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), thermokarst lake sediments (SB), and lake water (WB). Our study aimed to investigate the paired PB, SB, and WB 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 than in SB and PB. However, up to 41% of the OTUs were shared by PB, SB, and WB, suggesting that many taxa originate from the same sources via dispersal. SB and WB had reciprocal dispersal effects and both were correlated with PB. Dispersal limitation was the most dominant assembly process shaping PB and SB while homogeneous selection was the most dominant for WB. 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

39   is currently present in the atmosphere (Schuur et al., 2009; Hugelius et al., 2014; Mishra et

40   al., 2021). Permafrost is highly sensitive to climate warming (Wu et al., 2007; Jorgenson

41   et al., 2010; Biskaborn et al., 2019), which is expected to reduce 50-90% of permafrost

42   cover by 2100 (Lawrence et al., 2012; Chadburn et al., 2017). As a result of ice-rich

43   permafrost thaw, thermokarst lakes and ponds are formed (Kokelj and Jorgenson, 2013;

44   Farquharson et al., 2016) and extensively distributed across the Arctic and sub-Arctic

45   regions (de Jong et al., 2018) as well as the QTP (Niu et al., 2011; Luo et al., 2020). The

46   initial sediment and water in thermokarst lakes originate from the melting of permafrost,

47   and they are continuously replenished through the collapse of permafrost and precipitation

48   (West and Plug, 2008; de Jong et al., 2018). Thus, thermokarst lake sediments and water,

49   as well as the surrounding degraded permafrost soil, represent three distinct habitats

50   derived from the original permafrost during the process of thermokarst formation (Figure

51   1). It is well known that thermokarst processes substantially influence regional

52   hydrological, ecological, and biogeochemical processes (Chin et al., 2016; In'T Zandt et

53   al., 2020; Manasypov et al., 2021) and initiate a strong positive climate feedback to global

54   warming (Walter et al., 2006; Schuur et al., 2008; Schaefer et al., 2011; Anthony et al.,

55   2018). However, the microbial differences and relationships among these distinct habitats

56   in thermokarst landscapes are largely unknown.

57   Understanding microbes in thermokarst landscapes, and elsewhere, is important because

58   microbial communities play pivotal roles in driving biogeochemical and ecological

59   processes. To understand thermokarst microbial communities, we need to understand the

60   assembly mechanisms structuring them, a central research topic in microbial ecology

61   (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 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 1). Our aims were to (1) assess the spatial

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

108 community assembly mechanisms and environmental responses of the bacterial

109 communities in degraded permafrost soil, as well as in the sediment and water of

110 thermokarst lakes.

111 **2 Methods**

112 *2.1 Study area, field sampling, and chemical analysis*

113 This work was conducted across the QTP in July 2021 (Figure S1). In total, 44 sites were

114 investigated by collecting paired samples of lake water, lake sediment, and surrounding

115 permafrost soil (Figure 1a) (Ren et al., 2022a). The sampling strategy and chemical

116 analysis methods were described in detail in our previous publications (Ren et al., 2022a,

117 b). For water sampling of each lake, surface water samples were collected at a depth of 0.3

118 to 0.5 m with three replicates. For microbial analysis, 200 mL of water was filtered using

119 a 0.2-μm polycarbonate membrane filter (Whatman, UK) for DNA extraction. The

120 remaining water samples were transported to the lab for other physicochemical

121 measurements. For sediment sampling, the top 15 cm of sediment was collected from 3

122 points. Sediment samples for microbial analysis were collected in a 45-mL sterile

123 centrifuge tube, and the remaining samples were air-dried for analyzing physicochemical

124 properties. For permafrost sampling, five topsoil cores were collected along three 25-m

125 transects with increasing distances to the lake shore, respectively. The soils from one

126 transects were homogenized. Soil samples for microbial analysis were stored in 45-mL

127 sterile centrifuge tubes and the remaining soils were used for analyzing physicochemical

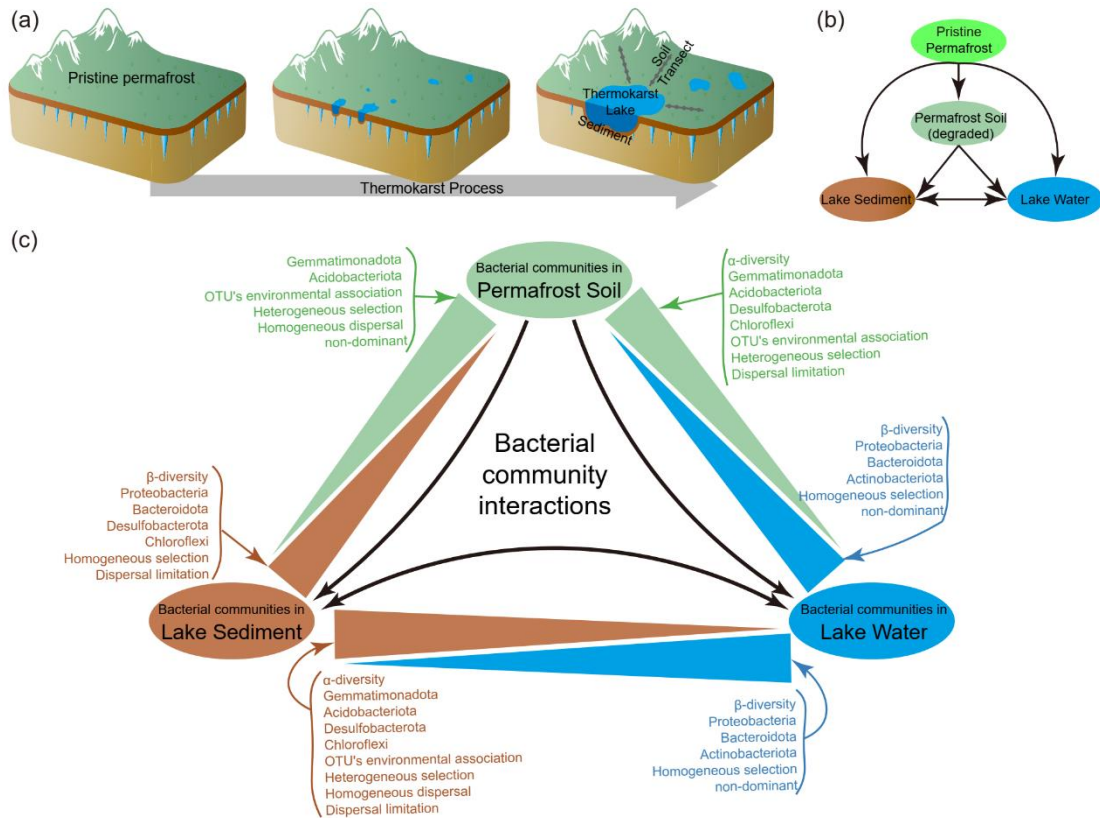
128 properties. For each sampling site, pH, conductivity (Cond), organic carbon (DOC in water

129 and SOC in sediment and soil), total nitrogen (TN), and total phosphorus (TP) were

130 measured according to our previous publications (Ren et al., 2022a, b). Moreover, the QTP

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

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

133 and mean annual precipitation (MAP) for each of the study sites.

134

135 Figure 1 (a) The process of thermokarst lake formation in ice-rich permafrost (modified

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

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

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

139 *2.2 DNA extraction, PCR, and sequencing*

140 The Magen Hipure Soil DNA Kit (Magen, China) was used to extract DNA from soil (0.5

141 g frozen soil), sediment (0.5 g frozen sediment), and water (membrane filter) samples

142 according to the manufacturer's protocols. Extraction blanks were routinely performed in

143   parallel. The prokaryotic 16S rRNA gene's V3-V4 hypervariable regions were amplified

144   using   universal   primers   343F-TACGGRAGGCAGCAG   and   798R-

145   AGGGTATCTAATCCT (Nossa et al., 2010). PCRs were conducted in 25 μl reaction

146   mixture containing 2.5 μl of TransStart buffer, 2 μl of dNTPs, 1 μl of each primer, 0.5 μl

147   of TransStart Taq DNA polymerase, and 20 ng template DNA. The PCR reactions were

148   conducted on a thermal cycler (ABI GeneAmp® 9700, USA) using the followed procedure:

149   initial denaturation at 94 °C for 5 min, 24 cycles of denaturation at 94 °C for 30 s followed

150   by annealing at 56 °C for 30 s and extension at 72 °C for 20 s, and a final extension at

151   72 °C for 5 min. To reduce amplification bias, three individual PCR amplifications were

152   performed for each sample and the triplicate PCR products were combined and purified.

153   DNA libraries were verified on 2% agarose gels and quantified using a Qubit 4 Fluorometer

154   (Thermo Fisher Scientific, Waltham, USA). Next generation sequencing of the amplicon

155   products was conducted on an Illumina Miseq Platform (Illumina, San Diego, CA, USA).

156   Automated cluster generation and 250/300 paired-end sequencing with dual reads were

157   performed following the manufacturer's instructions. The forward and reverse reads were

158   joined and assigned to samples based on barcode and truncated by cutting off the barcode

159   and primer sequence. Raw sequences were trimmed of ambiguous bases and low-quality

160   sequences (quality score lower than 20). After trimming, the paired-end reads were joined

161   and de-noised using QIIME1.9.1 (Caporaso et al., 2010). The sequences were subjected to

162   the following denoising criteria: sequences with ambiguous or homologous regions, as well

163   as those below 200 bp in length, were excluded; sequences with at least 75% of bases

164   having a quality score above Q20 were retained; and chimeric sequences were identified

165   and eliminated. All sequences from extraction blanks were removed. The effective

166 sequences were grouped into Operational Taxonomic Units (OTUs) using a 97% sequence

167 similarity threshold and annotated the taxonomic classifications against the SILVA 138

168 database (released on 02-Nov-2020) (Quast et al., 2013). The singletons were removed,

169 and the sequences were rarefied to the lowest number of sequences per sample (24,251

170 sequences) to eliminate the bias from the sampling effort.

171 *2.3 Analyses*

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

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

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

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

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

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

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

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

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

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

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

183 communities in different habitats, including permafrost soil bacterial communities (PB),

184 lake sediment bacterial communities (SB), and lake water bacterial communities (WB),

185 were assessed using Wilcoxon rank-sum test. The relationships between taxonomic and

186 environmental variables were assessed using Spearman correlation, and the P-values were

187 corrected using the FDR method (Benjamini and Hochberg, 1995). Mantel tests were

188 performed to examine the correlation between environmental variables and β-diversity. A

189   Non-metric Multidimensional Scaling (NMDS) analysis was conducted to examine the

190   distribution of PB, SB, and WB using the "metaMDS" function in the vegan 2.5-7 package

191   based on the Bray-Curtis distance using the relative abundance of OTUs. (Oksanen et al.,

192   2020). The distinctiveness of these communities was confirmed through a non-parametric

193   statistical test (ANOSIM) using the "anosim" function in the vegan package. The habitat

194   niche occupied by each species was estimated by calculating Levin's niche breadth (Levins,

195   1968) with the use of the spa 0.2.2 package (Zhang, 2016). Species with a broader niche

196   breadth were distributed more evenly across a wider range of habitats, compared to those

197   with a narrower niche breadth.

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

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

200   temperature and mean annual precipitation), and physicochemical variables (including pH,

201   conductivity, nutrients concentrations and stoichiometric ratios) of each habitat

202   (permafrost soil, lake sediment, and lake water), as well as their bacterial communities (PB,

203   SB, and WB). In model building, the SEM incorporated prior knowledges: (a) location and

204   climate factors potentially influence all the studied bacterial communities, (b)

205   physicochemical factor of each habitat potentially influences the corresponding bacterial

206   communities, and (c) permafrost soil potentially influences thermokarst lake sediment and

207   water, while lake sediment and water interact with each other. In the SEM, location, climate,

208   and physicochemical environments were reduced in dimensions by principal component

209   analysis (PCA), respectively, using the "prcomp" function of the vegan package, and the

210   first axis (PCA1) was used in SEM. For community structure, the first axis of NMDS was

211   used. SEM was constructed using the lavaan package (Rosseel, 2012). The fit of SEM was

212   assessed using standard indices, including chi-square (χ^2), goodness-of-fit index (GFI),

213   comparative fit index (CFI), root mean square residual (RMR), and root mean squared error

214   of approximation (RMSEA) (Hu and Bentler, 1999; Barrett, 2007).

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

216   2017) using the top 1000 abundant OTUs in PB, SB, and WB, respectively. For each

217   phylogenetic tree, a heatmap was built in the inner ring represents Spearman's correlation

218   between OTUs and environmental variables. The middle ring was built to represent the

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

220   abundance of the OTUs.

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

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

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

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

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

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

227   2013; Zhou and Ning, 2017). Because homogeneous selection results in communities that

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

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

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

231   measured as the fraction of pairwise comparisons with βNTI > +2. Because homogeneous

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

233   its impact was measured as the proportion of pairwise comparisons with −2 < βNTI < 2

234   and RC_{Bray} < −0.95. Conversely, communities constrained by dispersal limitation display

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

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

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

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

239 environmental variables and βNTI.

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

241 **3 Results**

242 *3.1 General distribution patterns of α-diversity*

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

244 9,361 OTUs, of which, 3870 OTUs were core OTUs shared by bacterial communities in

245 permafrost soil, lake sediment, and lake water (Figure S2). Moreover, a large number of

246 OTUs were shared by PB and SB (n=7053), of which, 16.4% were enriched in lake

247 sediment and 19.3% were enriched in permafrost soil (Figure S2). However, a relatively

248 small number of OTUs were shared by PB and WB (n=4007) and by SB and WB (n=4431),

249 and only a very small proportion of OTUs were enriched in lake water (Figure S2).

250 Bacterial communities had a significantly lower α-diversity in lake water than in lake

251 sediment and permafrost soil (Figure 2a). α-diversity was not significantly different

252 between PB and SB (Figure 2a). Correlation analyses showed that phylogenetic diversity

253 of PB was positively correlated with SOC, TN, and C:N:P ratios (Figure 2b). For SB, α-

254 diversity indices were positively correlated with MAT, MAP, SOC, TN, C:P and N:P,

255 while negatively correlated with latitude and pH (Figure 2b). For WB, α-diversity indices

256 were negatively correlated with pH, and Shannon diversity was negatively correlated with

257 DOC, TN, and C:P (Figure 2b).

258   PB and SB had a significantly greater phylogenetic diversity than WB (Figure 2a and

259   Figure 3). The OTUs in PB had significantly higher frequency than that of SB and WB

260   (Figure 3). The top 1000 abundant OTUs in PB were highly correlated with environmental

261   variables, particularly with latitude, MAP, SOC, TN, TP, and C:N:P ratios (Figure 3a). The

262   top 1000 abundant OTUs in SB were more commonly positively correlated with MAP,

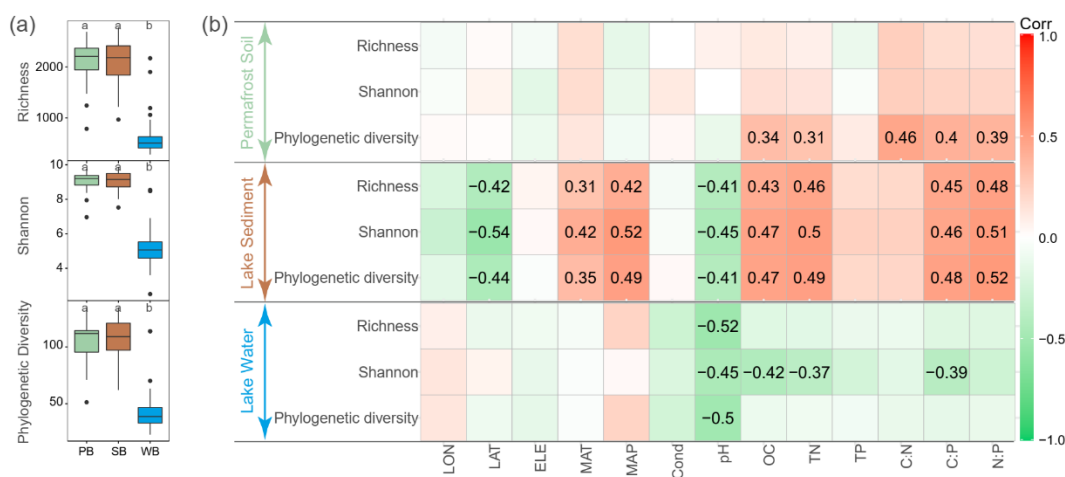
263   SOC, TN, and C:N:P ratios, but more commonly negatively correlated with latitude and

264   pH (Figure 3b). The top 1000 abundant OTUs in WB had relatively fewer significant

265   relationships with environmental variables in general, but were negatively correlated with

266   latitude, conductivity, pH, DOC, TN, and C:N:P ratios, while more positively correlated

267   with MAP (Figure 3c). In addition, WB had significantly higher SES.MNTD than PB and

268   SB (Figure 3d), suggesting higher phylogenetic clustering of bacterial taxa in WB.

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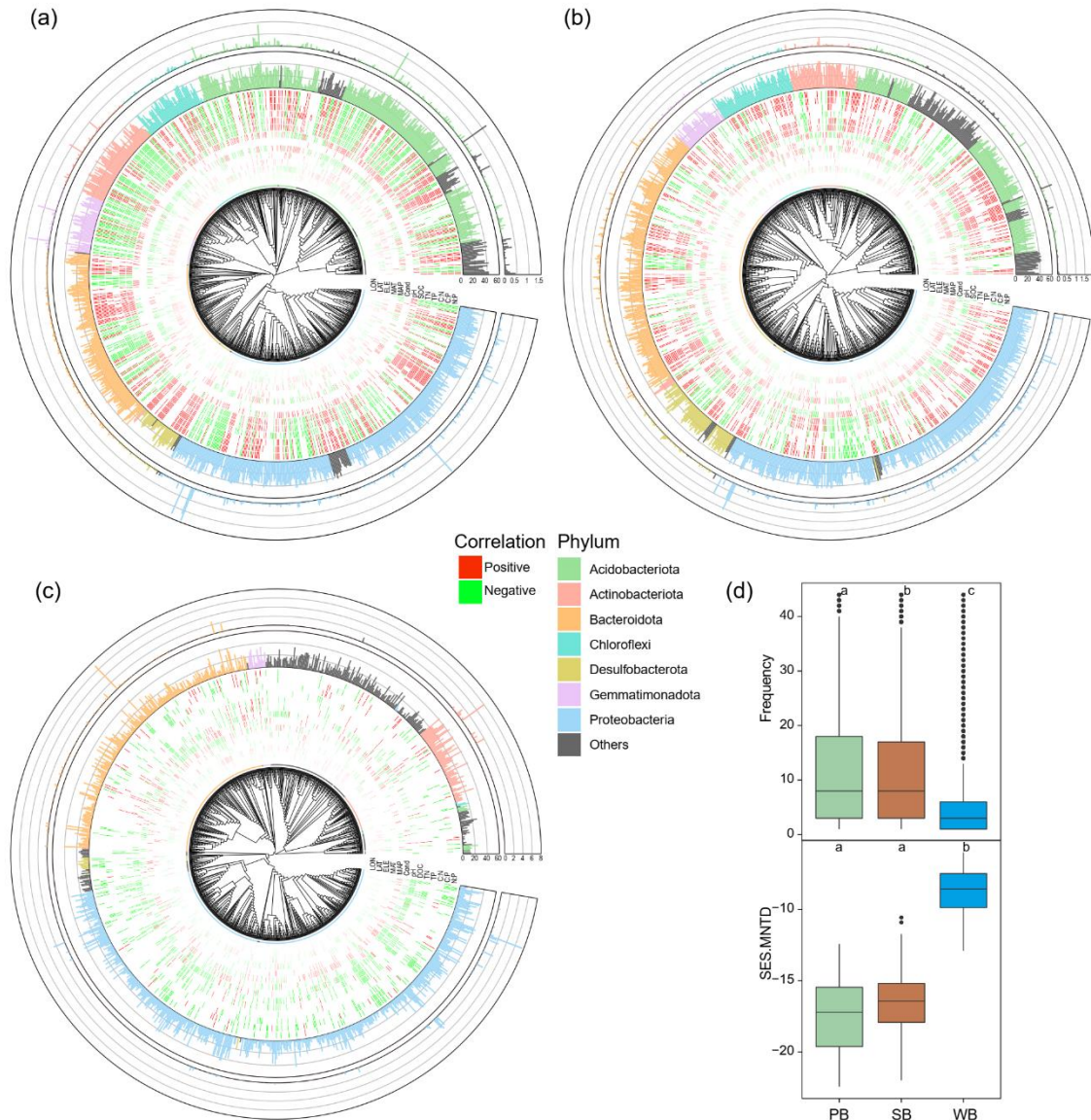
270   Figure 2 (a) Alpha diversity of bacterial communities in permafrost soil (PB), lake

271   sediment (SB), and lake water (WB). The different low-case letters represent significant

272   differences assessed using Wilcoxon rank-sum test. (b) Spearman correlations show the

273   relationships between alpha diversity and environmental factors. The color represents the

274 correlation coefficient, which is shown in number when the result is statistically significant

275 (p<0.05).

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277 Figure 3 Phylogenetic tree of the top 1000 OTUs in bacterial communities in (a) permafrost

278 soil, (b) lake sediment, and (c) lake water. Tree tips are colored by major phylum. The

279 inner ring of the heatmap represents spearman's correlation between OTUs and

280 environmental variables. Only significant (p<0.05) results are shown. The middle ring

281 represents the frequency of the OTUs in our studied sites. The outer ring represents the

282 relative abundance of the OTUs. (d) Boxplots showing differences of OTU's frequency and

283 SES.MNTD values among bacterial communities in permafrost soil (PB), lake sediment

284 (SB), and lake water (WB). The different lower-case letters represent significant

285 differences assessed using Wilcoxon rank-sum test.

286 *3.2 Community composition and β-diversity patterns*

287 PB were dominated by Proteobacteria (30.4%), Acidobacteriota (25.3%), Bacteroidota

288 (11.4%), Actinobacteriota (6.8%), Chloroflexi (5.2%), and Gemmatimonadota (5.2%)

289 (Figure 4a). SB were dominated by Proteobacteria (35.2%), Bacteroidota (20.0%),

290 Acidobacteriota (11.3%), Desulfobacterota (6.4%), Chloroflexi (6.3%), and

291 Actinobacteriota (5.8%) (Figure 4a). WB were dominated by Proteobacteria (46.9%),

292 Bacteroidota (29.2%), and Actinobacteriota (17.4%) (Figure 4a). While Proteobacteria

293 were predominant in all three habitat types, these dominant phyla had significantly

294 different relative abundances among these habitats. Proteobacteria and Bacteroidota had a

295 significantly higher relative abundance in WB than in SB and PB (Figure 4a). The relative

296 abundance of Actinobacteriota was the highest in WB but was not significantly different

297 between PB and SB (Figure 4a). Gemmatimonadota and Acidobacteriota were

298 significantly enriched in PB than in SB and WB. Desulfobacterota and Chloroflexi were

299 significantly enriched in SB than in PB and WB (Figure 4a).

300 These phyla responded differently to environmental variables (Figure 4b). For example,

301 Actinobacteriota and Gemmatimonadota in PB and Actinobacteriota and Desulfobacterota

302 in SB were negatively correlated with nutrient concentrations and ratios, while

303 Desulfobacterota in PB and Acidobacteriota in SB were positively correlated with nutrient

304   concentrations and ratios (Figure 4b). pH frequently correlated with taxa in various

305   taxonomic groups across all three habitats (Figure 4b)

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

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

308   (Figure 5a). The extent of difference was larger for WB vs PB (β=0.98; R_{ANOSIM} = 0.989,

309   P<0.001) than the differences for WB vs SB (β=0.96; R_{ANOSIM} = 0.967, P<0.001). There

310   was the least dissimilarity between PB and SB (β=0.81; R_{ANOSIM} = 0.384, P<0.001). The

311   fitted SEM model showed that PB had direct effects on SB and WB, and the latter two had

312   reciprocal effects on each other (Figure 5b). In addition, location, climate, and permafrost

313   soil physicochemical environments had direct effects on PB. Climate had direct effects on

314   SB while lake water physicochemical environments had direct effects on WB (Figure 5b).

315   WB had a higher β-diversity than SB and PB, suggesting that bacterial communities were

316   more spatially heterogeneous in lake water than in lake sediment and permafrost soil

317   (Figure 5c). Taxa in PB had higher habitat niche breadths than taxa in SB and WB (Figure

318   5d). We estimated the distance decay relationship of bacterial community similarity.

319   Significant distance-decay relationships were observed for all communities but the fitness

320   values were relatively low (Figure S3), indicating weak decay of community similarity

321   with geographic distance in thermokarst landscape. We also explored the main

322   environmental variables that influence the variations of the bacterial communities (Figure

323   6). β-diversities of PB, SB, and WB were all significantly correlated with latitude, MAP,

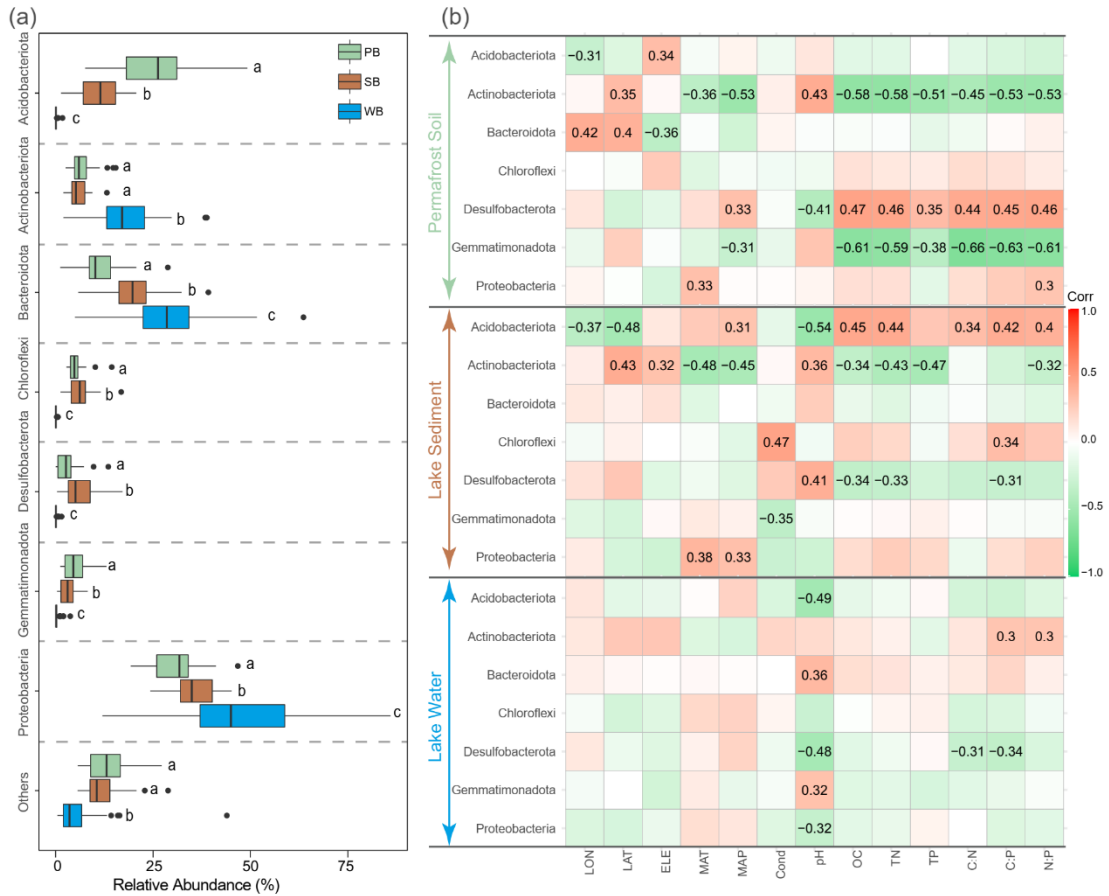
324   and pH (Figure 6). In addition, β-diversity of PB was also significantly correlated with all

325   the other environmental variables except MAT and conductivity. β-diversity of SB was

326   also significantly correlated with conductivity and C:N (Figure 6). β-diversity of WB was

327   also significantly correlated with elevation, MAT, conductivity, DOC, TN, and TP (Figure

328   6). The results suggested that the compositional variation among PB, SB, and WB was

329   differentially structured by spatial, climatic, and physicochemical variables.

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331   Figure 4 (a) Relative abundances of major phyla in bacterial communities in permafrost

332   soil (PB), lake sediment (SB), and lake water (WB). The different low-case letters represent

333   significant differences assessed using Wilcoxon rank-sum test. (b) Spearman correlations

334   show the relationships between the relative abundance of major phyla and environmental

335   factors. The color represents the correlation coefficient, which shown in number when the

336   result is significant (p<0.05).
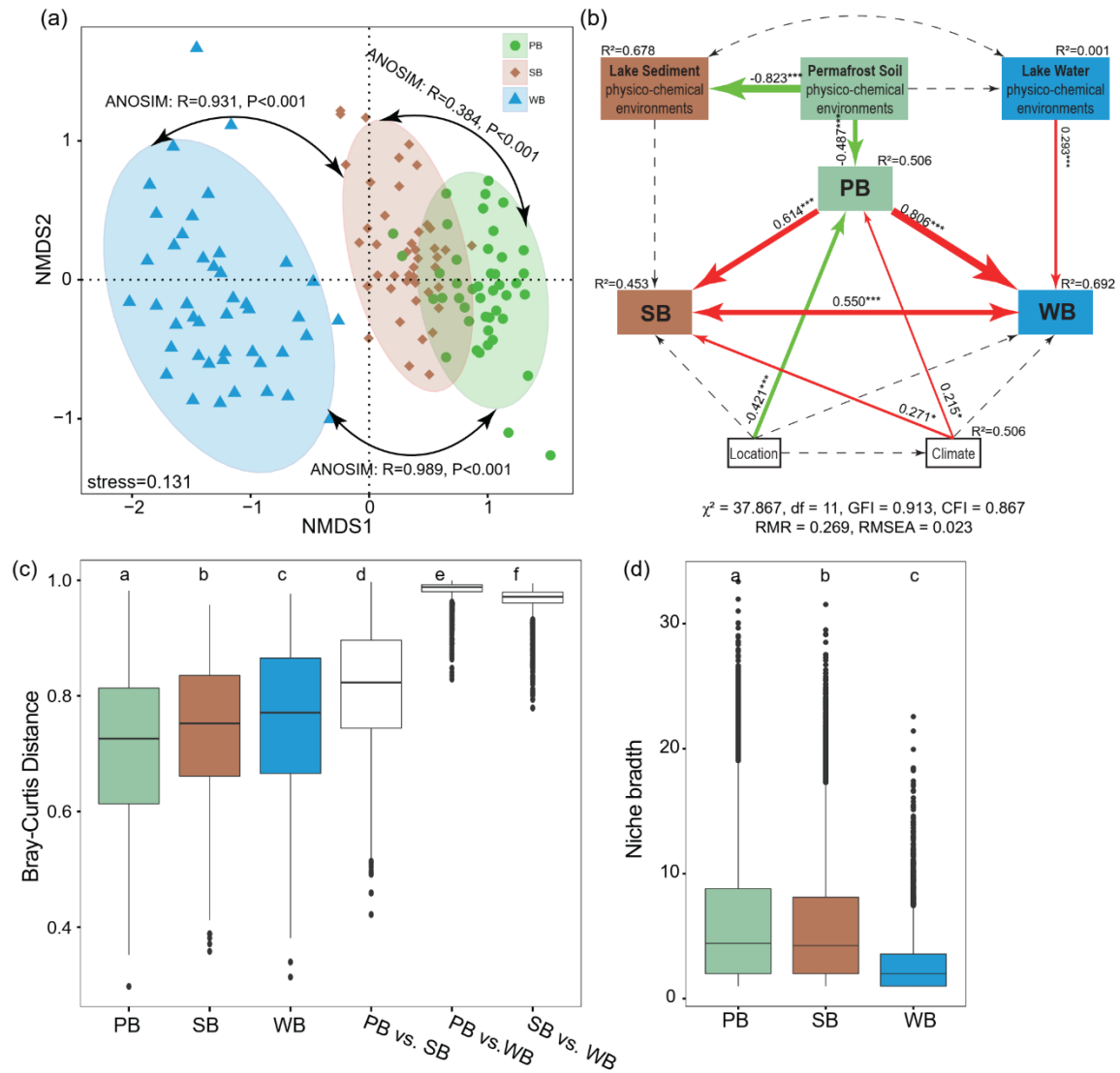
Figure 5 (a) Non-metric multidimensional scaling (NMDS) ordination showing the distribution of bacterial communities in permafrost soil (PB), lake sediment (SB), and lake water (WB). The differences between these communities are confirmed by the non-parametric statistical test (ANOSIM). (b) Structural equation modeling analysis depicting the relationships between location (including latitude, longitude, and elevation), climate (including mean annual temperature and mean annual precipitation), physicochemical

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

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

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

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

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

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

351 communities.

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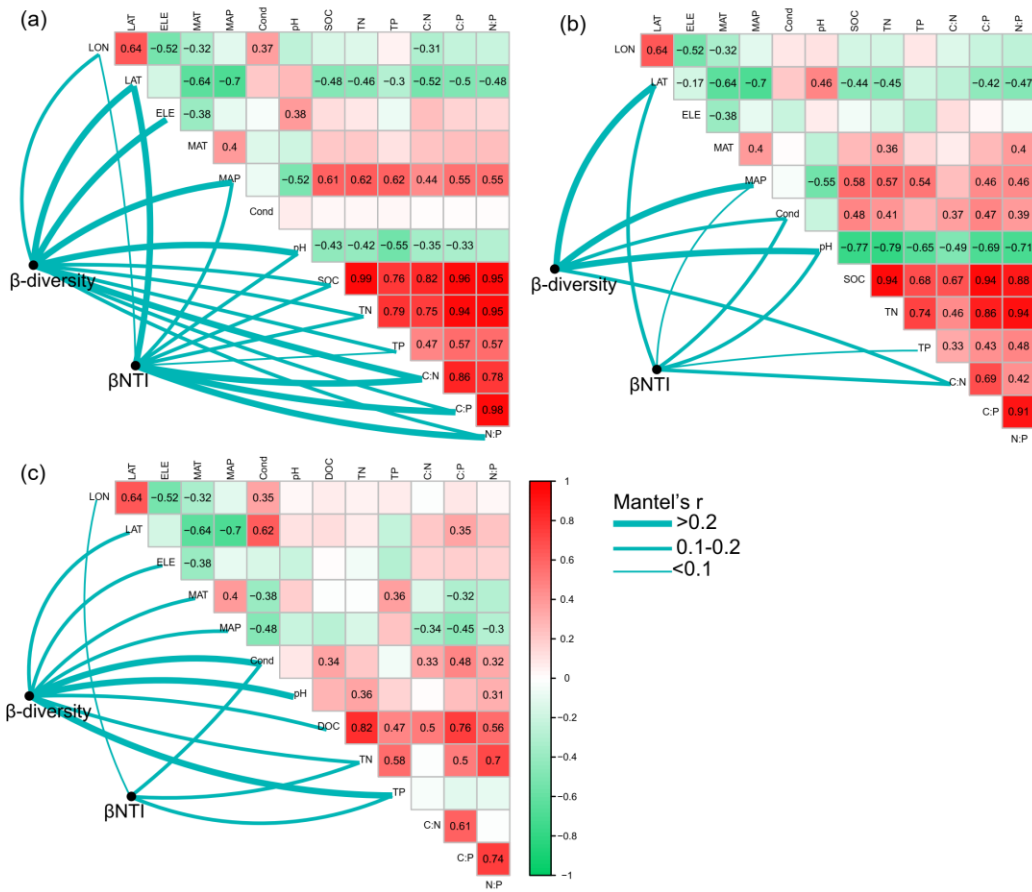
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355   Figure 6 Pairwise correlations between environmental variables as well as the Mantel tests

356   between environmental variables and β-diversity and beta nearest taxon index (βNTI) for

357   (a) bacterial communities in permafrost soil, (b) bacterial communities in lake sediment,

358   and (c) bacterial communities in lake water. β-diversity was calculated as Bray-Curtis

359   distance. The lines denote significant relationships while the line width represents the

360   Mantel's r statistic. Pairwise correlations between environmental variables are shown in

361   color gradient matrix. The color represents Pearson's correlation coefficient, which shown

362   in number when the result is significant (p<0.05). The abbreviations of the environmental

363   variables are explained in the Methods section.

364   *3.3 Assembly processes*

365   To explore the mechanisms underlining the observed distribution patterns, a null-model-

366   based framework was employed to quantify the deviation of phylogenetic turnover. PB had

367   significantly higher βNTI than SB and WB (Figure 7a). Deterministic processes

368   contributed 51.3%, 41.2%, and 44.9% to community variations for the bacterial

369   communities in permafrost soil, lake sediment, and lake water, respectively (Figure 7b). In

370   particular, the results showed that homogeneous selection contributed a larger fraction to

371   the assembly of the WB (44.8%), followed by SB (35.2%) and PB (29.7%) (Figure 7b).

372   Heterogeneous selection influenced PB (21.6%) more than SB (6.0%) and WB (0.1%).

373   Dispersal limitation contributed a larger fraction to SB (57.2%) than to PB (45%) and WB

374   (37.5%).

375   The relationships between βNTI and major environmental variables were used to estimate

376   changes in the relative influences of deterministic and stochastic assembly processes.

377   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). Particularly, differences of TP were significantly associated with βNTI of PB, SB, and WB, 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 was also significantly associated with other environmental variables, except elevation, MAT, and conductivity. βNTI of SB was also significantly associated with latitude, MAP, conductivity, pH, and C:N, while βNTI of WB was significantly associated with longitude, conductivity, and TN.
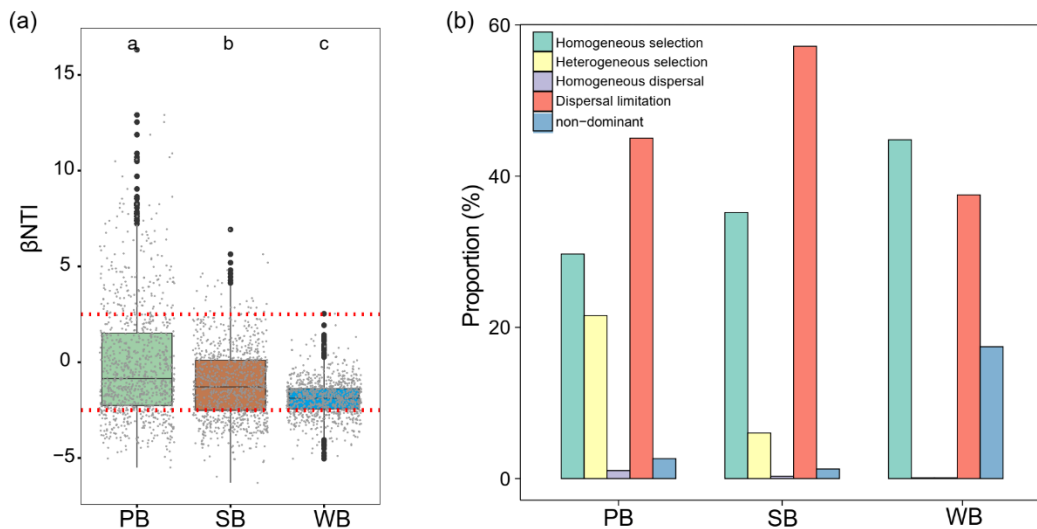
Figure 7 (a) The values of βNTI with horizontal dashed red lines indicate upper and lower significance thresholds at βNTI = +2 and −2, respectively in the three habitat types. (b) The contribution of deterministic (homogeneous and heterogeneous selection) and stochastic (dispersal limitations and homogenizing dispersal) processes to turnover in the assembly of bacterial communities in permafrost soil (PB), lake sediment (SB), and lake water (WB). "Non-dominant" indicates that the fraction was not dominated by any single process.

4 Discussion

4.1 Alpha diversity and community composition

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 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. In our study, alpha diversity and the dominant phyla found in PB and SB 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.

415   Bacterial communities in lake water had significantly lower alpha diversity as well as

416   distinct community composition and structure in comparison to bacterial communities in

417   permafrost soil and lake sediment. However, PB and SB had direct influence on WB. For

418   thermokarst lakes, the water first originates from the thawing of the ice-rich permafrost

419   and the lake is then fed by precipitation-derived and permafrost-derived water (Yang et al.,

420   2016a; Narancic et al., 2017; Wan et al., 2019). Microorganisms present in lake water have

421   a diverse range of sources, including terrestrial inputs and other sources such as bacteria

422   distributed with the atmosphere, associated with plants and animals, and carried by

423   migratory birds and animals (Ruiz-Gonzalez et al., 2015). Thus, there was a relatively small

424   proportion of OTUs shared between permafrost soil and lake water, as well as between

425   lake sediment and water, and only a few shared OTUs were enriched in lake water. It is a

426   well-established fact that different habitats often support distinct microbial communities

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

428   community composition between lake sediments and water has been extensively

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

430   2017). In addition, sediment generally harbor a higher species-level diversity of bacteria

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

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

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

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

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

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

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

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

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

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

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

442   and distinct assembly mechanisms.

443   Despite connections driven by dispersal, distinct thermokarst habitats had different

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

445   dominant phyla were significantly different in relative abundance among PB, SB, and WB.

446   In this study, Proteobacteria, Bacteroidota, Actinobacteriota, Gemmatimonadota,

447   Acidobacteriota, Desulfobacterota, and Chloroflexi dominated bacterial communities in

448   permafrost soil and/or thermokarst lakes despite high variabilities. Similar dominance of

449   these taxa has also been found in permafrost and thermokarst landscapes in other areas

450   (Aszalós et al., 2020; Belov et al., 2020; Wu et al., 2022). The most commonly reported

451   bacterial groups in permafrost environments include members of Proteobacteria,

452   Acidobacteria, Actinobacteria, Bacteroidetes, Firmicutes, and Chloroflexi (Steven et al.,

453   2009; Altshuler et al., 2017; Ottoni et al., 2022), as observed in our samples.

454   *4.2 Beta diversity and assembly processes*

455   In our studied regions across the QTP, PB, SB, and WB all had a high beta diversity

456   (average values > 0.7), with WB showing the highest, suggesting that bacterial

457   communities shifted substantially across the large spatial scale of our sampling. Moreover,

458   beta diversities of PB, SB, and WB were significantly correlated with each other, further

459   suggesting that the bacteria in different habitats had a considerable proportion of members

460   from the same source, the original pristine permafrost soil. The significantly lower mean

461   SES.MNTD for PB indicate that bacterial communities in permafrost soil were more

462   closely phylogenetically clustered and suffered stronger environmental filtering than those

463   in lake sediment and water (Langenheder et al., 2017), consistent with the observation that

464   PB had lower beta diversity than SB and WB. SES.MNTD is sensitive to changes in lineage

465   close to the phylogenetic tips (Kembel et al., 2010). The higher SES.MNTD observed for

466   SB and WB suggest the possibility that the bacteria in lake sediment and water exhibit a

467   substantial divergence in the co-occurring species, and thermokarst lakes have experienced

468   colonization by bacterial species originating from distinct clades or lineages from external

469   sources following permafrost thaw (Webb et al., 2002; Stegen et al., 2013).

470   The structure of bacterial communities can vary across spatiotemporal scales and different

471   habitats (Ren et al., 2017; Aguilar and Sommaruga, 2020; Pearman et al., 2020). A key

472   objective in the field of microbial ecology is to determine the relative influence of

473   stochastic and deterministic processes in shaping the assembly of communities (Stegen et

474   al., 2013; Zhou and Ning, 2017). In this study, bacterial communities in lake water

475   displayed a higher influence of homogeneous selection but lower influence of

476   heterogeneous selection compared to those in lake sediments and permafrost soil. Long-

477   term changes in thermokarst lakes result in homogenized habitats and consequently strong

478   homogenous selection on bacterial communities (Ning et al., 2019). In contrast, permafrost

479   soil is a highly heterogeneous environment across spatial scales (Etzelmüller, 2013;

480   Nitzbon et al., 2021), creating a wide range of habitats which can impose strong

481   heterogeneous selection pressures on bacterial communities. Furthermore, permafrost soil

482   is characterized by limited nutrient availability due to the frozen state of organic matters

483   (Beermann et al., 2017; Zhang et al., 2023), while lake water offers a more diverse and

484 abundant array of dissolved organic compounds and nutrients. As a result, bacterial

485 communities in permafrost soil might be more sensitive to variations in resource

486 availability, rendering them more strongly influenced by heterogeneous selection. In

487 addition, dispersal limitation contributed a larger fraction to SB (57.2%) than to PB (45%)

488 and WB (37.5%). The dispersal of microorganisms is often considered as a passive process

489 that results in community variation and turnover coupled with the function of

490 environmental filtering (Cline and Zak, 2014; Stegen et al., 2015; Custer et al., 2022). The

491 high dispersal limitation of microbial communities in thermokarst lakes may be attributed

492 to several factors. These include the isolated nature of thermokarst lakes, which are

493 endorheic and therefore have limited connectivity, as well as the strong restriction on

494 microbial dispersal and the presence of strong environmental filtering processes.

495 Additionally, the prolonged frozen phase of thermokarst lakes and permafrost soil restrict

496 the movement of microorganisms (Vargas Medrano, 2019; Vigneron et al., 2019).

497 Furthermore, geographical barriers, exemplified by prominent mountain ranges like the

498 Tanggula Mountains, Kunlun Mountains, Nyenchen Tanglha Mountains, and Bayan Har

499 Mountains, serve as impediments to the dispersal of both macro- and microorganisms (Wan

500 et al., 2016; Yu et al., 2019; Ren et al., 2022c). Particularly in lake sediment, where

501 bacterial communities are more isolated over distances and will not disperse as far as those

502 in lake water and permafrost soil, resulting in stronger influence of dispersal limitation

503 (Martiny et al., 2006; Xiong et al., 2012). Although the "everything is everywhere"

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

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

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

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

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

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

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

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

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

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

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

515   forest landscape (Bottos et al., 2018).

516   *4.3 Environmental influences*

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

518   in the field of microbial ecology (Fierer and Jackson, 2006; Pla-Rabes et al., 2011). In our

519   study, bacterial communities were differentially correlated with various measured

520   environmental variables. In our data, pH was consistently identified as a strong correlate

521   of microbial community structure and diversity, as is often observed in terrestrial and

522   aquatic ecosystems worldwide (Fierer and Jackson, 2006; Xiong et al., 2012). While such

523   correlations between pH and bacterial communities have been widely found, the regulation

524   mechanisms still remain unknown (Malard and Pearce, 2018). Moreover, the influences of

525   pH are often species- and location-specific (Malard and Pearce, 2018; Egelberg et al.,

526   2021). In this study, pH had significantly negative relationships with alpha diversity of

527   bacterial communities in lake sediment and water, and had negative or positive correlations

528   with some phyla. Moreover, differences in pH might drive community variation observed

529   between PB, SB, and WB, and shift community assembly processes for PB and SB.

Moreover, with permafrost degrading and thermokarst developing, nutrient status will be strongly altered in permafrost areas. Organic carbon and nutrient stocks in permafrost are decreasing (Turetsky et al., 2020; Wu et al., 2021) and thermokarst lakes are developing, leading to dynamic environmental change (Luo et al., 2015; Vucic et al., 2020). These environmental disruptions likely impose strong influences on bacterial communities in thermokarst landscapes. Our study showed that nutrient (C, N, and P) concentrations and stoichiometric ratios were strongly related to alpha diversity (particularly for SB) and community variation and assembly (particularly for PB). 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). 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, 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

553 deterministic processes had stronger influences on PB and WB than on SB. In addition,

554 thermokarst lakes have sediment directly formed from permafrost soil, and thus, permafrost

555 soil environments and bacterial communities had strong associations with that of lake

556 sediment.

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

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

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

560 2018). Warming and altered precipitation regimes under climate change have been

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

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

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

564 communities in thermokarst landscapes. Particularly for bacterial communities in

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

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

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

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

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

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

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

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

573 bacterial communities in thermokarst landscapes.

5 Conclusion

In this study, we investigated bacterial communities in paired water and sediment samples in thermokarst lakes as well as permafrost soil around the lakes across the QTP. esults showed that each habitat had distinct bacterial assemblages, with lower alpha diversity in lake water and higher beta diversity in lake sediment and permafrost soil. There was considerable overlap in OTUs across habitats. Bacterial communities in permafrost soil and lake sediment were influenced by dispersal limitation, while those in lake water were driven by homogeneous selection. Environmental variables, including latitude, mean annual precipitation, and pH, affected bacterial community variations in all habitats. The study highlights the unique bacterial communities and ecological impacts of permafrost degradation in diverse habitats created by thermokarst processes.

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Data availability statement:

Raw sequences were uploaded to the China National Center for Bioinformation (PRJCA009850, CRA007082).

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Conflict of interest disclosure:

596   The authors declare no competing interests.

597   **Author's contributions:**

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

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

600   **Ethics approval statement:**

601   Not applicable

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

603   Not applicable

604   **Originality-Significance Statement:**

605   This is our original study and not submitted to elsewhere

606   **Supplementary Information**

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

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

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

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

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

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

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

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

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

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

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

618   regressions.

619

620   **References**

621   Aguilar, P. and Sommaruga, R.: The balance between deterministic and stochastic
622   processes in structuring lake bacterioplankton community over time, Mol. Ecol., 29,
623   3117-3130, doi:10.1111/mec.15538, 2020.
624   Altshuler, I., Goordial, J. and Whyte, L. G.: Microbial life in permafrost. In Psychrophiles:
625   from biodiversity to biotechnology (pp. 153-179). Springer, Cham,2017.
626   Anthony, K. M., von Deimling, T. S., Nitze, I., Frolking, S., Emond, A., Daanen, R.,
627   Anthony, P., Lindgren, P., Jones, B. and Grosse, G.: 21st-century modeled permafrost
628   carbon emissions accelerated by abrupt thaw beneath lakes, Nat. Commun., 9, 3262,
629   doi:10.1038/s41467-018-05738-9, 2018.
630   Aszalós, J. M., Szabó, A., Megyes, M., Anda, D., Nagy, B. and Borsodi, A. K.: Bacterial
631   Diversity of a High-Altitude Permafrost Thaw Pond Located on Ojos del Salado (Dry
632   Andes,   Altiplano-Atacama   Region),   Astrobiology,   20,   754-765,
633   doi:10.1089/ast.2018.2012, 2020.
634   Baas-Becking, L. G. M.: Geobiologie of Inleiding Tot de Milieukunde, Van Stockkum &
635   Zoon, The Hague,1934.
636   Barbato, R. A., Jones, R. M., Douglas, T. A., Doherty, S. J., Messan, K., Foley, K. L.,
637   Perkins, E. J., Thurston, A. K. and Garcia-Reyero, N.: Not all permafrost microbiomes
638   are created equal: Influence of permafrost thaw on the soil microbiome in a laboratory
639   incubation   study,   Soil   Biology   and   Biochemistry,   167,   108605,
640   doi:https://doi.org/10.1016/j.soilbio.2022.108605, 2022.
641   Barrett, P.: Structural equation modelling: Adjudging model fit, Pers. Individ. Differ., 42,
642   815-824, doi:10.1016/j.paid.2006.09.018, 2007.
643   Beermann, F., Langer, M., Wetterich, S., Strauss, J., Boike, J., Fiencke, C., Schirrmeister,
644   L., Pfeiffer, E. M. and Kutzbach, L.: Permafrost thaw and liberation of inorganic
645   nitrogen in Eastern Siberia, Permafrost and Periglacial Processes, 28, 605-618,2017.
646   Belov, A. A., Cheptsov, V. S., Manucharova, N. A. and Ezhelev, Z. S.: Bacterial
647   Communities of Novaya Zemlya Archipelago Ice and Permafrost, Geosciences, 10, 67,
648   doi:10.3390/geosciences10020067, 2020.
649   Benjamini, Y. and Hochberg, Y.: Controlling the false discovery rate: a practical and
650   powerful approach to multiple testing, Journal of the Royal Statistical Society: Series
651   B (Methodological), 57, 289-300, doi:10.2307/2346101, 1995.
652   Bier, R. L., Bernhardt, E. S., Boot, C. M., Graham, E. B., Hall, E. K., Lennon, J. T.,
653   Nemergut, D. R., Osborne, B. B., Ruiz-Gonzalez, C., Schimel, J. P., Waldrop, M. P.
654   and Wallenstein, M. D.: Linking microbial community structure and microbial
655   processes: an empirical and conceptual overview, FEMS Microbiol. Ecol., 91,
656   doi:10.1093/femsec/fiv113, 2015.
657   Biskaborn, B. K., Smith, S. L., Noetzli, J., Matthes, H., Vieira, G., Streletskiy, D. A.,
658   Schoeneich, P., Romanovsky, V. E., Lewkowicz, A. G., Abramov, A., Allard, M.,
659   Boike, J., Cable, W. L., Christiansen, H. H., Delaloye, R., Diekmann, B., Drozdov, D.,

Etzelmuller, B., Grosse, G., Guglielmin, M., Ingeman-Nielsen, T., Isaksen, K., Ishikawa, M., Johansson, M., Johannsson, H., Joo, A., Kaverin, D., Kholodov, A., Konstantinov, P., Kroger, T., Lambiel, C., Lanckman, J. P., Luo, D., Malkova, G., Meiklejohn, I., Moskalenko, N., Oliva, M., Phillips, M., Ramos, M., Sannel, A., Sergeev, D., Seybold, C., Skryabin, P., Vasiliev, A., Wu, Q., Yoshikawa, K., Zheleznyak, M. and Lantuit, H.: Permafrost is warming at a global scale, Nat. Commun., 10, 264, doi:10.1038/s41467-018-08240-4, 2019.

Bottos, E. M., Kennedy, D. W., Romero, E. B., Fansler, S. J., Brown, J. M., Bramer, L. M., Chu, R. K., Tfaily, M. M., Jansson, J. K. and Stegen, J. C.: Dispersal limitation and thermodynamic constraints govern spatial structure of permafrost microbial communities, FEMS Microbiol. Ecol., 94, doi:10.1093/femsec/fiy110, 2018.

Briee, C., Moreira, D. and Lopez-Garcia, P.: Archaeal and bacterial community composition of sediment and plankton from a suboxic freshwater pond, Res. Microbiol., 158, 213-227, doi:10.1016/j.resmic.2006.12.012, 2007.

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., Fierer, N., Pena, A. G., Goodrich, J. K., Gordon, J. I., Huttley, G. A., Kelley, S. T., Knights, D., Koenig, J. E., Ley, R. E., Lozupone, C. A., Mcdonald, D., Muegge, B. D., Pirrung, M., Reeder, J., Sevinsky, J. R., Tumbaugh, P. J., Walters, W. A., Widmann, J., Yatsunenko, T., Zaneveld, J. and Knight, R.: QIIME allows analysis of high-throughput community sequencing data, Nat. Methods, 7, 335-336, doi:10.1038/nmeth.f.303, 2010.

Chadburn, S. E., Burke, E. J., Cox, P. M., Friedlingstein, P. and Hugelius, G.: An observation-based constraint on permafrost loss as a function of global warming, Nat. Clim. Chang., 7, 340-344, doi:10.1038/NCLIMATE3262, 2017.

Chase, J. M.: Stochastic community assembly causes higher biodiversity in more productive environments, Science, 328, 1388-1391, doi:10.1126/science.1187820, 2010.

Chave, J.: Neutral theory and community ecology, Ecol. Lett., 7, 241-253,2004.

Chin, K. S., Lento, J., Culp, J. M., Lacelle, D. and Kokelj, S. V.: Permafrost thaw and intense thermokarst activity decreases abundance of stream benthic macroinvertebrates, Glob. Change Biol., 22, 2715-2728, doi:10.1111/gcb.13225, 2016.

Cline, L. C. and Zak, D. R.: Dispersal limitation structures fungal community assembly in a long-term glacial chronosequence, Environ. Microbiol., 16, 1538-1548, doi:10.1111/1462-2920.12281, 2014.

Custer, G. F., Bresciani, L. and Dini-Andreote, F.: Ecological and Evolutionary Implications of Microbial Dispersal, Front. Microbiol., 13, 855859, doi:10.3389/fmicb.2022.855859, 2022.

de Jong, A., In, T. Z. M., Meisel, O. H., Jetten, M., Dean, J. F., Rasigraf, O. and Welte, C. U.: Increases in temperature and nutrient availability positively affect methane-cycling microorganisms in Arctic thermokarst lake sediments, Environ. Microbiol., 20, 4314-4327, doi:10.1111/1462-2920.14345, 2018.

Egelberg, J., Pena, N., Rivera, R. and Andruk, C.: Assessing the geographic specificity of pH prediction by classification and regression trees, PLoS One, 16, e0255119, doi:10.1371/journal.pone.0255119, 2021.

Elder, C. D., Thompson, D. R., Thorpe, A. K., Chandanpurkar, H. A., Hanke, P. J., Hasson, N., James, S. R., Minsley, B. J., Pastick, N. J., Olefeldt, D., Walter Anthony, K. M. and Miller, C. E.: Characterizing Methane Emission Hotspots From Thawing Permafrost,

706   Glob. Biogeochem. Cycle, 35, e2020GB006922, doi:10.1029/2020GB006922, 2021.

707   Etzelmüller, B.: Recent advances in mountain permafrost research, Permafrost and
708   Periglacial Processes, 24, 99-107,2013.

709   Farquharson, L. M., Mann, D. H., Grosse, G., Jones, B. M. and Romanovsky, V. E.: Spatial
710   distribution of thermokarst terrain in Arctic Alaska, Geomorphology, 273, 116-133,
711   doi:10.1016/j.geomorph.2016.08.007, 2016.

712   Fierer, N. and Jackson, R. B.: The diversity and biogeography of soil bacterial communities,
713   Proceedings of the National Academy of Sciences, 103, 626-631,
714   doi:10.1073/pnas.0507535103, 2006.

715   Fierer, N., Leff, J. W., Adams, B. J., Nielsen, U. N., Bates, S. T., Lauber, C. L., Owens, S.,
716   Gilbert, J. A., Wall, D. H. and Caporaso, J. G.: Cross-biome metagenomic analyses of
717   soil microbial communities and their functional attributes, Proceedings of the National
718   Academy of Sciences, 109, 21390-21395, doi:10.1073/pnas.1215210110, 2012.

719   Fu, L., Xie, R., Ma, D., Zhang, M. and Liu, L.: Variations in soil microbial community
720   structure and extracellular enzymatic activities along a forest‐wetland ecotone in
721   high‐latitude permafrost regions, Ecol. Evol., 13, e10205-n/a,
722   doi:10.1002/ece3.10205, 2023.

723   Gao, H., Wang, J., Yang, Y., Pan, X., Ding, Y. and Duan, Z.: Permafrost Hydrology of the
724   Qinghai-Tibet Plateau: A Review of Processes and Modeling, Front. Earth Sci., 8,
725   e576838, doi:10.3389/feart.2020.576838, 2021.

726   Garrido-Benavent, I., Pérez-Ortega, S., Durán, J., Ascaso, C., Pointing, S. B., Rodríguez-
727   Cielos, R., Navarro, F. and de Los Ríos, A.: Differential colonization and succession of
728   microbial communities in rock and soil substrates on a maritime antarctic glacier
729   forefield, Front. Microbiol., 11, e126, doi:10.3389/fmicb.2020.00126, 2020.

730   Gough, H. L. and Stahl, D. A.: Microbial community structures in anoxic freshwater lake
731   sediment along a metal contamination gradient, The ISME Journal, 5, 543-558,
732   doi:10.1038/ismej.2010.132, 2011.

733   Graham, E. B., Crump, A. R., Resch, C. T., Fansler, S., Arntzen, E., Kennedy, D. W.,
734   Fredrickson, J. K. and Stegen, J. C.: Coupling spatiotemporal community assembly
735   processes to changes in microbial metabolism, Front. Microbiol., 7, 1949,
736   doi:10.3389/fmicb.2016.01949, 2016.

737   Graham, E. and Stegen, J.: Dispersal-based microbial community assembly decreases
738   biogeochemical function, Processes, 5, 65, doi:10.3390/pr5040065, 2017.

739   Hu, L. and Bentler, P. M.: Cutoff criteria for fit indexes in covariance structure analysis:
740   Conventional criteria versus new alternatives, Structural equation modeling, 6, 1-55,
741   doi:10.1080/10705519909540118, 1999.

742   Hugelius, G., Strauss, J., Zubrzycki, S., Harden, J. W., Schuur, E. A. G., Ping, C. L.,
743   Schirrmeister, L., Grosse, G., Michaelson, G. J., Koven, C. D., O'Donnell, J. A.,
744   Elberling, B., Mishra, U., Camill, P., Yu, Z., Palmtag, J., Kuhry, P. and Argonne
745   National Lab. Anl, A. I. U. S.: Estimated stocks of circumpolar permafrost carbon with
746   quantified uncertainty ranges and identified data gaps, Biogeosciences, 11, 6573-6593,
747   doi:10.5194/bg-11-6573-2014, 2014.

748   Hugerth, L. W., Larsson, J., Alneberg, J., Lindh, M. V., Legrand, C., Pinhassi, J. and
749   Andersson, A. F.: Metagenome-assembled genomes uncover a global brackish
750   microbiome, Genome Biol., 16, 279, doi:10.1186/s13059-015-0834-7, 2015.

751   In'T Zandt, M. H., Liebner, S. and Welte, C. U.: Roles of thermokarst lakes in a warming

752   world, Trends Microbiol., 28, 769-779, doi:10.1016/j.tim.2020.04.002, 2020.
753 Jiao, S. and Lu, Y.: Abundant fungi adapt to broader environmental gradients than rare
754   fungi in agricultural fields, Glob. Change Biol., 26, 4506-4520, doi:10.1111/gcb.15130,
755   2020.
756 Jorgenson, M. T., Romanovsky, V., Harden, J., Shur, Y., O'Donnell, J., Schuur, E. A. G.,
757   Kanevskiy, M. and Marchenko, S.: Resilience and vulnerability of permafrost to climate
758   change, Can. J. For. Res., 40, 1219,2010.
759 Kembel, S. W., Cowan, P. D., Helmus, M. R., Cornwell, W. K., Morlon, H., Ackerly, D.
760   D., Blomberg, S. P. and Webb, C. O.: Picante: R tools for integrating phylogenies and
761   ecology, Bioinformatics, 26, 1463-1464, doi:10.1093/bioinformatics/btq166, 2010.
762 Kokelj, S. V. and Jorgenson, M. T.: Advances in thermokarst research, Permafrost and
763   Periglacial Processes, 24, 108-119, doi:10.1002/ppp.1779, 2013.
764 Langenheder, S., Wang, J., Karjalainen, S. M., Laamanen, T. M., Tolonen, K. T., Vilmi,
765   A. and Heino, J.: Bacterial metacommunity organization in a highly connected aquatic
766   system, FEMS Microbiol. Ecol., 93, fiw225, doi:10.1093/femsec/fiw225, 2017.
767 Lawrence, D. M., Slater, A. G. and Swenson, S. C.: Simulation of Present-Day and Future
768   Permafrost and Seasonally Frozen Ground Conditions in CCSM4, J. Clim., 25, 2207-
769   2225, doi:10.1175/JCLI-D-11-00334.1, 2012.
770 Le Moigne, A., Bartosiewicz, M., Schaepman Strub, G., Abiven, S. and Pernthaler, J.: The
771   biogeochemical variability of Arctic thermokarst ponds is reflected by stochastic and
772   niche‐driven microbial community assembly processes, Environ. Microbiol., 22,
773   4847-4862, doi:10.1111/1462-2920.15260, 2020.
774 Lee, Z. M. P., Poret-Peterson, A. T., Siefert, J. L., Kaul, D., Moustafa, A., Allen, A. E.,
775   Dupont, C. L., Eguiarte, L. E., Souza, V. and Elser, J. J.: Nutrient stoichiometry shapes
776   microbial community structure in an evaporitic shallow pond, Front. Microbiol., 8, 949,
777   doi:10.3389/fmicb.2017.00949, 2017.
778 Leibold, M. A., Chase, J. M. and Ernest, S. K.: Community assembly and the functioning
779   of ecosystems: how metacommunity processes alter ecosystems attributes, Ecology, 98,
780   909-919, doi:10.1002/ecy.1697, 2017.
781 Levins, R.: Evolution in changing environments: some theoretical explorations (No. 2).
782   Princeton University Press, Princeton, USA,1968.
783 Lindstrom, E. S. and Langenheder, S.: Local and regional factors influencing bacterial
784   community assembly, Environ. Microbiol. Rep., 4, 1-9, doi:10.1111/j.1758-
785   2229.2011.00257.x, 2012.
786 Liu, K., Yao, T., Pearce, D. A., Jiao, N., Zeng, Y., Guo, B. and Liu, Y.: Bacteria in the
787   lakes of the Tibetan Plateau and polar regions, Sci. Total Environ., 754, 142248,
788   doi:10.1016/j.scitotenv.2020.142248, 2021.
789 Louca, S., Parfrey, L. W. and Doebeli, M.: Decoupling function and taxonomy in the global
790   ocean microbiome, Science, 353, 1272-1277, doi:10.1126/science.aaf4507, 2016.
791 Lozupone, C. A. and Knight, R.: Global patterns in bacterial diversity, Proceedings of the
792   National Academy of Sciences, 104, 11436-11440, doi:10.1073/pnas.0611525104,
793   2007.
794 Lu, W., Wang, W., Shao, Q., Yu, Z., Hao, Z., Xing, W., Yong, B. and Li, J.: Hydrological
795   projections of future climate change over the source region of Yellow River and
796   Yangtze River in the Tibetan Plateau; a comprehensive assessment by coupling
797   RegCM4 and VIC model, Hydrol. Process., 32, 2096-2117, doi:10.1002/hyp.13145,

798   2018.

799   Luan, L., Liang, C., Chen, L., Wang, H., Xu, Q., Jiang, Y. and Sun, B.: Coupling Bacterial
800   Community Assembly to Microbial Metabolism across Soil Profiles, mSystems, 5,
801   e00298-20, doi:10.1128/mSystems.00298-20, 2020.

802   Luo, D., Jin, H., Du, H., Li, C., Ma, Q., Duan, S. and Li, G.: Variation of alpine lakes from
803   1986 to 2019 in the Headwater Area of the Yellow River, Tibetan Plateau using Google
804   Earth Engine, Adv. Clim. Chang. Res., 11, 11-21, doi:10.1016/j.accre.2020.05.007,
805   2020.

806   Luo, J., Niu, F., Lin, Z., Liu, M. and Yin, G.: Thermokarst lake changes between 1969 and
807   2010 in the Beilu River Basin, Qinghai-Tibet Plateau, China, Sci. Bull., 60, 556-564,
808   doi:10.1007/s11434-015-0730-2, 2015.

809   Mackelprang, R., Burkert, A., Haw, M., Mahendrarajah, T., Conaway, C. H., Douglas, T.
810   A. and Waldrop, M. P.: Microbial survival strategies in ancient permafrost: insights
811   from metagenomics, The ISME Journal, 11, 2305-2318, doi:10.1038/ismej.2017.93,
812   2017.

813   Makoto, K., Wilson, S. D., Turner, B. and Turner, B.: When and where does dispersal
814   limitation matter in primary succession? J. Ecol., 107, 559-565, doi:10.1111/1365-
815   2745.12988, 2019.

816   Malard, L. A. and Pearce, D. A.: Microbial diversity and biogeography in Arctic soils,
817   Environ. Microbiol. Rep., 10, 611-625, doi:10.1111/1758-2229.12680, 2018.

818   Manasypov, R. M., Pokrovsky, O. S., Shirokova, L. S., Auda, Y., Zinner, N. S., Vorobyev,
819   S. N. and Kirpotin, S. N.: Biogeochemistry of macrophytes, sediments and porewaters
820   in thermokarst lakes of permafrost peatlands, western Siberia, Sci. Total Environ., 763,
821   144201, doi:10.1016/j.scitotenv.2020.144201, 2021.

822   Martiny, J., Bohannan, B., Brown, J. H., Colwell, R. K., Fuhrman, J. A., Green, J. L.,
823   Horner-Devine, M. C., Kane, M., Krumins, J. A., Kuske, C. R., Morin, P. J., Naeem, S.,
824   Ovreas, L., Reysenbach, A. L., Smith, V. H. and Staley, J. T.: Microbial biogeography:
825   putting microorganisms on the map, Nat. Rev. Microbiol., 4, 102-112,
826   doi:10.1038/nrmicro1341, 2006.

827   Mishra, U., Hugelius, G., Shelef, E., Yang, Y., Strauss, J., Lupachev, A., Harden, J. W.,
828   Jastrow, J. D., Ping, C. L., Riley, W. J., Schuur, E., Matamala, R., Siewert, M., Nave,
829   L. E., Koven, C. D., Fuchs, M., Palmtag, J., Kuhry, P., Treat, C. C., Zubrzycki, S.,
830   Hoffman, F. M., Elberling, B., Camill, P., Veremeeva, A. and Orr, A.: Spatial
831   heterogeneity and environmental predictors of permafrost region soil organic carbon
832   stocks, Sci. Adv., 7, 5236, doi:10.1126/sciadv.aaz5236, 2021.

833   Mori, A. S., Isbell, F. and Seidl, R.: β -Diversity, Community Assembly, and Ecosystem
834   Functioning, Trends Ecol. Evol., 33, 549-564, doi:10.1016/j.tree.2018.04.012, 2018.

835   Mu, C., Abbott, B. W., Norris, A. J., Mu, M., Fan, C., Chen, X., Jia, L., Yang, R., Zhang,
836   T., Wang, K., Peng, X., Wu, Q., Guggenberger, G. and Wu, X.: The status and stability
837   of permafrost carbon on the Tibetan Plateau, Earth-Sci. Rev., 211, 103433,
838   doi:10.1016/j.earscirev.2020.103433, 2020.

839   Narancic, B., Wolfe, B. B., Pienitz, R., Meyer, H. and Lamhonwah, D.: Landscape-gradient
840   assessment of thermokarst lake hydrology using water isotope tracers, J. Hydrol., 545,
841   327-338,2017.

842   Nemergut, D. R., Schmidt, S. K., Fukami, T., O'Neill, S. P., Bilinski, T. M., Stanish, L. F.,
843   Knelman, J. E., Darcy, J. L., Lynch, R. C., Wickey, P. and Ferrenberg, S.: Patterns and

Processes of Microbial Community Assembly, Microbiol. Mol. Biol. Rev., 77, 342-356, doi:10.1128/MMBR.00051-12, 2013.

Ning, D., Deng, Y., Tiedje, J. M. and Zhou, J.: A general framework for quantitatively assessing ecological stochasticity, Proceedings of the National Academy of Sciences, 116, 16892-16898, doi:10.1073/pnas.1904623116, 2019.

Nitzbon, J., Langer, M., Martin, L. C. P., Westermann, S., Schneider Von Deimling, T. and Boike, J.: Effects of multi-scale heterogeneity on the simulated evolution of ice-rich permafrost lowlands under a warming climate, The cryosphere, 15, 1399-1422, doi:10.5194/tc-15-1399-2021, 2021.

Niu, F., Lin, Z., Liu, H. and Lu, J.: Characteristics of thermokarst lakes and their influence on permafrost in Qinghai – Tibet Plateau, Geomorphology, 132, 222-233, doi:10.1016/j.geomorph.2011.05.011, 2011.

Nossa, C. W., Oberdorf, W. E., Yang, L., Aas, J. A., Paster, B. J., Desantis, T. Z., Brodie, E. L., Malamud, D., Poles, M. A. and Pei, Z.: Design of 16S rRNA gene primers for 454 pyrosequencing of the human foregut microbiome, World J. Gastroenterol., 16, 4135-4144, doi:10.3748/wjg.v16.i33.4135, 2010.

Obu, J.: How much of the Earth's surface is underlain by permafrost? Journal of Geophysical Research: Earth Surface, 126, 2021JF006123, doi:10.1029/2021JF006123, 2021.

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., Mcglinn, D., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs, E. and Wagner, H.: vegan: Community Ecology Package. R package version 2.5-7. https://CRAN.R-project.org/package=vegan,2020.

Ottoni, J. R., de Oliveira, V. M. and Passarini, M. R. Z.: Microbes in thawing permafrost: contributions to climate change. In Microbiome Under Changing Climate (pp. 1-28). Woodhead Publishing, doi:10.1016/B978-0-323-90571-8.00001-8, 2022.

Payette, S., Delwaide, A., Caccianiga, M. and Beauchemin, M.: Accelerated thawing of subarctic peatland permafrost over the last 50 years, Geophys. Res. Lett., 31, L18208,2004.

Pearman, J. K., Biessy, L., Thomson-Laing, G., Waters, S., Vandergoes, M. J., Howarth, J. D., Rees, A., Moy, C., Pochon, X. and Wood, S. A.: Local factors drive bacterial and microeukaryotic community composition in lake surface sediment collected across an altitudinal gradient, FEMS Microbiol. Ecol., 96, doi:10.1093/femsec/fiaa070, 2020.

Pholchan, M. K., Baptista, J. D. C., Davenport, R. J., Sloan, W. T. and Curtis, T. P.: Microbial community assembly, theory and rare functions, Front. Microbiol., 4, e68, doi:10.3389/fmicb.2013.00068, 2013.

Piao, S., Tan, K., Nan, H., Ciais, P., Fang, J., Wang, T., Vuichard, N. and Zhu, B.: Impacts of climate and CO2 changes on the vegetation growth and carbon balance of Qinghai – Tibetan grasslands over the past five decades, Glob. Planet. Change, 98-99, 73-80, doi:https://doi.org/10.1016/j.gloplacha.2012.08.009, 2012.

Picazo, F., Vilmi, A., Aalto, J., Soininen, J., Casamayor, E. O., Liu, Y., Wu, Q., Ren, L., Zhou, J., Shen, J. and Wang, J.: Climate mediates continental scale patterns of stream microbial functional diversity, Microbiome, 8, e92, doi:10.1186/s40168-020-00873-2, 2020.

Pla-Rabes, S., Flower, R. J., Shilland, E. M. and Kreiser, A. M.: Assessing microbial diversity using recent lake sediments and estimations of spatio-temporal diversity, J.

890   Biogeogr., 38, 2033-2040, doi:10.1111/j.1365-2699.2011.02530.x, 2011.

891   Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J. and
892   Glockner, F. O.: The SILVA ribosomal RNA gene database project: improved data
893   processing and web-based tools, Nucleic. Acids. Res., 41, 590-596,
894   doi:10.1093/nar/gks1219, 2013.

895   R Core Team: R: A language and environment for statistical computing, R Foundation for
896   Statistical Computing, Vienna, Austria. https://www.R-project.org,2020.

897   Ren, Z. and Gao, H. K.: Abundant and rare soil fungi exhibit distinct succession patterns
898   in the forefield of Dongkemadi glacier on the central Qinghai-Tibet Plateau, Sci. Total
899   Environ., 828, e154563, doi:10.1016/j.scitotenv.2022.154563, 2022.

900   Ren, Z., Cao, S. K., Chen, T., Zhang, C. and Yu, J. L.: Bacterial functional redundancy and
901   carbon metabolism potentials in soil, sediment, and water of thermokarst landscapes
902   across the Qinghai-Tibet Plateau: implications for the fate of permafrost carbon, Sci.
903   Total Environ., 852, e158340, doi:10.1016/j.scitotenv.2022.158340, 2022a.

904   Ren, Z., Jia, X., Zhang, Y. T., Ma, K., Zhang, C. and Li, X.: Biogeography and
905   environmental drivers of zooplankton communities in permafrost-affected lakes on the
906   Qinghai-Tibet Plateau, Glob. Ecol. Conserv., 38, e02191,
907   doi:10.1016/j.gecco.2022.e02191, 2022c.

908   Ren, Z., Li, X., Zhang, C., Wang, Q., Fang, L., Cao, S. K. and Yu, J. L.: From permafrost
909   soil to thermokarst lake sediment: A view from C:N:P stoichiometry, Front. Environ.
910   Sci., 10, e986879,2022b.

911   Ren, Z., Luo, W. and Zhang, C.: Rare bacterial biosphere is more environmental controlled
912   and deterministically governed than abundant one in sediment of thermokarst lakes
913   across the Qinghai-Tibet Plateau, Front. Microbiol., 13, e944646,
914   doi:10.3389/fmicb.2022.944646, 2022d.

915   Ren, Z., Niu, D., Ma, P., Wang, Y., Fu, H. and Elser, J. J.: Cascading influences of
916   grassland degradation on nutrient limitation in a high mountain lake and its inflow
917   streams, Ecology, 100, e02755, doi:10.1002/ecy.2755, 2019a.

918   Ren, Z., Qu, X. D., Peng, W. Q., Yu, Y. and Zhang, M.: Nutrients drive the structures of
919   bacterial communities in sediments and surface waters in the river-lake system of
920   Poyang Lake, Water, 11, e930, doi:10.3390/w11050930, 2019b.

921   Ren, Z., Wang, F., Qu, X., Elser, J. J., Liu, Y. and Chu, L.: Taxonomic and functional
922   differences between microbial communities in Qinghai Lake and its input streams,
923   Front. Microbiol., 8, e2319, doi:10.3389/fmicb.2017.02319, 2017.

924   Ren, Z., Wang, Z. M., Wang, Y., Ma, P. P., Niu, D. C., Fu, H. and Elser, J. J.: Soil bacterial
925   communities vary with grassland degradation in the Qinghai Lake watershed, Plant Soil,
926   460, 541-557, doi:10.1007/s11104-020-04823-7, 2021.

927   Romanowicz, K. J. and Kling, G. W.: Summer thaw duration is a strong predictor of the
928   soil microbiome and its response to permafrost thaw in arctic tundra, Environ.
929   Microbiol., 24, 6220-6237, doi:10.1111/1462-2920.16218, 2022.

930   Rosseel, Y.: lavaan: An R Package for Structural Equation Modeling, J. Stat. Softw., 48,
931   1-36, doi:10.18637/jss.v048.i02, 2012.

932   Ruiz-Gonzalez, C., Pablo Nino-Garcia, J. and Del Giorgio, P. A.: Terrestrial origin of
933   bacterial communities in complex boreal freshwater networks, Ecol. Lett., 18, 1198-
934   1206, doi:10.1111/ele.12499, 2015.

935   Schaefer, K., Zhang, T., Bruhwiler, L. and Barrett, A. P.: Amount and timing of permafrost

936   carbon release in response to climate warming, Tellus B: Chemical and Physical
937   Meteorology, 63, 168-180,2011.
938 Schuur, E. A. G., Bockheim, J., Canadell, J. G., Euskirchen, E., Field, C. B., Goryachkin,
939   S. V., Hagemann, S., Kuhry, P., Lafleur, P. M., Lee, H., Mazhitova, G., Nelson, F. E.,
940   Rinke, A., Romanovsky, V. E., Shiklomanov, N., Tarnocai, C., Venevsky, S., Vogel, J.
941   G. and Zimov, S. A.: Vulnerability of permafrost carbon to climate change:
942   Implications for the global carbon cycle, Bioscience, 58, 701-714,
943   doi:10.1641/B580807, 2008.
944 Schuur, E. A., Vogel, J. G., Crummer, K. G., Lee, H., Sickman, J. O. and Osterkamp, T.
945   E.: The effect of permafrost thaw on old carbon release and net carbon exchange from
946   tundra, Nature, 459, 556-569, doi:10.1038/nature08031, 2009.
947 She, Z., Pan, X., Wang, J., Shao, R., Wang, G., Wang, S. and Yue, Z.: Vertical
948   environmental gradient drives prokaryotic microbial community assembly and species
949   coexistence in a stratified acid mine drainage lake, Water Res., 206, 117739,
950   doi:10.1016/j.watres.2021.117739, 2021.
951 Stegen, J. C., Lin, X., Fredrickson, J. K. and Konopka, A. E.: Estimating and mapping
952   ecological processes influencing microbial community assembly, Front. Microbiol., 6,
953   e370, doi:10.3389/fmicb.2015.00370, 2015.
954 Stegen, J. C., Lin, X., Fredrickson, J. K., Chen, X., Kennedy, D. W., Murray, C. J.,
955   Rockhold, M. L. and Konopka, A.: Quantifying community assembly processes and
956   identifying features that impose them, The ISME Journal, 7, 2069-2079,
957   doi:10.1038/ismej.2013.93, 2013.
958 Stegen, J. C., Lin, X., Konopka, A. E. and Fredrickson, J. K.: Stochastic and deterministic
959   assembly processes in subsurface microbial communities, ISME J., 6, 1653-1664,
960   doi:10.1038/ismej.2012.22, 2012.
961 Steven, B., Niederberger, T. D. and Whyte, L. G.: Bacterial and archaeal diversity in
962   permafrost. In Permafrost soils (pp. 59-72). Springer, Berlin, Heidelberg,2009.
963 Strickland, M. S., Lauber, C., Fierer, N. and Bradford, M. A.: Testing the Functional
964   Significance of Microbial Community Composition, Ecology, 90, 441-451,
965   doi:10.1890/08-0296.1, 2009.
966 Taş, N., Prestat, E., Wang, S., Wu, Y., Ulrich, C., Kneafsey, T., Tringe, S. G., Torn, M. S.,
967   Hubbard, S. S., Jansson, J. K., Pacific Northwest National Laboratory Pnnl, R. W. U.
968   S. and Lawrence Berkeley National Laboratory Lbnl, B. C. U. S.: Landscape
969   topography structures the soil microbiome in arctic polygonal tundra, Nat. Commun.,
970   9, 777-13, doi:10.1038/s41467-018-03089-z, 2018.
971 Telford, R. J., Vandvik, V. and Birks, H. J. B.: Dispersal Limitations Matter for Microbial
972   Morphospecies, Science, 312, 1015-1015, doi:10.1126/science.1125669, 2006.
973 Torsvik, V., Ovreas, L. and Thingstad, T. F.: Prokaryotic diversity - Magnitude, dynamics,
974   and controlling factors, Science, 296, 1064-1066, doi:10.1126/science.1071698, 2002.
975 Tripathi, B. M., Stegen, J. C., Kim, M., Dong, K., Adams, J. M. and Lee, Y. K.: Soil pH
976   mediates the balance between stochastic and deterministic assembly of bacteria, The
977   ISME Journal, 12, 1072-1083, doi:10.1038/s41396-018-0082-4, 2018.
978 Turetsky, M. R., Abbott, B. W., Jones, M. C., Anthony, K. W., Olefeldt, D., Schuur, E. A.
979   G., Grosse, G., Kuhry, P., Hugelius, G., Koven, C., Lawrence, D. M., Gibson, C.,
980   Sannel, A. B. K. and Mcguire, A. D.: Carbon release through abrupt permafrost thaw,
981   Nat. Geosci., 13, 138-143, doi:10.1038/s41561-019-0526-0, 2020.

982 Vargas Medrano, M.: Recent Changes in the Zooplankton Communities of Arctic Tundra
983   Ponds in Response to Warmer Temperatures and Nutrient Enrichment, Dissertation:
984   University of Texas at El Paso,2019.

985 Vellend, M., Srivastava, D. S., Anderson, K. M., Brown, C. D., Jankowski, J. E., Kleynhans,
986   E. J., Kraft, N. J. B., Letaw, A. D., Macdonald, A. A. M., Maclean, J. E., Myers-Smith,
987   I. H., Norris, A. R. and Xue, X.: Assessing the relative importance of neutral
988   stochasticity in ecological communities, Oikos, 123, 1420-1430,
989   doi:10.1111/oik.01493, 2014.

990 Veremeeva, A., Nitze, I., Günther, F., Grosse, G. and Rivkina, E.: Geomorphological and
991   climatic drivers of thermokarst lake area increase trend (1999-2018) in the Kolyma
992   Lowland Yedoma Region, North-Eastern Siberia, Remote Sens., 13, 178,
993   doi:10.3390/rs13020178, 2021.

994 Vigneron, A., Lovejoy, C., Cruaud, P., Kalenitchenko, D., Culley, A. and Vincent, W. F.:
995   Contrasting Winter Versus Summer Microbial Communities and Metabolic Functions
996   in a Permafrost Thaw Lake, Front. Microbiol., 10, 1656, doi:10.3389/fmicb.2019.01656,
997   2019.

998 Vucic, J. M., Gray, D. K., Cohen, R. S., Syed, M., Murdoch, A. D. and Sharma, S.: Changes
999   in water quality related to permafrost thaw may significantly impact zooplankton in
1000   small Arctic lakes, Ecol. Appl., 30, e02186, doi:10.1002/eap.2186, 2020.

1001 Walter, K. M., Zimov, S. A., Chanton, J. P., Verbyla, D. and Chapin, F. S.: Methane
1002   bubbling from Siberian thaw lakes as a positive feedback to climate warming, Nature,
1003   443, 71-75, doi:10.1038/nature05040, 2006.

1004 Wan, C., Gibson, J. J., Shen, S., Yi, Y., Yi, P. and Yu, Z.: Using stable isotopes paired with
1005   tritium analysis to assess thermokarst lake water balances in the Source Area of the
1006   Yellow River, northeastern Qinghai-Tibet Plateau, China, Sci. Total Environ., 689,
1007   1276-1292, doi:10.1016/j.scitotenv.2019.06.427, 2019.

1008 Wan, D. S., Feng, J. J., Jiang, D. C., Mao, K. S., Duan, Y. W., Miehe, G. and Opgenoorth,
1009   L.: The Quaternary evolutionary history, potential distribution dynamics, and
1010   conservation implications for a Qinghai-Tibet Plateau endemic herbaceous perennial,
1011   Anisodus tanguticus (Solanaceae), Ecol. Evol., 6, 1977-95, doi:10.1002/ece3.2019,
1012   2016.

1013 Webb, C. O., Ackerly, D. D., Mcpeek, M. A. and Donoghue, M. J.: Phylogenies and
1014   community ecology, Annual review of ecology and systematics, 33, 475-505,2002.

1015 West, J. J. and Plug, L. J.: Time-dependent morphology of thaw lakes and taliks in deep
1016   and shallow ground ice, Journal of Geophysical Research, 113, 1009,
1017   doi:10.1029/2006JF000696, 2008.

1018 Wu, L., Yang, F., Feng, J., Tao, X., Qi, Q., Wang, C., Schuur, E. A. G., Bracho, R., Huang,
1019   Y., Cole, J. R., Tiedje, J. M. and Zhou, J.: Permafrost thaw with warming reduces
1020   microbial metabolic capacities in subsurface soils, Mol. Ecol., 31, 1403-1415,
1021   doi:10.1111/mec.16319, 2022.

1022 Wu, M., Chen, S., Chen, J., Xue, K., Chen, S., Wang, X., Chen, T., Kang, S., Rui, J., Thies,
1023   J. E., Bardgett, R. D. and Wang, Y.: Reduced microbial stability in the active layer is
1024   associated with carbon loss under alpine permafrost degradation, Proceedings of the
1025   National Academy of Sciences, 118, e2025321118, doi:10.1073/pnas.2025321118,
1026   2021.

1027 Wu, Q., Dong, X., Liu, Y., Jin, H. and Zhang, T.: Responses of permafrost on the Qinghai-

1028   Tibet Plateau, China, to climate change and engineering construction, Arctic, Antarctic,
1029   and Alpine Research, 39, 682-687, doi:10.1657/1523-0430(07-508)[WU]2.0.CO;2,
1030   2007.
1031 Xiong, J., Liu, Y., Lin, X., Zhang, H., Zeng, J., Hou, J., Yang, Y., Yao, T., Knight, R. and
1032   Chu, H.: Geographic distance and pH drive bacterial distribution in alkaline lake
1033   sediments across Tibetan Plateau, Environ. Microbiol., 14, 2457-2466,
1034   doi:10.1111/j.1462-2920.2012.02799.x, 2012.
1035 Xu, Z. X., Gong, T. L. and Li, J. Y.: Decadal trend of climate in the Tibetan Plateau-
1036   regional temperature and precipitation, Hydrol. Process., 22, 3056-3065,
1037   doi:10.1002/hyp.6892, 2008.
1038 Yang, J., Jiang, H., Dong, H. and Liu, Y.: A comprehensive census of lake microbial
1039   diversity on a global scale, Science China Life Sciences, 62, 1320-1331,
1040   doi:10.1007/s11427-018-9525-9, 2019.
1041 Yang, J., Ma, L., Jiang, H., Wu, G. and Dong, H.: Salinity shapes microbial diversity and
1042   community structure in surface sediments of the Qinghai-Tibetan Lakes, Sci. Rep., 6,
1043   e25078, doi:10.1038/srep25078, 2016b.
1044 Yang, Y., Wu, Q., Yun, H., Jin, H. and Zhang, Z.: Evaluation of the hydrological
1045   contributions of permafrost to the thermokarst lakes on the Qinghai-Tibet Plateau using
1046   stable isotopes, Glob. Planet. Change, 140, 1-8, doi:10.1016/j.gloplacha.2016.03.006,
1047   2016a.
1048 Yao, T., Thompson, L. G., Mosbrugger, V., Zhang, F., Ma, Y., Luo, T., Xu, B., Yang, X.,
1049   Joswiak, D. R., Wang, W., Joswiak, M. E., Devkota, L. P., Tayal, S., Jilani, R. and
1050   Fayziev, R.: Third Pole Environment (TPE), Environ. Dev., 3, 52-64,
1051   doi:10.1016/j.envdev.2012.04.002, 2012.
1052 Yu, G., Smith, D. K., Zhu, H., Guan, Y. and Lam, T. T. Y.: ggtree: an R package for
1053   visualization and annotation of phylogenetic trees with their covariates and other
1054   associated data, Methods Ecol. Evol., 8, 28-36,2017.
1055 Yu, H., Favre, A., Sui, X., Chen, Z., Qi, W., Xie, G., Kleunen, M. and van Kleunen, M.:
1056   Mapping the genetic patterns of plants in the region of the Qinghai‐Tibet Plateau:
1057   Implications for conservation strategies, Diversity & distributions, 25, 310-324,
1058   doi:10.1111/ddi.12847, 2019.
1059 Zhang, D., Wang, L., Qin, S., Kou, D., Wang, S., Zheng, Z., Peñuelas, J. and Yang, Y.:
1060   Microbial nitrogen and phosphorus co‐limitation across permafrost region, Glob.
1061   Change Biol., 29, 3910-3923, doi:10.1111/gcb.16743, 2023.
1062 Zhang, J. L.: Species association analysis, Version 0.2.2. https://CRAN.R-
1063   project.org/package=spaa,2016.
1064 Zhang, Z., Chang, J., Xu, C., Zhou, Y., Wu, Y., Chen, X., Jiang, S. and Duan, Z.: The
1065   response of lake area and vegetation cover variations to climate change over the
1066   Qinghai-Tibetan Plateau during the past 30 years, The Science of the total environment,
1067   635, 443-451, doi:10.1016/j.scitotenv.2018.04.113, 2018.
1068 Zhou, C. P.: The spatial dataset of climate on the Tibetan Plateau (1961-2020). National
1069   Tibetan Plateau Data Center, doi:10.11888/AtmosphericPhysics.tpe.49.file, 2018.
1070 Zhou, J. and Ning, D.: Stochastic Community Assembly: Does It Matter in Microbial
1071   Ecology? Microbiol. Mol. Biol. Rev., 81, e00002-17, doi:10.1128/MMBR.00002-17,
1072   2017.
1073 Zhou, J., Deng, Y., Zhang, P., Xue, K., Liang, Y., Van Nostrand, J. D., Yang, Y., He, Z.,

1074   Wu, L., Stahl, D. A., Hazen, T. C., Tiedje, J. M. and Arkin, A. P.: Stochasticity,
1075   succession, and environmental perturbations in a fluidic ecosystem, Proceedings of the
1076   National Academy of Sciences, 111, E836-E845, doi:10.1073/pnas.1324044111, 2014.
1077   Zhou, J., Liu, W., Deng, Y., Jiang, Y., Xue, K., He, Z., Van Nostrand, J. D., Wu, L., Yang,
1078   Y., Wang, A., Lawrence Berkeley National Lab. Lbnl, B. C. U. S. and Handelsman, J.:
1079   Stochastic assembly leads to alternative communities with distinct functions in a
1080   bioreactor microbial community, mBio, 4, e00584-12, doi:10.1128/mBio.00584-12,
1081   2013.
1082   Zhou, L., Zhou, Y., Yao, X., Cai, J., Liu, X., Tang, X., Zhang, Y., Jang, K. and Jeppesen,
1083   E.: Decreasing diversity of rare bacterial subcommunities relates to dissolved organic
1084   matter along permafrost thawing gradients, Environ. Int., 134, 105330,
1085   doi:10.1016/j.envint.2019.105330, 2020.
1086   Zou, D., Zhao, L., Sheng, Y., Chen, J., Hu, G., Wu, T., Wu, J., Xie, C., Wu, X., Pang, Q.,
1087   Wang, W., Du, E., Li, W., Liu, G., Li, J., Qin, Y., Qiao, Y., Wang, Z., Shi, J. and Cheng,
1088   G.: A new map of permafrost distribution on the Tibetan Plateau, Cryosphere, 11, 2527-
1089   2542, doi:10.5194/tc-11-2527-2017, 2017.

1090