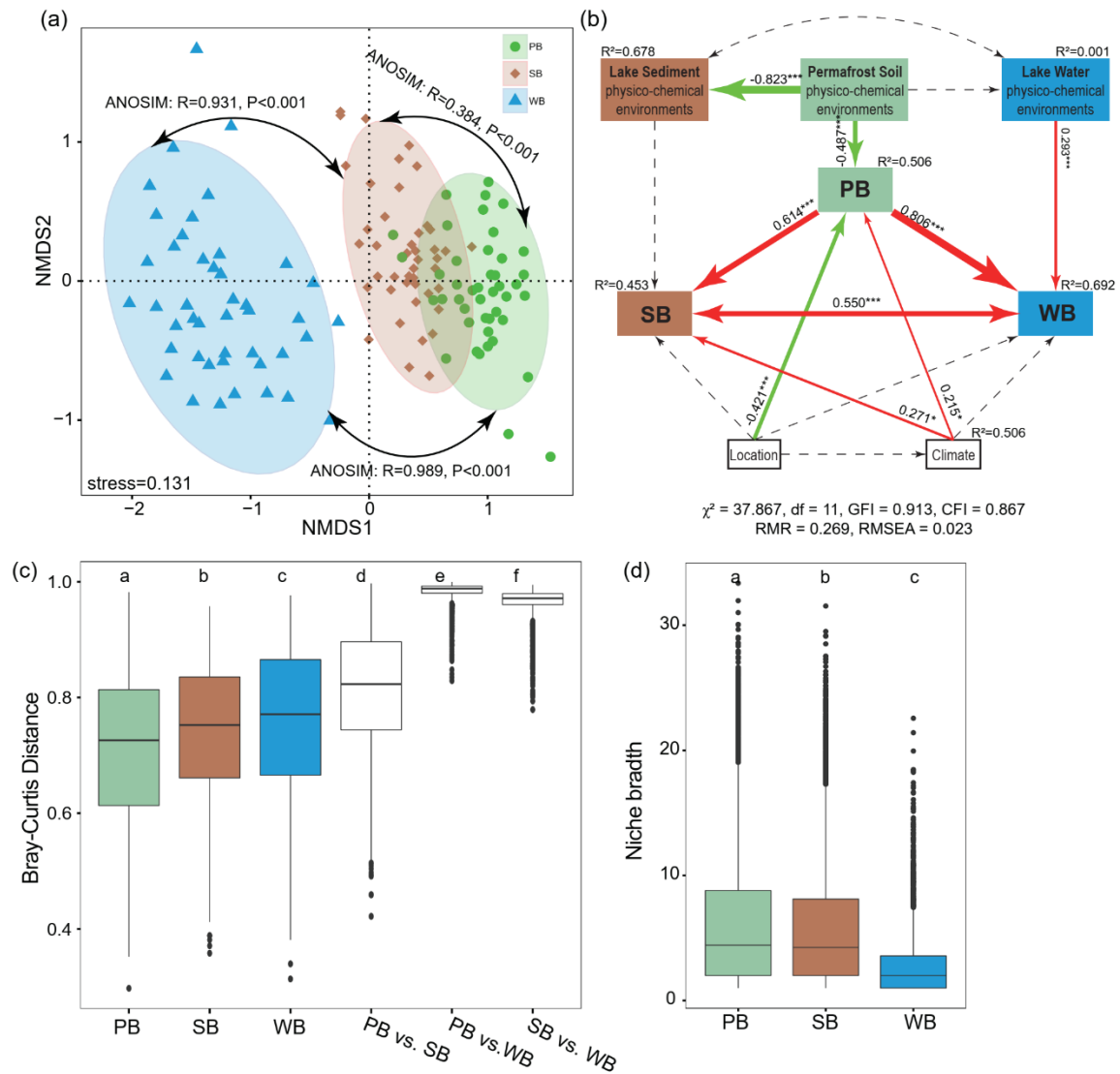
352   Figure 5 (a) Non-metric multidimensional scaling (NMDS) ordination showing the

353   distribution of bacterial communities in permafrost soil (PBCs), lake sediment (SBCs), and

354   lake water (WBCs). The differences between these communities are confirmed by the non-

355   parametric statistical test (ANOSIM). (b) Structural equation modeling analysis depicting

356   the relationships between location (including latitude, longitude, and elevation), climate

357   (including mean annual temperature and mean annual precipitation), physicochemical

358   environments (pH, conductivity, nutrients concentrations and stoichiometric ratios) of each

359   habitat. Solid and dashed arrows represent the significant and nonsignificant relationships,

360   respectively. Red and green arrows represent positive and negative relationships,

361   respectively. Significant path coefficients are shown adjacent to the path with *, **, and

362   *** denoting the significant level of p<0.05, p<0.01, and p<0.001, respectively. (c) β-

363   diversities within and between PB, SB, and WB. (d) Habitat niche breadth of the bacterial

364   communities.

365
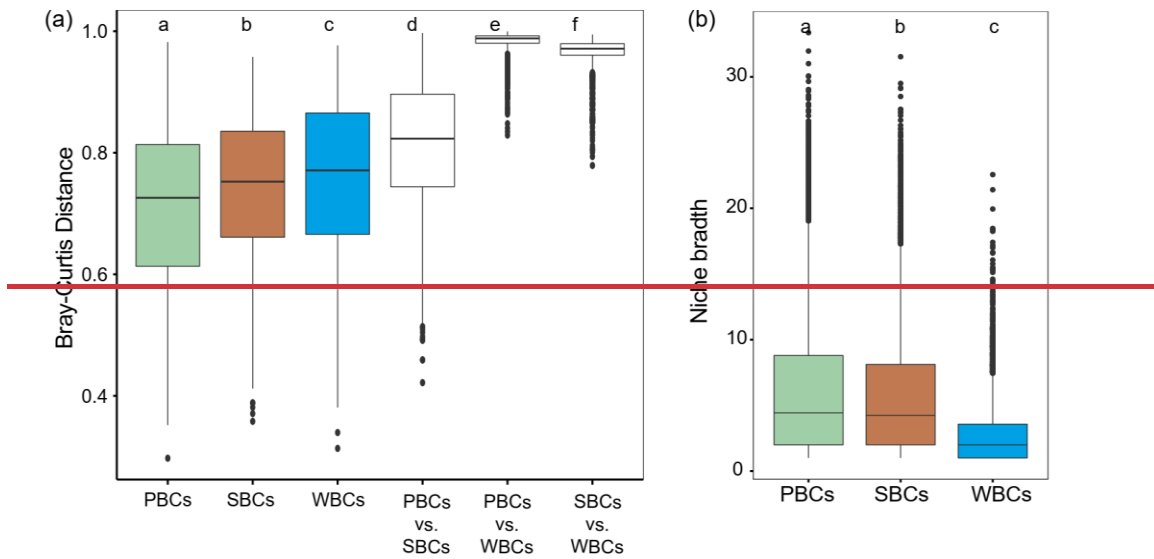
366   Figure 6 (a) β-diversities within and between PBCs, SBCs and WBCs. (b) Habitat niche

367   breadth of the bacterial communities.

368
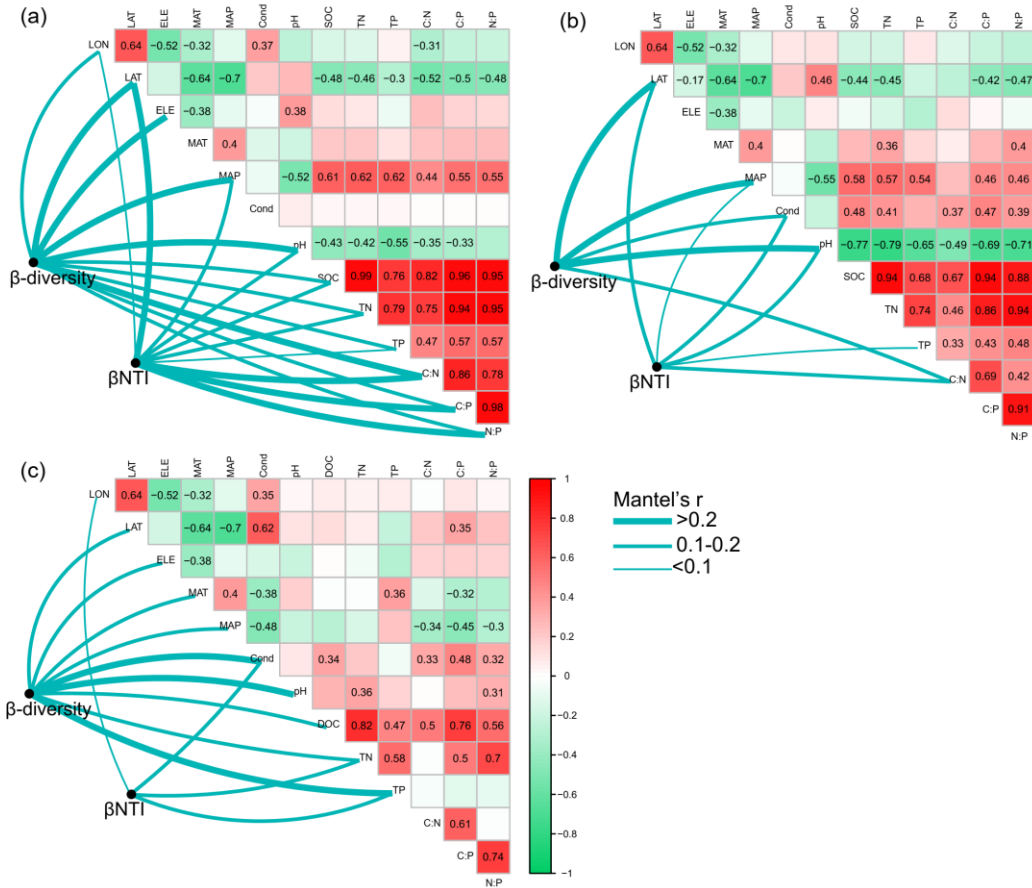
Figure 67 Pairwise correlations between environmental variables as well as the Mantel tests between environmental variables and β-diversity and beta nearest taxon index (βNTI) for (a) bacterial communities in permafrost soil, (b) bacterial communities in lake sediment, and (c) bacterial communities in lake water. β-diversity was calculated as Bray-Curtis distance. The lines denote significant relationships while the line width represents the Mantel's r statistic. Pairwise correlations between environmental variables are shown in color gradient matrix. The color represents Pearson's correlation coefficient, which shown in number when the result is significant (p<0.05). The abbreviations of the environmental variables are explained in the Methods section.

3.3 Assembly processes

To explore the mechanisms underlining the observed distribution patterns, a null-model-based framework was employed to quantify the deviation of phylogenetic turnover. PB~~Cs~~ had significantly higher βNTI than SB~~Cs~~ and WB~~Cs~~ (Figure 7~~Figure 8~~a). Deterministic processes contributed 51.3%, 41.2%, and 44.9% to community variations for the bacterial communities in permafrost soil, lake sediment, and lake water, respectively (Figure 7~~Figure 8~~b). In particular, the results showed that homogeneous selection contributed a larger fraction to the assembly of the WB~~Cs~~ (44.8%), followed by SB~~Cs~~ (35.2%) and PB~~Cs~~ (29.7%) (Figure 7~~Figure 8~~b). Heterogeneous selection influenced PB~~Cs~~ (21.6%) more than SB~~Cs~~ (6.0%) and WB~~Cs~~ (0.1%). Dispersal limitation contributed a larger fraction to SB~~Cs~~ (57.2%) than to PB~~Cs~~ (45%) and WB~~Cs~~ (37.5%).

The relationships between βNTI and major environmental variables were used to estimate changes in the relative influences of deterministic and stochastic assembly processes. Mantel tests showed that the assembly processes of bacterial communities in permafrost soil, lake sediment, and lake water had similarities and differences in the responses to environmental variables (Figure 6~~Figure 7~~). Particularly, differences of TP were significantly associated with βNTI of PB~~Cs~~, SB~~Cs~~, and WB~~Cs~~, implying that an increasing divergence of TP could contribute to a shift from homogeneous selection to heterogeneous selection in the assembly of bacterial communities in the QTP thermokarst landscape. Moreover, βNTI of PB~~Cs~~ was also significantly associated with other environmental variables, except elevation, MAT, and conductivity. βNTI of SB~~Cs~~ was also significantly associated with latitude, MAP, conductivity, pH, and C:N, while βNTI of WB~~Cs~~ was significantly associated with longitude, conductivity, and TN.

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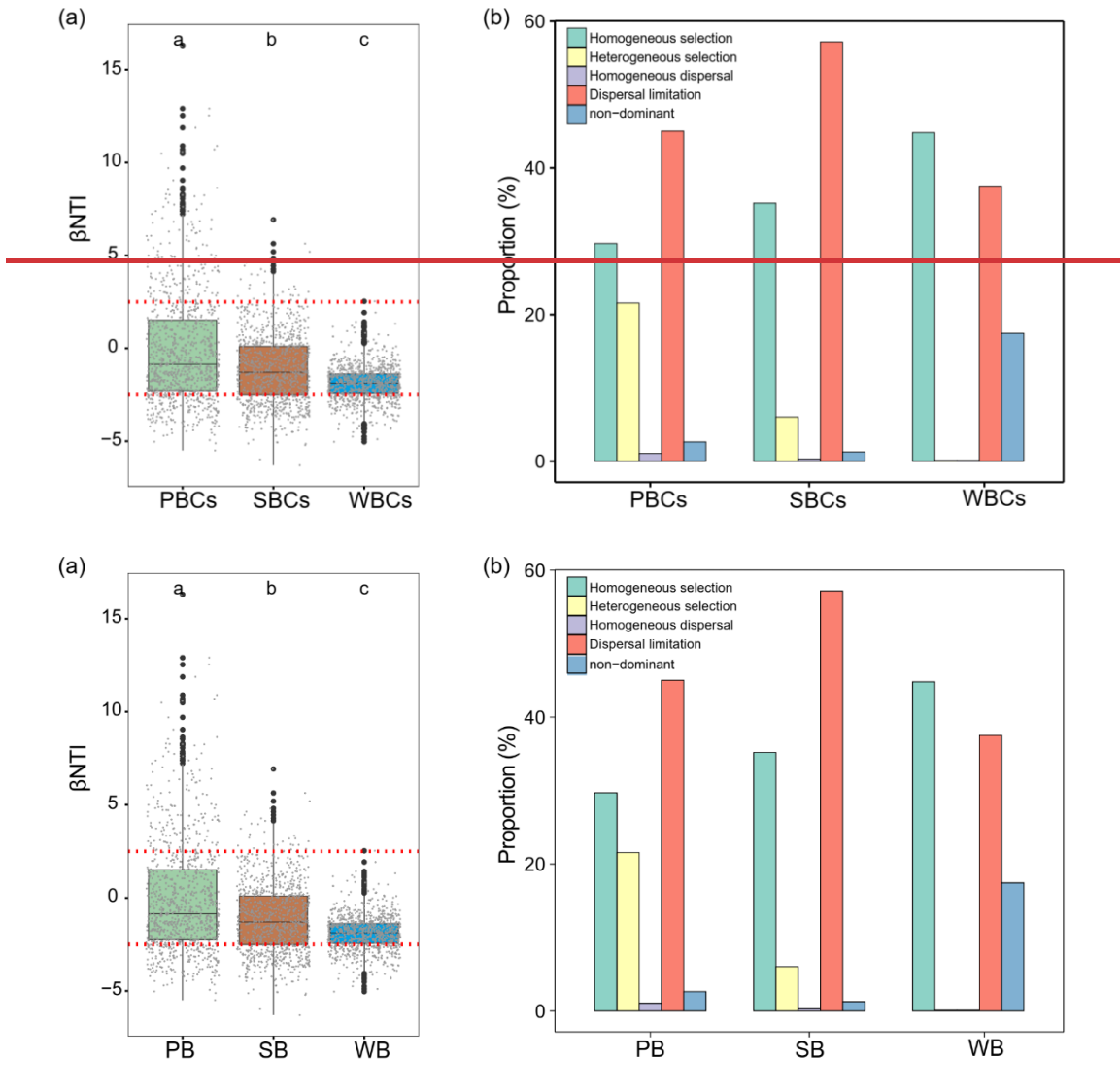
Figure 78 (a) The values of βNTI with horizontal dashed red lines indicate upper and lower significance thresholds at βNTI = +2 and −2, respectively in the three habitat types. (b) The contribution of deterministic (homogeneous and heterogeneous selection) and stochastic (dispersal limitations and homogenizing dispersal) processes to turnover in the assembly of bacterial communities in permafrost soil (PB), lake sediment (SB), and lake water (WB)PBCs, SBCs and WBCs. "Non-dominant" indicates that the fraction was not dominated by any single process.

4 Discussion

Thermokarst lakes and degraded permafrost are distinct habitats derived from original permafrost during the process of thermokarst formation. Degraded permafrost can be further converted to thermokarst lake sediment during the continuous process of thermokarst formation (Figure 1). In our studied thermokarst landscapes across the QTP, bacterial communities in degraded permafrost soil (PCBs), thermokarst lake sediment (SBCs), and thermokarst lake water (WCBs) differed in multiple aspects, such as α-diversity, β-diversity, community composition, community assembly rules, and environmental responses (Figure 1c), supporting a view in which thermokarst formation generates novel habitat conditions and microbial communities in landscapes formerly occupied by permafrost.

4.1 Alpha diversity and community composition

Permafrost soil and lake sediments on the QTP had significantly higher alpha diversity than lake water. A considerable proportion (41%) of OTUs were shared among PCBs, SCBs, and WCBs. However, besides a small number of unique OTUs, only a small proportion of OTUs were enriched in lake water. In addition, bacterial communities were also significantly different in composition and structure among permafrost soil, lake sediment, and lake water, but with lower dissimilarities between PCBs and SBCs. Due to the origin of thermokarst lakes from permafrost, there is no doubt that permafrost soil, lake sediments, and lake water should share a certain number of OTUs.

Thermokarst lakes are known to have sediments that derive from the permafrost soil and are constantly replenished by the collapse of nearby permafrost (Payette et al., 2004; West and Plug, 2008; Veremeeva et al., 2021). This suggests that permafrost soil and lake

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sediments are likely to have high levels of similarity in bacterial diversity and community composition. Thus, there is no doubt that permafrost soil, lake sediments, and lake water should share a certain number of bacteria. Indeed, our study showed that 41% OTUs were shared among PB, SB, and WB, while 75% OTUs were shared between PB and SB. Additionally, our prior research has shown that there are close correlations between the abiotic features of the two environments (Ren et al., 2022b). However, despite these similarities and connections, we found substantial differences in the bacterial communities of permafrost soil and lake sediments. As proposed by the Baas-Becking hypothesis (Baas-Becking, 1934), environmental selection is partially responsible for variation in microbial communities, which are also shaped by other ecological processes, such as diversification and dispersal limitation. Indeed, iIn our study, alpha diversity and the dominant phyla found in PBCs and SBCs responded differently to various environmental variables. In addition, there were significant differences in composition and structure among PB, SB, and WB, while the dissimilarities between PB and SB were the lowest.

Bacterial communities in lake water had significantly lower alpha diversity as well as distinct community composition and structure in comparison to bacterial communities in permafrost soil and lake sediment. However, PBCs and SBCs had direct influence on WBCs. For thermokarst lakes, the water first originates from the thawing of the ice-rich permafrost and the lake is then fed by precipitation-derived and permafrost-derived water (Yang et al., 2016a; Narancic et al., 2017; Wan et al., 2019). Microorganisms present in lake water have a diverse range of sources, including terrestrial inputs and other sources such as bacteria distributed with the atmosphere, associated with plants and animals, and carried by migratory birds and animals (Ruiz-Gonzalez et al., 2015). Thus, there was a

456 relatively small proportion of OTUs shared between permafrost soil and lake water, as well

457 as between lake sediment and water, and only a few shared OTUs were enriched in lake

458 water. It is a well-established fact that different habitats often support distinct microbial

459 communities (Fierer et al., 2012; Hugerth et al., 2015; Louca et al., 2016). The contrast in

460 bacterial community composition between lake sediments and water has been extensively

461 documented (Briee et al., 2007; Gough and Stahl, 2011; Yang et al., 2016b; Ren et al.,

462 2017). In addition, sediment generally harbor a higher species-level diversity of bacteria

463 compared to lake water (Lozupone and Knight, 2007; Ren et al., 2019b). For example, in

464 a permafrost thaw pond of Andes, it was also found that water samples had lower alpha

465 diversity than lake sediment and permafrost samples (Aszalós et al., 2020). Permafrost soil

466 and lake sediment may provide more habitat heterogeneity for bacterial taxa than the water

467 column, supported by our observation that the bacterial taxa had higher niche breadth in

468 permafrost soil and lake sediment than in lake water. Moreover, in hydrologically

469 connected terrestrial-aquatic ecosystems, bacterial communities can present distinct but

470 directional spatial structure driven by terrestrial recruited taxa (Ruiz-Gonzalez et al., 2015).

471 Thus, these community similarities between distinct bacterial habitats might be the result

472 of common bacterial source (original permafrost) and the differences are likely caused by

473 subsequent environmental selection, colonization from multiple other bacterial sources,

474 and distinct assembly mechanisms.

475 Despite connections driven by dispersal, distinct thermokarst habitats had ~~distinct~~ different

476 bacterial community composition, as seen in previous work (Ottoni et al., 2022). All the

477 dominant phyla were significantly different in relative abundance among ~~permafrost soil~~

478 ~~(PBCs), lake sediment (SBCs), and lake water (WBCs)~~PB, SB, and WB. In this study,

Proteobacteria, Bacteroidota, Actinobacteriota, Gemmatimonadota, Acidobacteriota, Desulfobacterota, and Chloroflexi dominated bacterial communities in permafrost soil and/or thermokarst lakes despite high variabilitiesy. Similar dominance of these taxa has also been found in permafrost and thermokarst landscapes in other areas (Aszalós et al., 2020; Belov et al., 2020; Wu et al., 2022). The most commonly reported bacterial groups in permafrost environments include members of Proteobacteria, Acidobacteria, Actinobacteria, Bacteroidetes, Firmicutes, and Chloroflexi (Steven et al., 2009; Altshuler et al., 2017; Ottoni et al., 2022), as observed in our samples.

4.2 Beta diversity and assembly processes

In our studied regions across the QTP, PBCs, SBCs, and WBCs all had a high beta diversity (average values > 0.7), with WBCs showing the highest, suggesting that bacterial communities shifted substantially across the large spatial scale of our sampling. Moreover, beta diversities of PBCs, SBCs, and WBCs were significantly correlated with each other, further suggesting that the bacteria in different habitats had a considerable proportion of members from the same source, the original pristine permafrost soil. The significantly lower mean SES.MNTD for PBCs indicate that bacterial communities in permafrost soil were more closely phylogenetically clustered and suffered stronger environmental filtering than those in lake sediment and water (Langenheder et al., 2017), consistent with the observation that PBCs had lower beta diversity than SBCs and WBCs. SES.MNTD is sensitive to changes in lineage close to the phylogenetic tips (Kembel et al., 2010). The higher SES.MNTD observed for SB and WB suggest the possibility that the bacteria in lake sediment and water exhibit a substantial divergence in the co-occurring species, and thermokarst lakes have experienced colonization by bacterial species originating from

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distinct clades or lineages from external sources following permafrost thaw (Webb et al., 2002; Stegen et al., 2013).

The structure of bacterial communities can vary across spatiotemporal scales and different habitats (Ren et al., 2017; Aguilar and Sommaruga, 2020; Pearman et al., 2020). A key objective in the field of microbial ecology is to determine the relative influence of stochastic and deterministic processes in shaping the assembly of communities (Stegen et al., 2013; Zhou and Ning, 2017). In this study, deterministic processes contributed 51.3%, 41.2%, and 44.9% to community variation for the bacterial communities in permafrost soil, lake sediment, and lake water, respectively. Homogeneous selection contributed a larger fraction to the assembly of the WBCs (44.8%), followed by SBCs (35.2%) and PBCs (29.7%). Heterogeneous selection influenced PBCs (21.6%) more strongly than SBCs (6.0%) and WBCs (0.1%). In addition, bbacterial communities in lake water displayed a higher influence of homogeneous selection but lower influence of heterogeneous selection compared to those in lake sediments and permafrost soil in our study. The reason for this might be that lLong-term changes in thermokarst lakes result in homogenized habitats and consequently strong homogenous selection on bacterial communities (Ning et al., 2019). In contrast, permafrost soil is a highly heterogeneous environment across spatial scales (Etzelmüller, 2013; Nitzbon et al., 2021), creating a wide range of habitats which can impose strong heterogeneous selection pressures on bacterial communities. Furthermore, permafrost soil is characterized by limited nutrient availability due to the frozen state of organic matters (Beermann et al., 2017; Zhang et al., 2023), while lake water offers a more diverse and abundant array of dissolved organic compounds and nutrients. As a result, bacterial communities in permafrost soil might be more sensitive to variations in resource

availability, rendering them more strongly influenced by heterogeneous selection. ~~Deterministic processes could also cause the communities to be more dissimilar through heterogeneous selection, which also imposed strong control on PBCs.~~In addition, ~~D~~dispersal limitation contributed a larger fraction to SB~~Cs~~ (57.2%) than to PB~~Cs~~ (45%) and WB~~Cs~~ (37.5%). The dispersal of microorganisms is often considered as a passive process that results in community variation and turnover coupled with the function of environmental filtering (Cline and Zak, 2014; Stegen et al., 2015; Custer et al., 2022). The high dispersal limitation of microbial communities in thermokarst lakes may be attributed to several factors. These include the isolated nature of thermokarst lakes, which are endorheic and therefore have limited connectivity, as well as the strong restriction on microbial dispersal and the presence of strong environmental filtering processes. ~~The high dispersal limitation of microbial communities in thermokarst lakes could be potentially explained that the isolated nature of thermokarst lakes being endorheic results in limited connectivity and strong restriction of microbial dispersal, as well as strong environmental filtering.~~Additionally, the prolonged frozen phase of thermokarst lakes and permafrost soil restrict the movement of microorganisms (Vargas Medrano, 2019; Vigneron et al., 2019). Furthermore, geographical barriers, exemplified by prominent mountain ranges like the Tanggula Mountains, Kunlun Mountains, Nyenchen Tanglha Mountains, and Bayan Har Mountains, serve as impediments to the dispersal of both macro- and microorganisms (Wan et al., 2016; Yu et al., 2019; Ren et al., 2022c). Particularly in lake sediment, where bacterial communities are more isolated over distances and will not disperse as far as those in lake water and permafrost soil, resulting in stronger influence of dispersal limitation (Martiny et al., 2006; Xiong et al., 2012). Although the "everything is everywhere"

548   hypothesis suggests that many microorganisms have a cosmopolitan distribution, their

549   slow mobility allows for the development of regional phylogenetic differences and the

550   emergence of specialized, endemic taxa in isolated habitats, resulting in a low likelihood

551   of microorganisms dispersing to suitable distant sites (Telford et al., 2006). Therefore,

552   dispersal processes in this thermokarst landscape may be restricted by the lack of

553   hydrological connection, limited movement of water, short duration since thawing, and

554   strong environmental filtering, contributing to the observed high dispersal limitation in the

555   studied permafrost soil and thermokarst lakes. This inference is supported by many

556   previous studies showing that dispersal limitation plays a major role in structuring

557   microbial communities in lakes (Telford et al., 2006). Strong dispersal limitation for

558   bacterial communities in permafrost has also been documented across an Alaskan boreal

559   forest landscape (Bottos et al., 2018). ~~In addition, bacterial communities in lake water~~

560   ~~displayed a higher influence of homogeneous selection compared to those in lake~~

561   ~~sediments and permafrost soil in our study. The reason for this might be that long-term~~

562   ~~changes in thermokarst lakes result in homogenized habitats and consequently strong~~

563   ~~homogenous selection on bacterial communities. Deterministic processes could also cause~~

564   ~~the communities to be more dissimilar through heterogeneous selection, which also~~

565   ~~imposed strong control on PBCs.~~

566   *4.3 Environmental influences*

567   Understanding how environmental factors shape bacterial communities is a crucial aspect

568   in the field of microbial ecology (Fierer and Jackson, 2006; Pla-Rabes et al., 2011). ~~With~~

569   ~~global warming, climatic and physicochemical environments will be strongly altered in~~

570   ~~permafrost areas. On the QTP in particular, air temperature and precipitation are increasing~~

571 ~~in most regions (Xu et al., 2008; Lu et al., 2018). Moreover, organic carbon and nutrient~~

572 ~~stocks in permafrost are decreasing (Turetsky et al., 2020; Wu et al., 2021) and thermokarst~~

573 ~~lakes are developing, driving dynamic environmental change (Luo et al., 2015; Vucic et~~

574 ~~al., 2020). These environmental disruptions likely impose strong influences on bacterial~~

575 ~~communities in thermokarst landscapes.~~ In our study, bacterial communities were

576 differentially correlated with various measured environmental variables. In our data, pH

577 was consistently identified as a strong correlate of microbial community structure and

578 diversity, as is often observed in terrestrial and aquatic ecosystems worldwide (Fierer and

579 Jackson, 2006; Xiong et al., 2012). While such correlations between pH and bacterial

580 communities have been widely found, the regulation mechanisms still remain unknown

581 (Malard and Pearce, 2018). Moreover, the influences of pH are often species- and location-

582 specific (Malard and Pearce, 2018; Egelberg et al., 2021). In this study, pH had

583 significantly negative relationships with alpha diversity of bacterial communities in lake

584 sediment and water, and had negative or positive correlations with some phyla. Moreover,

585 differences in pH might drive community variation observed between PB~~Cs~~, SB~~Cs~~, and

586 WB~~Cs~~, and shift community assembly processes for PB~~Cs~~ and SB~~Cs~~. <u>Moreover, with</u>

587 <u>permafrost degrading and thermokarst developing, nutrient status will be strongly altered</u>

588 <u>in permafrost areas.</u> ~~On the QTP in particular, air temperature and precipitation are~~

589 ~~increasing in most regions (Xu et al., 2008; Lu et al., 2018)~~<u>Organic carbon and nutrient</u>

590 <u>stocks in permafrost are decreasing</u> (Turetsky et al., 2020; Wu et al., 2021) <u>and thermokarst</u>

591 <u>lakes are developing, leading to dynamic environmental change</u> (Luo et al., 2015; Vucic et

592 al., 2020). <u>These environmental disruptions likely impose strong influences on bacterial</u>

593 <u>communities in thermokarst landscapes.</u> Our study ~~also~~ showed that nutrient (C, N, and P)

concentrations and stoichiometric ratios were strongly related to alpha diversity (particularly for SB~~Cs~~) and community variation and assembly (particularly for PB~~Cs~~). High organic matter content, for instance, has been shown to support diverse and complex microbial communities (Garrido-Benavent et al., 2020; Ren and Gao, 2022). The role of nutrient availability in shaping bacterial communities has also been well established (Torsvik et al., 2002; Lee et al., 2017; Zhou et al., 2020). ~~High organic matter content, for instance, has been shown to support diverse and complex microbial communities (Garrido-Benavent et al., 2020; Ren and Gao, 2022).~~ For example, Actinobacteria and Gemmatimonadota have a negative, while Gemmatimonadota has a positive relationship with organic carbon and nutrients in permafrost (Romanowicz and Kling, 2022; Fu et al., 2023), in line with our results. The fact that different bacterial phyla exhibited varied responses to changes in organic carbon and nutrient further emphasizes the intricate interplay between microorganisms and their environment. Due to their ecological strategies, metabolic features, and environmental preferences, bacteria in permafrost respond differentially to nutrient status and other stressors ~~(Carbonero et al., 2014)~~, driving adaptive changes in community composition and function (Mackelprang et al., 2017). In addition, compared to permafrost soil and lake water, lake sediment can exhibit more stable physicochemical conditions. However, permafrost soil and lake water experience more dynamic and extreme environmental changes, which drive the bacterial communities. The results of SEM also in line with bacterial community assembly that deterministic processes had stronger influences on PB and WB than on SB. In addition, ~~T~~thermokarst lakes have sediment directly formed from permafrost soil, and thus, permafrost soil environments and bacterial communities had strong associations with that of lake sediment.

617   In addition to physicochemical environments, location and climate were also suggested to

618   influence bacterial communities in distinct habitats. On the QTP in particular, air

619   temperature and precipitation are increasing in most regions (Xu et al., 2008; Lu et al.,

620   2018). Warming and altered precipitation regimes under climate change have been

621   demonstrated to affect alpha diversity and composition of stream microbial communities

622   at continental scales (Picazo et al., 2020). Our study indicates that location (particularly

623   latitude) and climate (particularly MAP) factors are important in shifting bacterial

624   communities in thermokarst landscapes. Particularly for bacterial communities in

625   permafrost soil, location and climate have been evidenced as strong factors in shaping

626   microbial communities (Taş et al., 2018; Barbato et al., 2022). Understanding large-scale

627   pattern of bacterial communities is increasingly important to offer insights into the impacts

628   of climate change (Picazo et al., 2020; Ren et al., 2021). As global climate changes, QTP

629   is getting warmer and more humid (Xu et al., 2008; Lu et al., 2018). Therefore, significant

630   alterations to the physical, chemical, and biological properties of thermokarst lakes on the

631   QTP can be expected in the coming decades. Based on "space-for-time" substitution, our

632   study serves as a foundation for predicting the potential impact of climate change on

633   bacterial communities in thermokarst landscapes.

634   **5 Conclusion**

635   In this study, we investigated bacterial communities in paired water and sediment samples

636   in thermokarst lakes as well as permafrost soil around the lakes across the QTP. esults

637   showed that each habitat had distinct bacterial assemblages, with lower alpha diversity in

638   lake water and higher beta diversity in lake sediment and permafrost soil. There was

639   considerable overlap in OTUs across habitats. Bacterial communities in permafrost soil

640 and lake sediment were influenced by dispersal limitation, while those in lake water were

641 driven by homogeneous selection. Environmental variables, including latitude, mean

642 annual precipitation, and pH, affected bacterial community variations in all habitats. The

643 study highlights the unique bacterial communities and ecological impacts of permafrost

644 degradation in diverse habitats created by thermokarst processes.

648 **Data availability statement:**

649 Raw sequences were uploaded to the China National Center for Bioinformation

650 (PRJCA009850, CRA007082).

655 **Conflict of interest disclosure:**

656 The authors declare no competing interests.

657 **Author's contributions:**

658 Z.R. designed the study, did the analyses, and prepared the manuscript, performed the field

659 work and laboratory work. All the authors prepared the manuscript.

660 **Ethics approval statement:**

661   Not applicable

662   **Permission to reproduce material from other sources:**

663   Not applicable

664   **Originality-Significance Statement:**

665   This is our original study and not submitted to elsewhere

666   **Supplementary Information**

667   Figure S1 Map of the 44 sampling sites of permafrost soil and thermokarst lakes across the

668   Qinghai-Tibet Plateau. The distribution of the permafrost was cited from Zou et al., 2017.

669   This map was cited from Ren et al, 2022a.

670   Figure S2 (a) Venn diagram showing the unique and shared OTUs among distinct bacterial

671   communities in permafrost soil (PB), lake sediment (SB), and lake water (WB). (b) The

672   volcano plot showing the shared OTUs that significantly (t-test, $P < 0.05$) enriched in a

673   certain habitat. The volcano plot was constructed using $\log_2$ (fold change) on x-axis and $-$

674    $\log_{10}$ (p-values of t-test) on y-axis.

675   Figure S3 Distance-decay curves showing community similarity of bacterial communities

676   in permafrost soil (PB), lake sediment (SB), and lake water (WB) against geographic

677   distances between sampling sites. Solid lines denote the ordinary least-squares linear

678   regressions.

679

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