

16 **Abstract**

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

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

35 **1 Introduction**

36 Permafrost is an important landscape in high latitude and altitude regions, covering 15%
37 of the land area of the Northern Hemisphere (Obu, 2021) and 40% of the Qinghai-Tibet
38 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 1Figure-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~~Figure 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~~Figure 1~~). Our aims were to (1) assess
107 the spatial pattern of alpha and beta diversity of bacterial communities, and (2) evaluate

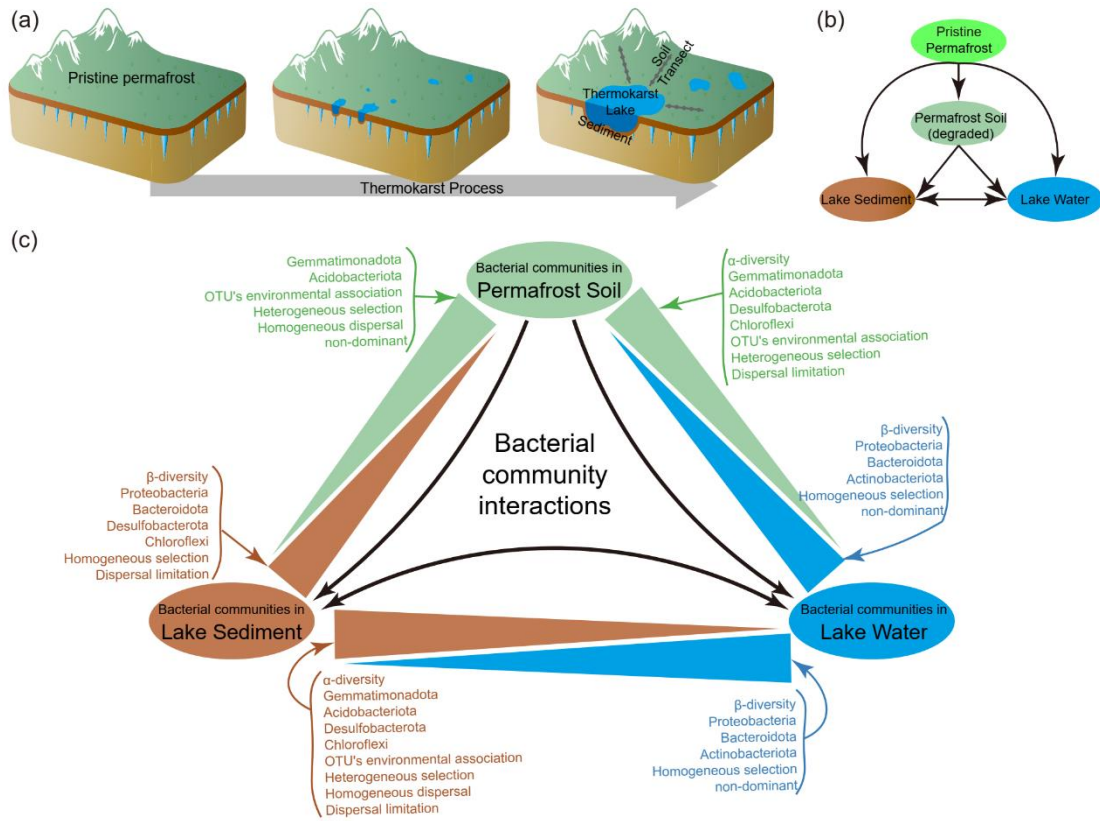
108 the community assembly mechanisms and environmental responses of the bacterial
109 communities in degraded permafrost soil, as well as in the sediment and water of
110 thermokarst lakes.

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](#)~~Figure S1~~). In total, 44
114 sites were investigated by collecting paired samples of lake water, lake sediment, and
115 surrounding permafrost soil ([Figure 1](#)~~Figure 1~~a) (Ren et al., 2022a). The sampling strategy
116 and chemical analysis methods were described in detail in our previous publications (Ren
117 et al., 2022a, b). For water sampling of each lake, surface water samples were collected at
118 a depth of 0.3 to 0.5 m with three replicates. For microbial analysis, 200 mL of water was
119 filtered using a 0.2- μ m polycarbonate membrane filter (Whatman, UK) for DNA extraction.
120 The 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 methods of DNA extraction, PCR, and sequencing were described in detail in our~~
 141 ~~previous publication (Ren et al., 2022a). In brief, t~~The Magen Hipure Soil DNA Kit
 142 (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 (PB ϵ s),
188 lake sediment bacterial communities (SB ϵ s), and lake water bacterial communities

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 (β NTI) 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 β NTI < -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 $\beta\text{NTI} > +2$. 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 $-2 < \beta\text{NTI} < 2$
239 and $\text{RC}_{\text{Bray}} < -0.95$. 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 $-2 < \beta\text{NTI} < 2$ and $\text{RC}_{\text{Bray}} > 0.95$. -Finally, the
242 fraction of the pairwise comparisons with $-2 < \beta\text{NTI} < 2$ and $-0.95 < \text{RC}_{\text{Bray}} < 0.95$ 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).

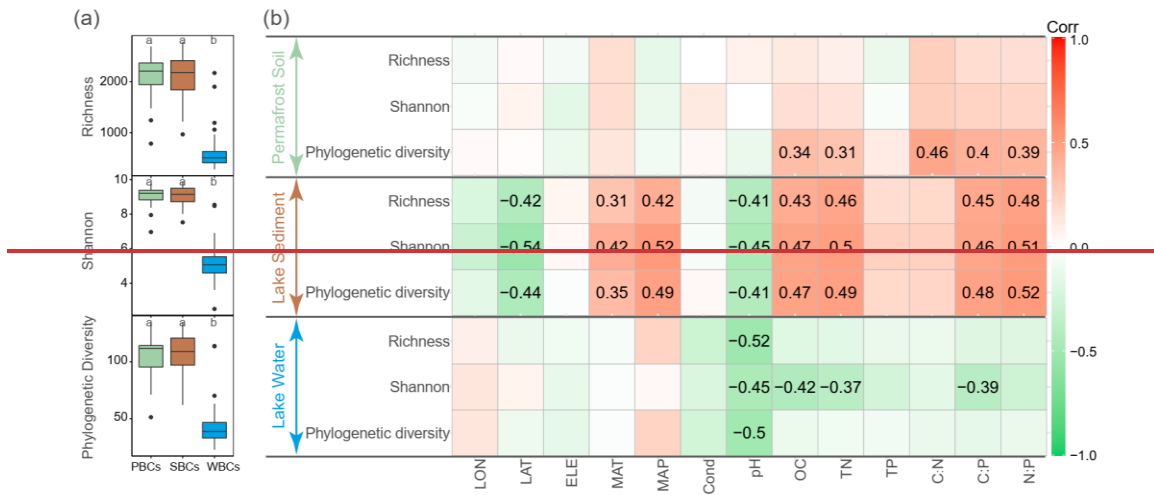
246 **3 Results**

247 *3.1 General distribution patterns of α -diversity*

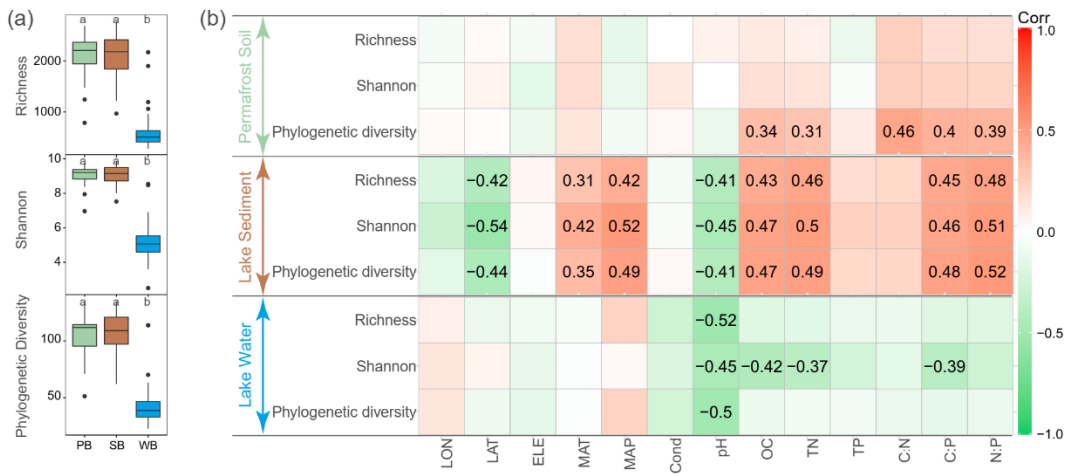
248 After quality filtering, 3,201,132 high quality sequences were obtained and clustered into
249 9,361 OTUs, of which, 3870 OTUs were core OTUs shared by bacterial communities in
250 permafrost soil, lake sediment, and lake water (Figure S2Figure-S2). Moreover, a large
251 number of OTUs were shared by PB_{Cs} and SB_{Cs} ($n=7053$), 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 PB_{Cs} and WB_{Cs} ($n=4007$)
254 and by SB_{Cs} and WB_{Cs} ($n=4431$), 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 PB_{Cs} and SB_{Cs} (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 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 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 2b).

264 PBCs and SBCs had a significantly greater phylogenetic diversity than WBCs (Figure
265 2a and Figure 3). The OTUs in PBCs had significantly higher frequency
266 than that of SBCs and WBCs (Figure 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 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 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 3c). In
274 addition, WBCs had significantly higher SES.MNTD than PBCs and SBCs (Figure
275 3d), suggesting higher phylogenetic clustering of bacterial taxa in WBCs.

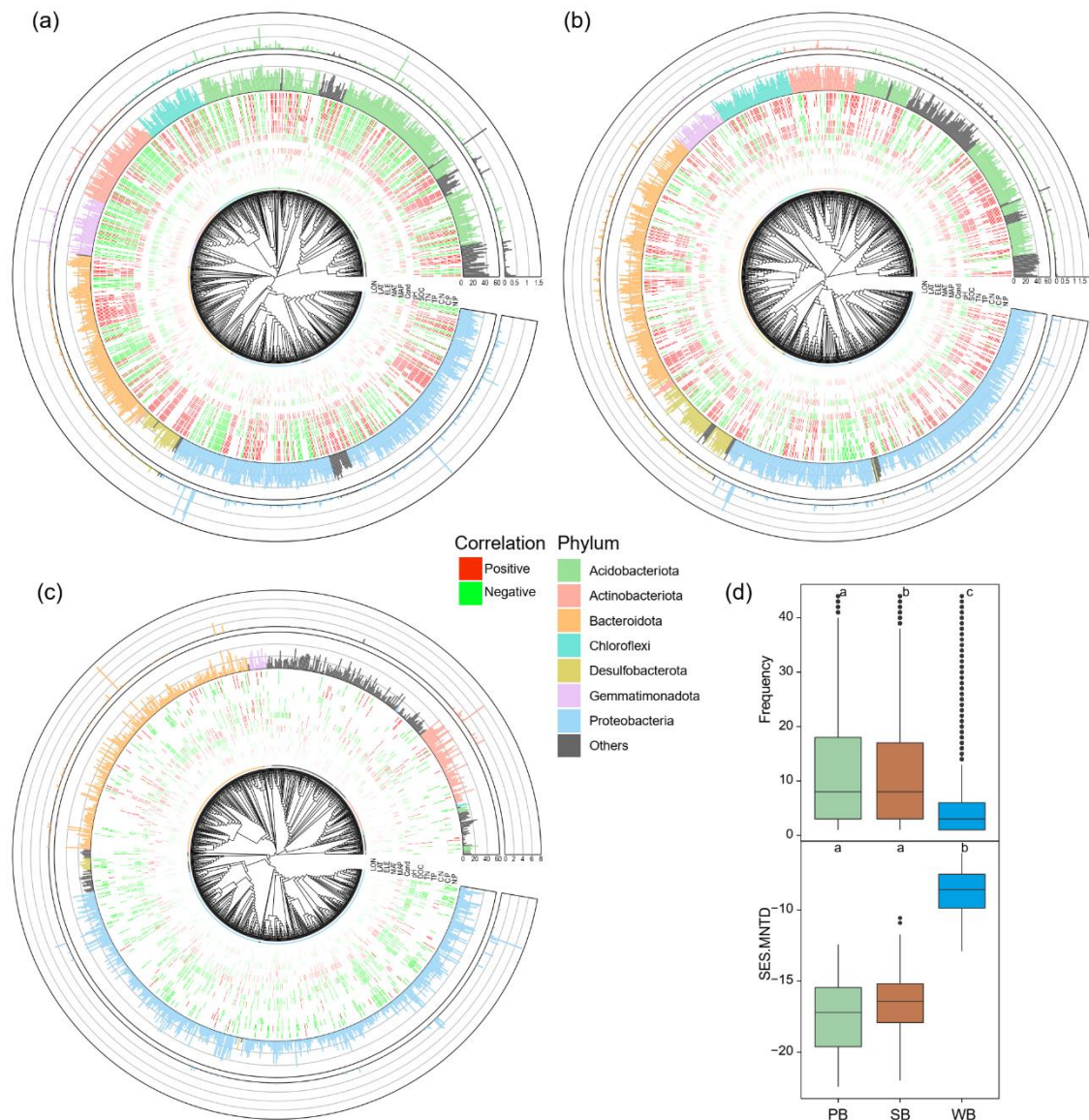


276



277

278 Figure 2 (a) Alpha diversity of bacterial communities in permafrost soil (PBE), lake
 279 sediment (SBE), and lake water (WBE). The different low-case letters represent significant
 280 differences assessed using Wilcoxon rank-sum test. (b) Spearman correlations show the
 281 relationships between alpha diversity and environmental factors. The color represents the
 282 correlation coefficient, which is shown in number when the result is statistically significant
 283 ($p < 0.05$).



284

285 Figure 3 Phylogenetic tree of the top 1000 OTUs in bacterial communities in (a) permafrost
 286 soil (~~PBC~~), (b) lake sediment (~~SBC~~), and (c) lake water (~~WBC~~). Tree tips are colored by
 287 major phylum. The inner ring of the heatmap represents spearman's correlation between
 288 OTUs and environmental variables. Only significant ($p < 0.05$) results are shown. The
 289 middle ring represents the frequency of the OTUs in our studied sites. The outer ring
 290 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 (PB~~Es~~),
292 lake sediment (SB~~Es~~), and lake water (WB~~Es~~). 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 PB~~Es~~ 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 4~~Figure 4a). SB~~Es~~ 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 4~~Figure 4a). WB~~Es~~ were dominated by Proteobacteria
300 (46.9%), Bacteroidota (29.2%), and Actinobacteriota (17.4%) (~~Figure 4~~Figure 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 WB~~Es~~ than in SB~~Es~~ and
304 PB~~Es~~ (~~Figure 4~~Figure 4a). The relative abundance of Actinobacteriota was the highest in
305 WB~~Es~~ but was not significantly different between PB~~Es~~ and SB~~Es~~ (~~Figure 4~~Figure 4a).
306 Gemmatimonadota and Acidobacteriota were significantly enriched in PB~~Es~~ than in SB~~Es~~
307 and WB~~Es~~. Desulfobacterota and Chloroflexi were significantly enriched in SB~~Es~~ than in
308 PB~~Es~~ and WB~~Es~~ (~~Figure 4~~Figure 4a).

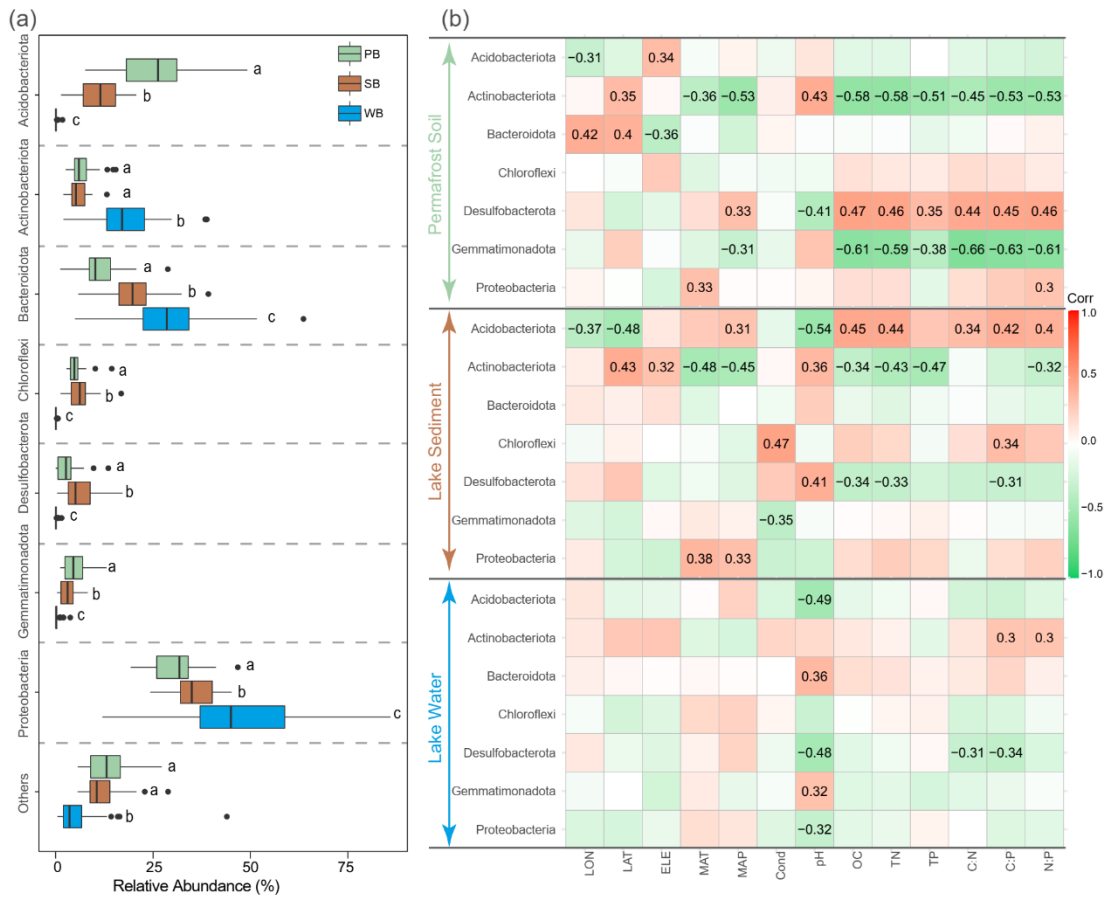
309 These phyla responded differently to environmental variables (~~Figure 4~~Figure 4b). For
310 example, Actinobacteriota and Gemmatimonadota in PB~~Es~~ and Actinobacteriota and
311 Desulfobacterota in SB~~Es~~ were negatively correlated with nutrient concentrations and
312 ratios, while Desulfobacterota in PB~~Es~~ and Acidobacteriota in SB~~Es~~ were positively
313 correlated with nutrient concentrations and ratios (~~Figure 4~~Figure 4b). pH was a frequently

314 correlated ~~with~~ ~~for~~ taxa in various taxonomic groups across all three habitats ([Figure](#)
315 [4b](#)~~Figure-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 5](#)~~Figure-5~~a). The extent of difference was larger for WB~~Es~~ vs PB~~Es~~ ($\beta=0.98$;
319 $R_{ANOSIM} = 0.989$, $P<0.001$) than the differences for WB~~Es~~ vs SB~~Es~~ ($\beta=0.96$; $R_{ANOSIM} =$
320 0.967 , $P<0.001$). There was the least dissimilarity between PB~~Es~~ and SB~~Es~~ ($\beta=0.81$;
321 $R_{ANOSIM} = 0.384$, $P<0.001$). The fitted SEM model showed that PB~~Es~~ had direct effects on
322 SB~~Es~~ and WB~~Es~~, and the latter two had reciprocal effects on each other ([Figure 5](#)~~Figure~~
323 [5b](#)). In addition, location, climate, and permafrost soil physicochemical environments had
324 direct effects on PB~~Es~~. Climate had direct effects on SB~~Es~~ while lake water
325 physicochemical environments had direct effects on WB~~Es~~ ([Figure 5](#)~~Figure-5~~b).

326 WB~~Es~~ had a higher β -diversity than SB~~Es~~ and PB~~Es~~, suggesting that bacterial
327 communities were more spatially heterogeneous in lake water than in lake sediment and
328 permafrost soil ([Figure 5c](#)~~Figure-6a~~). Taxa in PB~~Es~~ had higher habitat niche breadths than
329 taxa in SB~~Es~~ and WB~~Es~~ ([Figure 5d](#)~~Figure-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 [S3](#)~~Figure-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 6](#)~~Figure-7~~). β -diversities of PB~~Es~~, SB~~Es~~,
335 and WB~~Es~~ were all significantly correlated with latitude, MAP, and pH ([Figure 6](#)~~Figure-7~~).
336 In addition, β -diversity of PB~~Es~~ was also significantly correlated with all the other

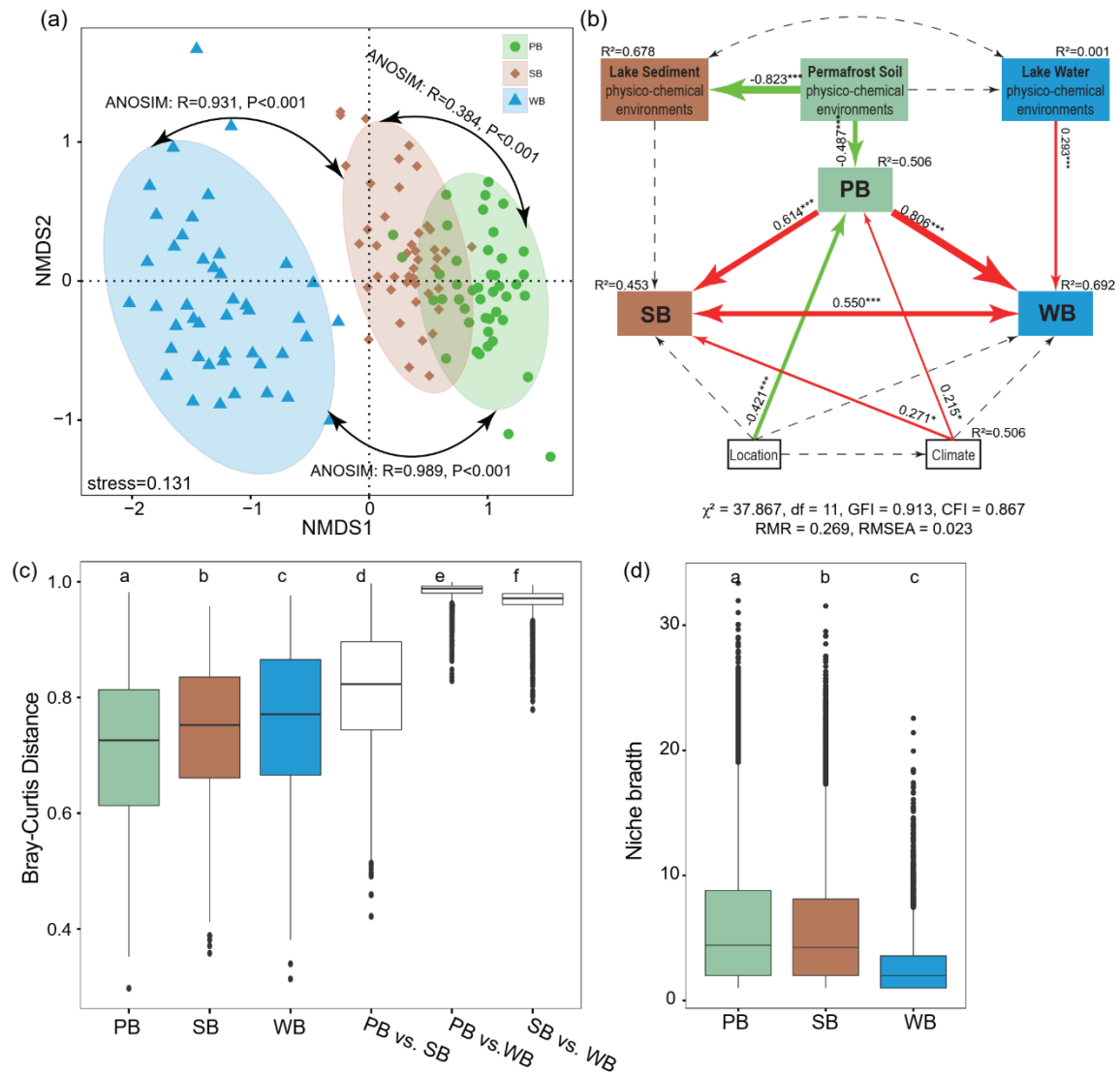
337 environmental variables except MAT and conductivity. β -diversity of SBCEs was also
 338 significantly correlated with conductivity and C:N (Figure 6Figure 7). β -diversity of WBCEs
 339 was also significantly correlated with elevation, MAT, conductivity, DOC, TN, and TP
 340 (Figure 6Figure 7). The results suggested that the compositional variation among PBCEs,
 341 SBCEs, and WBCEs was differentially structured by spatial, climatic, and physicochemical
 342 variables.



343
 344 Figure 4 (a) Relative abundances of major phyla in bacterial communities in permafrost
 345 soil (PBCEs), lake sediment (SBCEs), and lake water (WBCEs). 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 ($p < 0.05$).

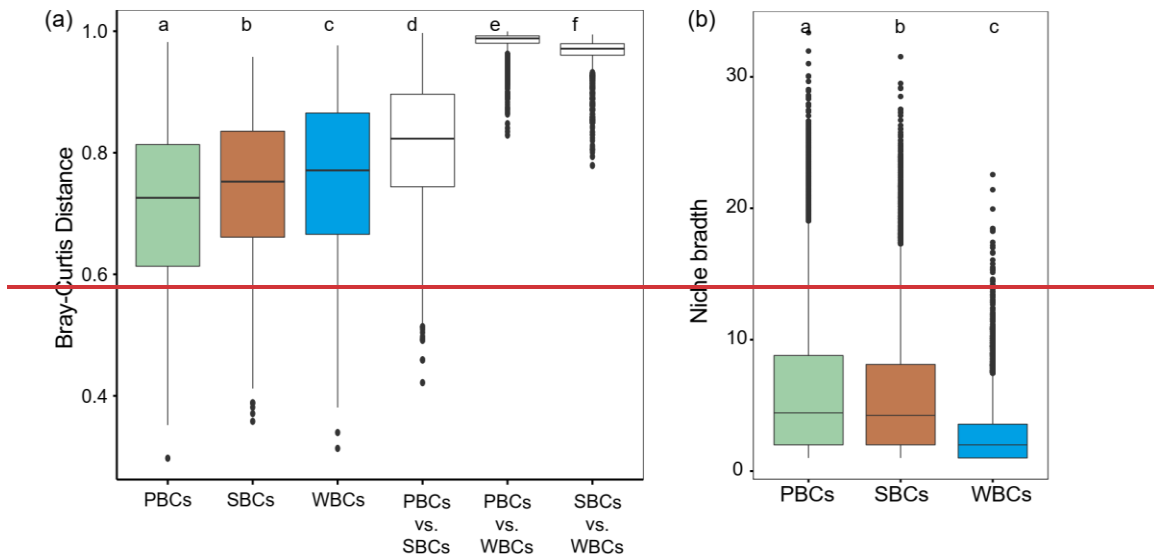
350



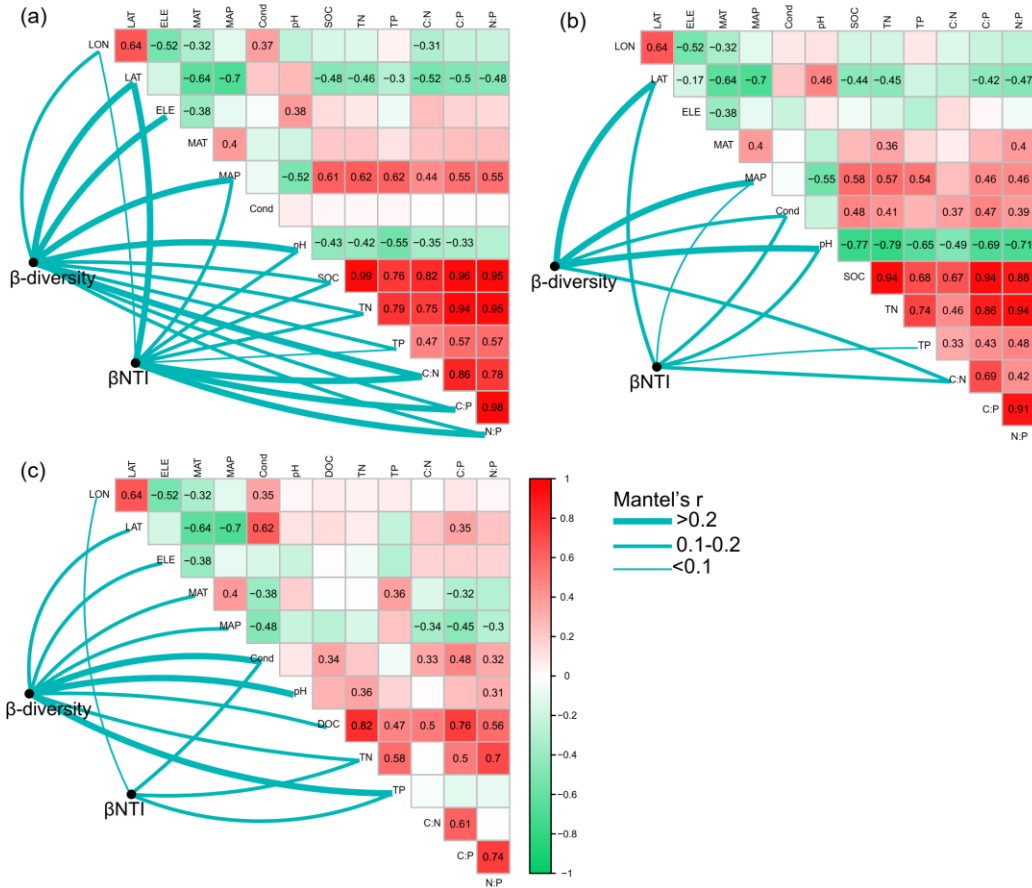
351

352 Figure 5 (a) Non-metric multidimensional scaling (NMDS) ordination showing the
 353 distribution of bacterial communities in permafrost soil (PB_{Cs}), lake sediment (SB_{Cs}), and
 354 lake water (WB_{Cs}). 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.



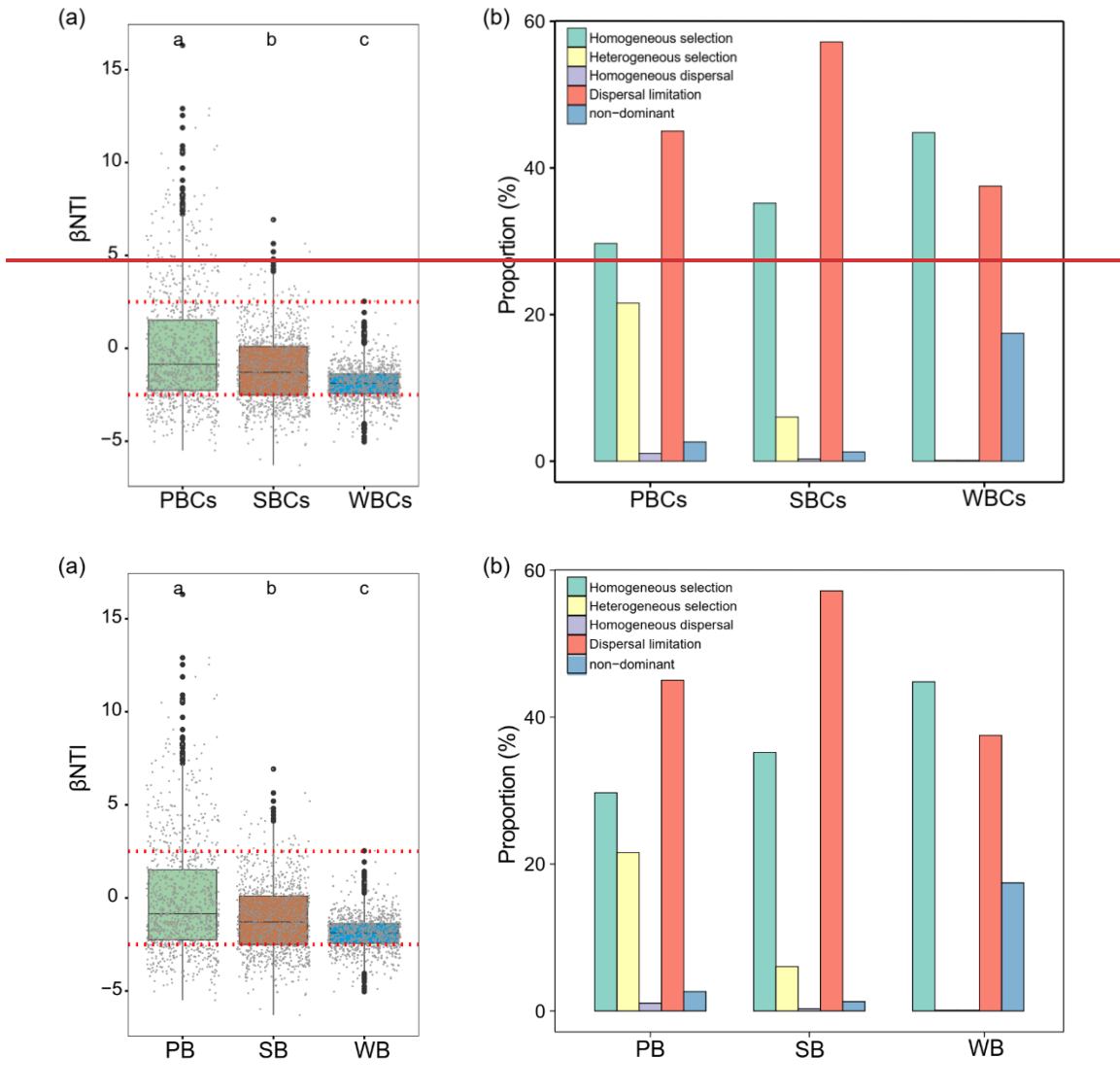
368

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

378 3.3 Assembly processes

379 To explore the mechanisms underlining the observed distribution patterns, a null-model-
380 based framework was employed to quantify the deviation of phylogenetic turnover. PBCEs
381 had significantly higher β NTI than SBCEs and WBCEs (Figure 7Figure-8a). Deterministic
382 processes contributed 51.3%, 41.2%, and 44.9% to community variations for the bacterial
383 communities in permafrost soil, lake sediment, and lake water, respectively (Figure
384 7Figure-8b). In particular, the results showed that homogeneous selection contributed a
385 larger fraction to the assembly of the WBCEs (44.8%), followed by SBCEs (35.2%) and PBCEs
386 (29.7%) (Figure 7Figure-8b). Heterogeneous selection influenced PBCEs (21.6%) more than
387 SBCEs (6.0%) and WBCEs (0.1%). Dispersal limitation contributed a larger fraction to SBCEs
388 (57.2%) than to PBCEs (45%) and WBCEs (37.5%).

389 The relationships between β NTI and major environmental variables were used to estimate
390 changes in the relative influences of deterministic and stochastic assembly processes.
391 Mantel tests showed that the assembly processes of bacterial communities in permafrost
392 soil, lake sediment, and lake water had similarities and differences in the responses to
393 environmental variables (Figure 6Figure-7). Particularly, differences of TP were
394 significantly associated with β NTI of PBCEs, SBCEs, and WBCEs, implying that an increasing
395 divergence of TP could contribute to a shift from homogeneous selection to heterogeneous
396 selection in the assembly of bacterial communities in the QTP thermokarst landscape.
397 Moreover, β NTI of PBCEs was also significantly associated with other environmental
398 variables, except elevation, MAT, and conductivity. β NTI of SBCEs was also significantly
399 associated with latitude, MAP, conductivity, pH, and C:N, while β NTI of WBCEs was
400 significantly associated with longitude, conductivity, and TN.



401

402

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

410 **4 Discussion**

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

421 *4.1 Alpha diversity and community composition*

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

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

433 sediments are likely to have high levels of similarity in bacterial diversity and community
434 composition. Thus, there is no doubt that permafrost soil, lake sediments, and lake water
435 should share a certain number of bacteria. Indeed, our study showed that 41% OTUs were
436 shared among PB, SB, and WB, while 75% OTUs were shared between PB and SB.
437 Additionally, our prior research has shown that there are close correlations between the
438 abiotic features of the two environments (Ren et al., 2022b). However, despite these
439 similarities and connections, we found substantial differences in the bacterial communities
440 of permafrost soil and lake sediments. As proposed by the Baas-Becking hypothesis (Baas-
441 Becking, 1934), environmental selection is partially responsible for variation in microbial
442 communities, which are also shaped by other ecological processes, such as diversification
443 and dispersal limitation. ~~Indeed, i~~In our study, alpha diversity and the dominant phyla
444 found in PB~~C~~s and SB~~C~~s responded differently to various environmental variables. In
445 addition, there were significant differences in composition and structure among PB, SB,
446 and WB, while the dissimilarities between PB and SB were the lowest.
447 Bacterial communities in lake water had significantly lower alpha diversity as well as
448 distinct community composition and structure in comparison to bacterial communities in
449 permafrost soil and lake sediment. However, PB~~C~~s and SB~~C~~s had direct influence on
450 WB~~C~~s. For thermokarst lakes, the water first originates from the thawing of the ice-rich
451 permafrost and the lake is then fed by precipitation-derived and permafrost-derived water
452 (Yang et al., 2016a; Narancic et al., 2017; Wan et al., 2019). Microorganisms present in
453 lake water have a diverse range of sources, including terrestrial inputs and other sources
454 such as bacteria distributed with the atmosphere, associated with plants and animals, and
455 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,

479 Proteobacteria, Bacteroidota, Actinobacteriota, Gemmatimonadota, Acidobacteriota,
480 Desulfobacterota, and Chloroflexi dominated bacterial communities in permafrost soil
481 and/or thermokarst lakes despite high variability^{iesy}. Similar dominance of these taxa has
482 also been found in permafrost and thermokarst landscapes in other areas (Aszalós et al.,
483 2020; Belov et al., 2020; Wu et al., 2022). The most commonly reported bacterial groups
484 in permafrost environments include members of Proteobacteria, Acidobacteria,
485 Actinobacteria, Bacteroidetes, Firmicutes, and Chloroflexi (Steven et al., 2009; Altshuler
486 et al., 2017; Ottoni et al., 2022), as observed in our samples.

487 ***4.2 Beta diversity and assembly processes***

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

502 distinct clades or lineages from external sources following permafrost thaw (Webb et al.,
503 2002; Stegen et al., 2013).

504 The structure of bacterial communities can vary across spatiotemporal scales and different
505 habitats (Ren et al., 2017; Aguilar and Sommaruga, 2020; Pearman et al., 2020). A key
506 objective in the field of microbial ecology is to determine the relative influence of
507 stochastic and deterministic processes in shaping the assembly of communities (Stegen et
508 al., 2013; Zhou and Ning, 2017). In this study, ~~deterministic processes contributed 51.3%,~~
509 ~~41.2%, and 44.9% to community variation for the bacterial communities in permafrost soil,~~
510 ~~lake sediment, and lake water, respectively. Homogeneous selection contributed a larger~~
511 ~~fraction to the assembly of the WBCs (44.8%), followed by SBCs (35.2%) and PBCs~~
512 ~~(29.7%). Heterogeneous selection influenced PBCs (21.6%) more strongly than SBCs~~
513 ~~(6.0%) and WBCs (0.1%).~~ In addition, bacterial communities in lake water displayed a
514 higher influence of homogeneous selection but lower influence of heterogeneous selection
515 compared to those in lake sediments and permafrost soil in our study. The reason for this
516 might be that long-term changes in thermokarst lakes result in homogenized habitats and
517 consequently strong homogenous selection on bacterial communities (Ning et al., 2019).
518 In contrast, permafrost soil is a highly heterogeneous environment across spatial scales
519 (Etzelmüller, 2013; Nitzbon et al., 2021), creating a wide range of habitats which can
520 impose strong heterogeneous selection pressures on bacterial communities. Furthermore,
521 permafrost soil is characterized by limited nutrient availability due to the frozen state of
522 organic matters (Beermann et al., 2017; Zhang et al., 2023), while lake water offers a more
523 diverse and abundant array of dissolved organic compounds and nutrients. As a result,
524 bacterial communities in permafrost soil might be more sensitive to variations in resource

525 availability, rendering them more strongly influenced by heterogeneous selection.
526 Deterministic processes could also cause the communities to be more dissimilar through
527 heterogeneous selection, which also imposed strong control on PBCs.In addition,
528 Dispersal limitation contributed a larger fraction to SBCEs (57.2%) than to PBCs (45%)
529 and WBCEs (37.5%). The dispersal of microorganisms is often considered as a passive
530 process that results in community variation and turnover coupled with the function of
531 environmental filtering (Cline and Zak, 2014; Stegen et al., 2015; Custer et al., 2022). The
532 high dispersal limitation of microbial communities in thermokarst lakes could be
533 potentially explained by that their isolated nature of thermokarst lakes being endorheic
534 results in limited connectivity and strong restriction of microbial dispersal, as well as strong
535 environmental filtering. Additionally, the prolonged frozen phase of thermokarst lakes and
536 permafrost soil restrict the movement of microorganisms (Vargas Medrano, 2019;
537 Vigneron et al., 2019). Furthermore, geographical barriers, exemplified by prominent
538 mountain ranges like the Tanggula Mountains, Kunlun Mountains, Nyenchen Tanglha
539 Mountains, and Bayan Har Mountains, serve as impediments to the dispersal of both
540 macro- and microorganisms (Wan et al., 2016; Yu et al., 2019; Ren et al., 2022c).
541 Particularly in lake sediment, where bacterial communities are more isolated over distances
542 and will not disperse as far as those in lake water and permafrost soil, resulting in stronger
543 influence of dispersal limitation (Martiny et al., 2006; Xiong et al., 2012). Although the
544 “everything is everywhere” hypothesis suggests that many microorganisms have a
545 cosmopolitan distribution, their slow mobility allows for the development of regional
546 phylogenetic differences and the emergence of specialized, endemic taxa in isolated
547 habitats, resulting in a low likelihood of microorganisms dispersing to suitable distant sites

548 (Telford et al., 2006). Therefore, dispersal processes in this thermokarst landscape may be
549 restricted by the lack of hydrological connection, limited movement of water, short
550 duration since thawing, and strong environmental filtering, contributing to the observed
551 high dispersal limitation in the studied permafrost soil and thermokarst lakes. This
552 inference is supported by many previous studies showing that dispersal limitation plays a
553 major role in structuring microbial communities in lakes (Telford et al., 2006). Strong
554 dispersal limitation for bacterial communities in permafrost has also been documented
555 across an Alaskan boreal forest landscape (Bottos et al., 2018). ~~In addition, bacterial~~
556 ~~communities in lake water displayed a higher influence of homogeneous selection~~
557 ~~compared to those in lake sediments and permafrost soil in our study. The reason for this~~
558 ~~might be that long term changes in thermokarst lakes result in homogenized habitats and~~
559 ~~consequently strong homogenous selection on bacterial communities. Deterministic~~
560 ~~processes could also cause the communities to be more dissimilar through heterogeneous~~
561 ~~selection, which also imposed strong control on PBCs.~~

562 ***4.3 Environmental influences***

563 Understanding how environmental factors shape bacterial communities is a crucial aspect
564 in the field of microbial ecology (Fierer and Jackson, 2006; Pla-Rabes et al., 2011). ~~With~~
565 ~~global warming, climatic and physicochemical environments will be strongly altered in~~
566 ~~permafrost areas. On the QTP in particular, air temperature and precipitation are increasing~~
567 ~~in most regions (Xu et al., 2008; Lu et al., 2018). Moreover, organic carbon and nutrient~~
568 ~~stocks in permafrost are decreasing (Turetsky et al., 2020; Wu et al., 2021) and thermokarst~~
569 ~~lakes are developing, driving dynamic environmental change (Luo et al., 2015; Vucic et~~
570 ~~al., 2020). These environmental disruptions likely impose strong influences on bacterial~~

571 ~~communities in thermokarst landscapes.~~ In our study, bacterial communities were
572 differentially correlated with various measured environmental variables. In our data, pH
573 was consistently identified as a strong correlate of microbial community structure and
574 diversity, as is often observed in terrestrial and aquatic ecosystems worldwide (Fierer and
575 Jackson, 2006; Xiong et al., 2012). While such correlations between pH and bacterial
576 communities have been widely found, the regulation mechanisms still remain unknown
577 (Malard and Pearce, 2018). Moreover, the influences of pH are often species- and location-
578 specific (Malard and Pearce, 2018; Egelberg et al., 2021). In this study, pH had
579 significantly negative relationships with alpha diversity of bacterial communities in lake
580 sediment and water, and had negative or positive correlations with some phyla. Moreover,
581 differences in pH might drive community variation observed between PBCs, SBCs, and
582 WBCs, and shift community assembly processes for PBCs and SBCs. Moreover, with
583 permafrost degrading and thermokarst developing, nutrient status will be strongly altered
584 in permafrost areas. On the QTP in particular, air temperature and precipitation are
585 increasing in most regions (Xu et al., 2008; Lu et al., 2018) Organic carbon and nutrient
586 stocks in permafrost are decreasing (Turetsky et al., 2020; Wu et al., 2021) and thermokarst
587 lakes are developing, leading to dynamic environmental change (Luo et al., 2015; Vucic et
588 al., 2020). These environmental disruptions likely impose strong influences on bacterial
589 communities in thermokarst landscapes. Our study ~~also~~ showed that nutrient (C, N, and P)
590 concentrations and stoichiometric ratios were strongly related to alpha diversity
591 (particularly for SBCs) and community variation and assembly (particularly for PBCs).
592 High organic matter content, for instance, has been shown to support diverse and complex
593 microbial communities (Garrido-Benavent et al., 2020; Ren and Gao, 2022). The role of

594 nutrient availability in shaping bacterial communities has also been well established
595 ([Torsvik et al., 2002](#); [Lee et al., 2017](#); [Zhou et al., 2020](#)). ~~High organic matter content, for~~
596 ~~instance, has been shown to support diverse and complex microbial communities~~ (~~[Garrido-](#)~~
597 ~~[Benavent et al., 2020](#); [Ren and Gao, 2022](#)~~). For example, Actinobacteria and
598 Gemmatimonadota have a negative, while Gemmatimonadota has a positive relationship
599 with organic carbon and nutrients in permafrost ([Romanowicz and Kling, 2022](#); [Fu et al.,](#)
600 [2023](#)), in line with our results. The fact that different bacterial phyla exhibited varied
601 responses to changes in organic carbon and nutrient further emphasizes the intricate
602 interplay between microorganisms and their environment. Due to their ecological strategies,
603 metabolic features, and environmental preferences, bacteria in permafrost respond
604 differentially to nutrient status and other stressors (~~[Carbonero et al., 2014](#)~~), driving adaptive
605 changes in community composition and function ([Mackelprang et al., 2017](#)). In addition,
606 compared to permafrost soil and lake water, lake sediment can exhibit more stable
607 physicochemical conditions. However, permafrost soil and lake water experience more
608 dynamic and extreme environmental changes, which drive the bacterial communities. The
609 results of SEM also in line with bacterial community assembly that deterministic processes
610 had stronger influences on PB and WB than on SB. In addition, ~~T~~ thermokarst lakes have
611 sediment directly formed from permafrost soil, and thus, permafrost soil environments and
612 bacterial communities had strong associations with that of lake sediment.

613 In addition to physicochemical environments, location and climate were also suggested to
614 influence bacterial communities in distinct habitats. On the QTP in particular, air
615 temperature and precipitation are increasing in most regions ([Xu et al., 2008](#); [Lu et al.,](#)
616 [2018](#)). Warming and altered precipitation regimes under climate change have been

617 demonstrated to affect alpha diversity and composition of stream microbial communities
618 at continental scales (Picazo et al., 2020). Our study indicates that location (particularly
619 latitude) and climate (particularly MAP) factors are important in shifting bacterial
620 communities in thermokarst landscapes. Particularly for bacterial communities in
621 permafrost soil, location and climate have been evidenced as strong factors in shaping
622 microbial communities (Taş et al., 2018; Barbato et al., 2022). Understanding large-scale
623 pattern of bacterial communities is increasingly important to offer insights into the impacts
624 of climate change (Picazo et al., 2020; Ren et al., 2021). As global climate changes, QTP
625 is getting warmer and more humid (Xu et al., 2008; Lu et al., 2018). Therefore, significant
626 alterations to the physical, chemical, and biological properties of thermokarst lakes on the
627 QTP can be expected in the coming decades. Based on “space-for-time” substitution, our
628 study serves as a foundation for predicting the potential impact of climate change on
629 bacterial communities in thermokarst landscapes.

630 **5 Conclusion**

631 In this study, we investigated bacterial communities in paired water and sediment samples
632 in thermokarst lakes as well as permafrost soil around the lakes across the QTP. results
633 showed that each habitat had distinct bacterial assemblages, with lower alpha diversity in
634 lake water and higher beta diversity in lake sediment and permafrost soil. There was
635 considerable overlap in OTUs across habitats. Bacterial communities in permafrost soil
636 and lake sediment were influenced by dispersal limitation, while those in lake water were
637 driven by homogeneous selection. Environmental variables, including latitude, mean
638 annual precipitation, and pH, affected bacterial community variations in all habitats. The

639 study highlights the unique bacterial communities and ecological impacts of permafrost
640 degradation in diverse habitats created by thermokarst processes.

641 **Acknowledgements**

642 We are grateful to Yongming Deng and Xuan Jia for their assistance in the field and
643 laboratory work.

644 **Data availability statement:**

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

647 **Funding statement:**

648 This study was supported by [the National Natural Science Foundation of China \(42301132\)](#)
649 [and](#) the open funding of the State Key Laboratory of Lake Science and Environment
650 (2022SKL010).

651 **Conflict of interest disclosure:**

652 The authors declare no competing interests.

653 **Author's contributions:**

654 Z.R. designed the study, did the analyses, and prepared the manuscript, performed the field
655 work and laboratory work. All the authors prepared the manuscript.

656 **Ethics approval statement:**

657 Not applicable

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

659 Not applicable

660 **Originality-Significance Statement:**

661 This is our original study and not submitted to elsewhere

662 **Supplementary Information**

663 Figure S1 Map of the 44 sampling sites of permafrost soil and thermokarst lakes across the
664 Qinghai-Tibet Plateau. The distribution of the permafrost was cited from Zou et al., 2017.

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

666 Figure S2 (a) Venn diagram showing the unique and shared OTUs among distinct bacterial
667 communities in permafrost soil (PB), lake sediment (SB), and lake water (WB). (b) The
668 volcano plot showing the shared OTUs that significantly (t-test, $P < 0.05$) enriched in a
669 certain habitat. The volcano plot was constructed using \log_2 (fold change) on x-axis and –
670 \log_{10} (p-values of t-test) on y-axis.

671 Figure S3 Distance-decay curves showing community similarity of bacterial communities
672 in permafrost soil (PB), lake sediment (SB), and lake water (WB) against geographic
673 distances between sampling sites. Solid lines denote the ordinary least-squares linear
674 regressions.

675

676 **References**

677 Aguilar, P. and Sommaruga, R.: The balance between deterministic and stochastic
678 processes in structuring lake bacterioplankton community over time, *Mol. Ecol.*, 29,
679 3117-3130, doi:10.1111/mec.15538, 2020.
680 Altshuler, I., Goordial, J. and Whyte, L. G.: Microbial life in permafrost. In *Psychrophiles:*
681 *from biodiversity to biotechnology* (pp. 153-179). Springer, Cham, 2017.
682 Anthony, K. M., von Deimling, T. S., Nitze, I., Frolking, S., Emond, A., Daanen, R.,
683 Anthony, P., Lindgren, P., Jones, B. and Grosse, G.: 21st-century modeled permafrost
684 carbon emissions accelerated by abrupt thaw beneath lakes, *Nat. Commun.*, 9, 3262,

685 doi:10.1038/s41467-018-05738-9, 2018.

686 Aszalós, J. M., Szabó, A., Megyes, M., Anda, D., Nagy, B. and Borsodi, A. K.: Bacterial
687 Diversity of a High-Altitude Permafrost Thaw Pond Located on Ojos del Salado (Dry
688 Andes, Altiplano-Atacama Region), *Astrobiology*, 20, 754-765,
689 doi:10.1089/ast.2018.2012, 2020.

690 Baas-Becking, L. G. M.: *Geobiologie of Inleiding Tot de Milieukunde*, Van Stockkum &
691 Zoon, The Hague, 1934.

692 Barbato, R. A., Jones, R. M., Douglas, T. A., Doherty, S. J., Messan, K., Foley, K. L.,
693 Perkins, E. J., Thurston, A. K. and Garcia-Reyero, N.: Not all permafrost microbiomes
694 are created equal: Influence of permafrost thaw on the soil microbiome in a laboratory
695 incubation study, *Soil Biology and Biochemistry*, 167, 108605,
696 doi:https://doi.org/10.1016/j.soilbio.2022.108605, 2022.

697 Barrett, P.: Structural equation modelling: Adjudging model fit, *Pers. Individ. Differ.*, 42,
698 815-824, doi:10.1016/j.paid.2006.09.018, 2007.

699 Beermann, F., Langer, M., Wetterich, S., Strauss, J., Boike, J., Fiencke, C., Schirrmeister,
700 L., Pfeiffer, E. M. and Kutzbach, L.: Permafrost thaw and liberation of inorganic
701 nitrogen in Eastern Siberia, *Permafrost and Periglacial Processes*, 28, 605-618, 2017.

702 Belov, A. A., Cheptsov, V. S., Manucharova, N. A. and Ezhelev, Z. S.: Bacterial
703 Communities of Novaya Zemlya Archipelago Ice and Permafrost, *Geosciences*, 10, 67,
704 doi:10.3390/geosciences10020067, 2020.

705 Benjamini, Y. and Hochberg, Y.: Controlling the false discovery rate: a practical and
706 powerful approach to multiple testing, *Journal of the Royal Statistical Society: Series*
707 *B (Methodological)*, 57, 289-300, doi:10.2307/2346101, 1995.

708 Bier, R. L., Bernhardt, E. S., Boot, C. M., Graham, E. B., Hall, E. K., Lennon, J. T.,
709 Nemergut, D. R., Osborne, B. B., Ruiz-Gonzalez, C., Schimel, J. P., Waldrop, M. P.
710 and Wallenstein, M. D.: Linking microbial community structure and microbial
711 processes: an empirical and conceptual overview, *FEMS Microbiol. Ecol.*, 91,
712 doi:10.1093/femsec/fiv113, 2015.

713 Biskaborn, B. K., Smith, S. L., Noetzli, J., Matthes, H., Vieira, G., Streletskiy, D. A.,
714 Schoeneich, P., Romanovsky, V. E., Lewkowicz, A. G., Abramov, A., Allard, M.,
715 Boike, J., Cable, W. L., Christiansen, H. H., Delaloye, R., Diekmann, B., Drozdov, D.,
716 Etmuller, B., Grosse, G., Guglielmin, M., Ingeman-Nielsen, T., Isaksen, K.,
717 Ishikawa, M., Johansson, M., Johannsson, H., Joo, A., Kaverin, D., Kholodov, A.,
718 Konstantinov, P., Kroger, T., Lambiel, C., Lanckman, J. P., Luo, D., Malkova, G.,
719 Meiklejohn, I., Moskalenko, N., Oliva, M., Phillips, M., Ramos, M., Sannel, A.,
720 Sergeev, D., Seybold, C., Skryabin, P., Vasiliev, A., Wu, Q., Yoshikawa, K.,
721 Zheleznyak, M. and Lantuit, H.: Permafrost is warming at a global scale, *Nat. Commun.*,
722 10, 264, doi:10.1038/s41467-018-08240-4, 2019.

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

727 Brie, C., Moreira, D. and Lopez-Garcia, P.: Archaeal and bacterial community
728 composition of sediment and plankton from a suboxic freshwater pond, *Res. Microbiol.*,
729 158, 213-227, doi:10.1016/j.resmic.2006.12.012, 2007.

730 Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E.

731 K., Fierer, N., Pena, A. G., Goodrich, J. K., Gordon, J. I., Huttley, G. A., Kelley, S. T.,
732 Knights, D., Koenig, J. E., Ley, R. E., Lozupone, C. A., Mcdonald, D., Muegge, B. D.,
733 Pirrung, M., Reeder, J., Sevinsky, J. R., Tumbaugh, P. J., Walters, W. A., Widmann, J.,
734 Yatsunenkov, T., Zaneveld, J. and Knight, R.: QIIME allows analysis of high-throughput
735 community sequencing data, *Nat. Methods*, 7, 335-336, doi:10.1038/nmeth.f.303, 2010.
736 Chadburn, S. E., Burke, E. J., Cox, P. M., Friedlingstein, P. and Hugelius, G.: An
737 observation-based constraint on permafrost loss as a function of global warming, *Nat.*
738 *Clim. Chang.*, 7, 340-344, doi:10.1038/NCLIMATE3262, 2017.
739 Chase, J. M.: Stochastic community assembly causes higher biodiversity in more
740 productive environments, *Science*, 328, 1388-1391, doi:10.1126/science.1187820,
741 2010.
742 Chave, J.: Neutral theory and community ecology, *Ecol. Lett.*, 7, 241-253, 2004.
743 Chin, K. S., Lento, J., Culp, J. M., Lacelle, D. and Kokelj, S. V.: Permafrost thaw and
744 intense thermokarst activity decreases abundance of stream benthic macroinvertebrates,
745 *Glob. Change Biol.*, 22, 2715-2728, doi:10.1111/gcb.13225, 2016.
746 Cline, L. C. and Zak, D. R.: Dispersal limitation structures fungal community assembly in
747 a long-term glacial chronosequence, *Environ. Microbiol.*, 16, 1538-1548,
748 doi:10.1111/1462-2920.12281, 2014.
749 Custer, G. F., Bresciani, L. and Dini-Andreote, F.: Ecological and Evolutionary
750 Implications of Microbial Dispersal, *Front. Microbiol.*, 13, 855859,
751 doi:10.3389/fmicb.2022.855859, 2022.
752 de Jong, A., In, T. Z. M., Meisel, O. H., Jetten, M., Dean, J. F., Rasigraf, O. and Welte, C.
753 U.: Increases in temperature and nutrient availability positively affect methane-cycling
754 microorganisms in Arctic thermokarst lake sediments, *Environ. Microbiol.*, 20, 4314-
755 4327, doi:10.1111/1462-2920.14345, 2018.
756 Egelberg, J., Pena, N., Rivera, R. and Andruk, C.: Assessing the geographic specificity of
757 pH prediction by classification and regression trees, *PLoS One*, 16, e0255119,
758 doi:10.1371/journal.pone.0255119, 2021.
759 Elder, C. D., Thompson, D. R., Thorpe, A. K., Chandanpurkar, H. A., Hanke, P. J., Hasson,
760 N., James, S. R., Minsley, B. J., Pastick, N. J., Olefeldt, D., Walter Anthony, K. M. and
761 Miller, C. E.: Characterizing Methane Emission Hotspots From Thawing Permafrost,
762 *Glob. Biogeochem. Cycle*, 35, e2020GB006922, doi:10.1029/2020GB006922, 2021.
763 Etzelmüller, B.: Recent advances in mountain permafrost research, *Permafrost and*
764 *Periglacial Processes*, 24, 99-107, 2013.
765 Farquharson, L. M., Mann, D. H., Grosse, G., Jones, B. M. and Romanovsky, V. E.: Spatial
766 distribution of thermokarst terrain in Arctic Alaska, *Geomorphology*, 273, 116-133,
767 doi:10.1016/j.geomorph.2016.08.007, 2016.
768 Fierer, N. and Jackson, R. B.: The diversity and biogeography of soil bacterial communities,
769 *Proceedings of the National Academy of Sciences*, 103, 626-631,
770 doi:10.1073/pnas.0507535103, 2006.
771 Fierer, N., Leff, J. W., Adams, B. J., Nielsen, U. N., Bates, S. T., Lauber, C. L., Owens, S.,
772 Gilbert, J. A., Wall, D. H. and Caporaso, J. G.: Cross-biome metagenomic analyses of
773 soil microbial communities and their functional attributes, *Proceedings of the National*
774 *Academy of Sciences*, 109, 21390-21395, doi:10.1073/pnas.1215210110, 2012.
775 Fu, L., Xie, R., Ma, D., Zhang, M. and Liu, L.: Variations in soil microbial community
776 structure and extracellular enzymatic activities along a forest - wetland ecotone in

777 high - latitude permafrost regions, *Ecol. Evol.*, 13, e10205-n/a,
778 doi:10.1002/ece3.10205, 2023.

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

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

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

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

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

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

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

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

807 In'T Zandt, M. H., Liebner, S. and Welte, C. U.: Roles of thermokarst lakes in a warming
808 world, *Trends Microbiol.*, 28, 769-779, doi:10.1016/j.tim.2020.04.002, 2020.

809 Jiao, S. and Lu, Y.: Abundant fungi adapt to broader environmental gradients than rare
810 fungi in agricultural fields, *Glob. Change Biol.*, 26, 4506-4520, doi:10.1111/gcb.15130,
811 2020.

812 Jorgenson, M. T., Romanovsky, V., Harden, J., Shur, Y., O'Donnell, J., Schuur, E. A. G.,
813 Kanevskiy, M. and Marchenko, S.: Resilience and vulnerability of permafrost to climate
814 change, *Can. J. For. Res.*, 40, 1219, 2010.

815 Kembel, S. W., Cowan, P. D., Helmus, M. R., Cornwell, W. K., Morlon, H., Ackerly, D.
816 D., Blomberg, S. P. and Webb, C. O.: Picante: R tools for integrating phylogenies and
817 ecology, *Bioinformatics*, 26, 1463-1464, doi:10.1093/bioinformatics/btq166, 2010.

818 Kokelj, S. V. and Jorgenson, M. T.: Advances in thermokarst research, *Permafrost and*
819 *Periglacial Processes*, 24, 108-119, doi:10.1002/ppp.1779, 2013.

820 Langenheder, S., Wang, J., Karjalainen, S. M., Laamanen, T. M., Tolonen, K. T., Vilmi,
821 A. and Heino, J.: Bacterial metacommunity organization in a highly connected aquatic
822 system, *FEMS Microbiol. Ecol.*, 93, fiw225, doi:10.1093/femsec/fiw225, 2017.

823 Lawrence, D. M., Slater, A. G. and Swenson, S. C.: Simulation of Present-Day and Future
824 Permafrost and Seasonally Frozen Ground Conditions in CCSM4, *J. Clim.*, 25, 2207-
825 2225, doi:10.1175/JCLI-D-11-00334.1, 2012.

826 Le Moigne, A., Bartosiewicz, M., Schaepman Strub, G., Abiven, S. and Pernthaler, J.: The
827 biogeochemical variability of Arctic thermokarst ponds is reflected by stochastic and
828 niche - driven microbial community assembly processes, *Environ. Microbiol.*, 22,
829 4847-4862, doi:10.1111/1462-2920.15260, 2020.

830 Lee, Z. M. P., Poret-Peterson, A. T., Siefert, J. L., Kaul, D., Moustafa, A., Allen, A. E.,
831 Dupont, C. L., Eguiarte, L. E., Souza, V. and Elser, J. J.: Nutrient stoichiometry shapes
832 microbial community structure in an evaporitic shallow pond, *Front. Microbiol.*, 8, 949,
833 doi:10.3389/fmicb.2017.00949, 2017.

834 Leibold, M. A., Chase, J. M. and Ernest, S. K.: Community assembly and the functioning
835 of ecosystems: how metacommunity processes alter ecosystems attributes, *Ecology*, 98,
836 909-919, doi:10.1002/ecy.1697, 2017.

837 Levins, R.: Evolution in changing environments: some theoretical explorations (No. 2).
838 Princeton University Press, Princeton, USA, 1968.

839 Lindstrom, E. S. and Langenheder, S.: Local and regional factors influencing bacterial
840 community assembly, *Environ. Microbiol. Rep.*, 4, 1-9, doi:10.1111/j.1758-
841 2229.2011.00257.x, 2012.

842 Liu, K., Yao, T., Pearce, D. A., Jiao, N., Zeng, Y., Guo, B. and Liu, Y.: Bacteria in the
843 lakes of the Tibetan Plateau and polar regions, *Sci. Total Environ.*, 754, 142248,
844 doi:10.1016/j.scitotenv.2020.142248, 2021.

845 Louca, S., Parfrey, L. W. and Doebeli, M.: Decoupling function and taxonomy in the global
846 ocean microbiome, *Science*, 353, 1272-1277, doi:10.1126/science.aaf4507, 2016.

847 Lozupone, C. A. and Knight, R.: Global patterns in bacterial diversity, *Proceedings of the*
848 *National Academy of Sciences*, 104, 11436-11440, doi:10.1073/pnas.0611525104,
849 2007.

850 Lu, W., Wang, W., Shao, Q., Yu, Z., Hao, Z., Xing, W., Yong, B. and Li, J.: Hydrological
851 projections of future climate change over the source region of Yellow River and
852 Yangtze River in the Tibetan Plateau; a comprehensive assessment by coupling
853 RegCM4 and VIC model, *Hydrol. Process.*, 32, 2096-2117, doi:10.1002/hyp.13145,
854 2018.

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

858 Luo, D., Jin, H., Du, H., Li, C., Ma, Q., Duan, S. and Li, G.: Variation of alpine lakes from
859 1986 to 2019 in the Headwater Area of the Yellow River, Tibetan Plateau using Google
860 Earth Engine, *Adv. Clim. Chang. Res.*, 11, 11-21, doi:10.1016/j.accre.2020.05.007,
861 2020.

862 Luo, J., Niu, F., Lin, Z., Liu, M. and Yin, G.: Thermokarst lake changes between 1969 and
863 2010 in the Beilu River Basin, Qinghai-Tibet Plateau, China, *Sci. Bull.*, 60, 556-564,
864 doi:10.1007/s11434-015-0730-2, 2015.

865 Mackelprang, R., Burkert, A., Haw, M., Mahendrarajah, T., Conaway, C. H., Douglas, T.
866 A. and Waldrop, M. P.: Microbial survival strategies in ancient permafrost: insights
867 from metagenomics, *The ISME Journal*, 11, 2305-2318, doi:10.1038/ismej.2017.93,
868 2017.

869 Makoto, K., Wilson, S. D., Turner, B. and Turner, B.: When and where does dispersal
870 limitation matter in primary succession? *J. Ecol.*, 107, 559-565, doi:10.1111/1365-
871 2745.12988, 2019.

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

874 Manasyopov, R. M., Pokrovsky, O. S., Shirokova, L. S., Auda, Y., Zinner, N. S., Vorobyev,
875 S. N. and Kirpotin, S. N.: Biogeochemistry of macrophytes, sediments and porewaters
876 in thermokarst lakes of permafrost peatlands, western Siberia, *Sci. Total Environ.*, 763,
877 144201, doi:10.1016/j.scitotenv.2020.144201, 2021.

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

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

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

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

895 Narancic, B., Wolfe, B. B., Pienitz, R., Meyer, H. and Lamhonwah, D.: Landscape-gradient
896 assessment of thermokarst lake hydrology using water isotope tracers, *J. Hydrol.*, 545,
897 327-338, 2017.

898 Nemergut, D. R., Schmidt, S. K., Fukami, T., O'Neill, S. P., Bilinski, T. M., Stanish, L. F.,
899 Knelman, J. E., Darcy, J. L., Lynch, R. C., Wickey, P. and Ferrenberg, S.: Patterns and
900 Processes of Microbial Community Assembly, *Microbiol. Mol. Biol. Rev.*, 77, 342-356,
901 doi:10.1128/MMBR.00051-12, 2013.

902 Ning, D., Deng, Y., Tiedje, J. M. and Zhou, J.: A general framework for quantitatively
903 assessing ecological stochasticity, *Proceedings of the National Academy of Sciences*,
904 116, 16892-16898, doi:10.1073/pnas.1904623116, 2019.

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

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

912 Nossa, C. W., Oberdorf, W. E., Yang, L., Aas, J. A., Paster, B. J., Desantis, T. Z., Brodie,
913 E. L., Malamud, D., Poles, M. A. and Pei, Z.: Design of 16S rRNA gene primers for
914 454 pyrosequencing of the human foregut microbiome, *World J. Gastroenterol.*, 16,

915 4135-4144, doi:10.3748/wjg.v16.i33.4135, 2010.

916 Obu, J.: How much of the Earth's surface is underlain by permafrost? *Journal of*
917 *Geophysical Research: Earth Surface*, 126, 2021JF006123, doi:10.1029/2021JF006123,
918 2021.

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

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

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

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

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

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

940 Picazo, F., Vilmi, A., Aalto, J., Soininen, J., Casamayor, E. O., Liu, Y., Wu, Q., Ren, L.,
941 Zhou, J., Shen, J. and Wang, J.: Climate mediates continental scale patterns of stream
942 microbial functional diversity, *Microbiome*, 8, e92, doi:10.1186/s40168-020-00873-2,
943 2020.

944 Pla-Rabes, S., Flower, R. J., Shilland, E. M. and Kreiser, A. M.: Assessing microbial
945 diversity using recent lake sediments and estimations of spatio-temporal diversity, *J.*
946 *Biogeogr.*, 38, 2033-2040, doi:10.1111/j.1365-2699.2011.02530.x, 2011.

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

951 R Core Team: *R: A language and environment for statistical computing*, R Foundation for
952 Statistical Computing, Vienna, Austria. <https://www.R-project.org>,2020.

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

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

960 Ren, Z., Jia, X., Zhang, Y. T., Ma, K., Zhang, C. and Li, X.: Biogeography and

961 environmental drivers of zooplankton communities in permafrost-affected lakes on the
962 Qinghai-Tibet Plateau, *Glob. Ecol. Conserv.*, 38, e02191,
963 doi:10.1016/j.gecco.2022.e02191, 2022c.

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

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

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

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

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

980 Ren, Z., Wang, Z. M., Wang, Y., Ma, P. P., Niu, D. C., Fu, H. and Elser, J. J.: Soil bacterial
981 communities vary with grassland degradation in the Qinghai Lake watershed, *Plant Soil*,
982 460, 541-557, doi:10.1007/s11104-020-04823-7, 2021.

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

986 Rosseel, Y.: lavaan: An R Package for Structural Equation Modeling, *J. Stat. Softw.*, 48,
987 1-36, doi:10.18637/jss.v048.i02, 2012.

988 Ruiz-Gonzalez, C., Pablo Nino-Garcia, J. and Del Giorgio, P. A.: Terrestrial origin of
989 bacterial communities in complex boreal freshwater networks, *Ecol. Lett.*, 18, 1198-
990 1206, doi:10.1111/ele.12499, 2015.

991 Schaefer, K., Zhang, T., Bruhwiler, L. and Barrett, A. P.: Amount and timing of permafrost
992 carbon release in response to climate warming, *Tellus B: Chemical and Physical*
993 *Meteorology*, 63, 168-180, 2011.

994 Schuur, E. A. G., Bockheim, J., Canadell, J. G., Euskirchen, E., Field, C. B., Goryachkin,
995 S. V., Hagemann, S., Kuhry, P., Lafleur, P. M., Lee, H., Mazhitova, G., Nelson, F. E.,
996 Rinke, A., Romanovsky, V. E., Shiklomanov, N., Tarnocai, C., Venevsky, S., Vogel, J.
997 G. and Zimov, S. A.: Vulnerability of permafrost carbon to climate change:
998 Implications for the global carbon cycle, *Bioscience*, 58, 701-714,
999 doi:10.1641/B580807, 2008.

1000 Schuur, E. A., Vogel, J. G., Crummer, K. G., Lee, H., Sickman, J. O. and Osterkamp, T.
1001 E.: The effect of permafrost thaw on old carbon release and net carbon exchange from
1002 tundra, *Nature*, 459, 556-569, doi:10.1038/nature08031, 2009.

1003 She, Z., Pan, X., Wang, J., Shao, R., Wang, G., Wang, S. and Yue, Z.: Vertical
1004 environmental gradient drives prokaryotic microbial community assembly and species
1005 coexistence in a stratified acid mine drainage lake, *Water Res.*, 206, 117739,
1006 doi:10.1016/j.watres.2021.117739, 2021.

1007 Stegen, J. C., Lin, X., Fredrickson, J. K. and Konopka, A. E.: Estimating and mapping
1008 ecological processes influencing microbial community assembly, *Front. Microbiol.*, 6,
1009 e370, doi:10.3389/fmicb.2015.00370, 2015.

1010 Stegen, J. C., Lin, X., Fredrickson, J. K., Chen, X., Kennedy, D. W., Murray, C. J.,
1011 Rockhold, M. L. and Konopka, A.: Quantifying community assembly processes and
1012 identifying features that impose them, *The ISME Journal*, 7, 2069-2079,
1013 doi:10.1038/ismej.2013.93, 2013.

1014 Stegen, J. C., Lin, X., Konopka, A. E. and Fredrickson, J. K.: Stochastic and deterministic
1015 assembly processes in subsurface microbial communities, *ISME J.*, 6, 1653-1664,
1016 doi:10.1038/ismej.2012.22, 2012.

1017 Steven, B., Niederberger, T. D. and Whyte, L. G.: Bacterial and archaeal diversity in
1018 permafrost. In *Permafrost soils* (pp. 59-72). Springer, Berlin, Heidelberg, 2009.

1019 Strickland, M. S., Lauber, C., Fierer, N. and Bradford, M. A.: Testing the Functional
1020 Significance of Microbial Community Composition, *Ecology*, 90, 441-451,
1021 doi:10.1890/08-0296.1, 2009.

1022 Taş, N., Prestat, E., Wang, S., Wu, Y., Ulrich, C., Kneafsey, T., Tringe, S. G., Torn, M. S.,
1023 Hubbard, S. S., Jansson, J. K., Pacific Northwest National Laboratory Pnnl, R. W. U.
1024 S. and Lawrence Berkeley National Laboratory Lbnl, B. C. U. S.: Landscape
1025 topography structures the soil microbiome in arctic polygonal tundra, *Nat. Commun.*,
1026 9, 777-13, doi:10.1038/s41467-018-03089-z, 2018.

1027 Telford, R. J., Vandvik, V. and Birks, H. J. B.: Dispersal Limitations Matter for Microbial
1028 Morphospecies, *Science*, 312, 1015-1015, doi:10.1126/science.1125669, 2006.

1029 Torsvik, V., Ovreas, L. and Thingstad, T. F.: Prokaryotic diversity - Magnitude, dynamics,
1030 and controlling factors, *Science*, 296, 1064-1066, doi:10.1126/science.1071698, 2002.

1031 Tripathi, B. M., Stegen, J. C., Kim, M., Dong, K., Adams, J. M. and Lee, Y. K.: Soil pH
1032 mediates the balance between stochastic and deterministic assembly of bacteria, *The*
1033 *ISME Journal*, 12, 1072-1083, doi:10.1038/s41396-018-0082-4, 2018.

1034 Turetsky, M. R., Abbott, B. W., Jones, M. C., Anthony, K. W., Olefeldt, D., Schuur, E. A.
1035 G., Grosse, G., Kuhry, P., Hugelius, G., Koven, C., Lawrence, D. M., Gibson, C.,
1036 Sannel, A. B. K. and Mcguire, A. D.: Carbon release through abrupt permafrost thaw,
1037 *Nat. Geosci.*, 13, 138-143, doi:10.1038/s41561-019-0526-0, 2020.

1038 Vargas Medrano, M.: Recent Changes in the Zooplankton Communities of Arctic Tundra
1039 Ponds in Response to Warmer Temperatures and Nutrient Enrichment, *Dissertation:*
1040 *University of Texas at El Paso*, 2019.

1041 Vellend, M., Srivastava, D. S., Anderson, K. M., Brown, C. D., Jankowski, J. E., Kleynhans,
1042 E. J., Kraft, N. J. B., Letaw, A. D., Macdonald, A. A. M., Maclean, J. E., Myers-Smith,
1043 I. H., Norris, A. R. and Xue, X.: Assessing the relative importance of neutral
1044 stochasticity in ecological communities, *Oikos*, 123, 1420-1430,
1045 doi:10.1111/oik.01493, 2014.

1046 Veremeeva, A., Nitze, I., Günther, F., Grosse, G. and Rivkina, E.: Geomorphological and
1047 climatic drivers of thermokarst lake area increase trend (1999-2018) in the Kolyma
1048 Lowland Yedoma Region, North-Eastern Siberia, *Remote Sens.*, 13, 178,
1049 doi:10.3390/rs13020178, 2021.

1050 Vigneron, A., Lovejoy, C., Cruaud, P., Kalenitchenko, D., Culley, A. and Vincent, W. F.:
1051 Contrasting Winter Versus Summer Microbial Communities and Metabolic Functions
1052 in a Permafrost Thaw Lake, *Front. Microbiol.*, 10, 1656, doi:10.3389/fmicb.2019.01656,

1053 2019.

1054 Vucic, J. M., Gray, D. K., Cohen, R. S., Syed, M., Murdoch, A. D. and Sharma, S.: Changes
1055 in water quality related to permafrost thaw may significantly impact zooplankton in
1056 small Arctic lakes, *Ecol. Appl.*, 30, e02186, doi:10.1002/eap.2186, 2020.

1057 Walter, K. M., Zimov, S. A., Chanton, J. P., Verbyla, D. and Chapin, F. S.: Methane
1058 bubbling from Siberian thaw lakes as a positive feedback to climate warming, *Nature*,
1059 443, 71-75, doi:10.1038/nature05040, 2006.

1060 Wan, C., Gibson, J. J., Shen, S., Yi, Y., Yi, P. and Yu, Z.: Using stable isotopes paired with
1061 tritium analysis to assess thermokarst lake water balances in the Source Area of the
1062 Yellow River, northeastern Qinghai-Tibet Plateau, China, *Sci. Total Environ.*, 689,
1063 1276-1292, doi:10.1016/j.scitotenv.2019.06.427, 2019.

1064 Wan, D. S., Feng, J. J., Jiang, D. C., Mao, K. S., Duan, Y. W., Mieke, G. and Opgenoorth,
1065 L.: The Quaternary evolutionary history, potential distribution dynamics, and
1066 conservation implications for a Qinghai-Tibet Plateau endemic herbaceous perennial,
1067 *Anisodus tanguticus* (Solanaceae), *Ecol. Evol.*, 6, 1977-95, doi:10.1002/ece3.2019,
1068 2016.

1069 Webb, C. O., Ackerly, D. D., Mcpeek, M. A. and Donoghue, M. J.: Phylogenies and
1070 community ecology, *Annual review of ecology and systematics*, 33, 475-505, 2002.

1071 West, J. J. and Plug, L. J.: Time-dependent morphology of thaw lakes and taliks in deep
1072 and shallow ground ice, *Journal of Geophysical Research*, 113, 1009,
1073 doi:10.1029/2006JF000696, 2008.

1074 Wu, L., Yang, F., Feng, J., Tao, X., Qi, Q., Wang, C., Schuur, E. A. G., Bracho, R., Huang,
1075 Y., Cole, J. R., Tiedje, J. M. and Zhou, J.: Permafrost thaw with warming reduces
1076 microbial metabolic capacities in subsurface soils, *Mol. Ecol.*, 31, 1403-1415,
1077 doi:10.1111/mec.16319, 2022.

1078 Wu, M., Chen, S., Chen, J., Xue, K., Chen, S., Wang, X., Chen, T., Kang, S., Rui, J., Thies,
1079 J. E., Bardgett, R. D. and Wang, Y.: Reduced microbial stability in the active layer is
1080 associated with carbon loss under alpine permafrost degradation, *Proceedings of the*
1081 *National Academy of Sciences*, 118, e2025321118, doi:10.1073/pnas.2025321118,
1082 2021.

1083 Wu, Q., Dong, X., Liu, Y., Jin, H. and Zhang, T.: Responses of permafrost on the Qinghai-
1084 Tibet Plateau, China, to climate change and engineering construction, *Arctic, Antarctic,*
1085 *and Alpine Research*, 39, 682-687, doi:10.1657/1523-0430(07-508)[WU]2.0.CO;2,
1086 2007.

1087 Xiong, J., Liu, Y., Lin, X., Zhang, H., Zeng, J., Hou, J., Yang, Y., Yao, T., Knight, R. and
1088 Chu, H.: Geographic distance and pH drive bacterial distribution in alkaline lake
1089 sediments across Tibetan Plateau, *Environ. Microbiol.*, 14, 2457-2466,
1090 doi:10.1111/j.1462-2920.2012.02799.x, 2012.

1091 Xu, Z. X., Gong, T. L. and Li, J. Y.: Decadal trend of climate in the Tibetan Plateau-
1092 regional temperature and precipitation, *Hydrol. Process.*, 22, 3056-3065,
1093 doi:10.1002/hyp.6892, 2008.

1094 Yang, J., Jiang, H., Dong, H. and Liu, Y.: A comprehensive census of lake microbial
1095 diversity on a global scale, *Science China Life Sciences*, 62, 1320-1331,
1096 doi:10.1007/s11427-018-9525-9, 2019.

1097 Yang, J., Ma, L., Jiang, H., Wu, G. and Dong, H.: Salinity shapes microbial diversity and
1098 community structure in surface sediments of the Qinghai-Tibetan Lakes, *Sci. Rep.*, 6,

1099 e25078, doi:10.1038/srep25078, 2016b.

1100 Yang, Y., Wu, Q., Yun, H., Jin, H. and Zhang, Z.: Evaluation of the hydrological
1101 contributions of permafrost to the thermokarst lakes on the Qinghai-Tibet Plateau using
1102 stable isotopes, *Glob. Planet. Change*, 140, 1-8, doi:10.1016/j.gloplacha.2016.03.006,
1103 2016a.

1104 Yao, T., Thompson, L. G., Mosbrugger, V., Zhang, F., Ma, Y., Luo, T., Xu, B., Yang, X.,
1105 Joswiak, D. R., Wang, W., Joswiak, M. E., Devkota, L. P., Tayal, S., Jilani, R. and
1106 Fayziev, R.: Third Pole Environment (TPE), *Environ. Dev.*, 3, 52-64,
1107 doi:10.1016/j.envdev.2012.04.002, 2012.

1108 Yu, G., Smith, D. K., Zhu, H., Guan, Y. and Lam, T. T. Y.: ggtree: an R package for
1109 visualization and annotation of phylogenetic trees with their covariates and other
1110 associated data, *Methods Ecol. Evol.*, 8, 28-36, 2017.

1111 Yu, H., Favre, A., Sui, X., Chen, Z., Qi, W., Xie, G., Kleunen, M. and van Kleunen, M.:
1112 Mapping the genetic patterns of plants in the region of the Qinghai - Tibet Plateau:
1113 Implications for conservation strategies, *Diversity & distributions*, 25, 310-324,
1114 doi:10.1111/ddi.12847, 2019.

1115 Zhang, D., Wang, L., Qin, S., Kou, D., Wang, S., Zheng, Z., Peñuelas, J. and Yang, Y.:
1116 Microbial nitrogen and phosphorus co - limitation across permafrost region, *Glob.*
1117 *Change Biol.*, 29, 3910-3923, doi:10.1111/gcb.16743, 2023.

1118 Zhang, J. L.: Species association analysis, Version 0.2.2. [https://CRAN.R-](https://CRAN.R-project.org/package=spaa)
1119 [project.org/package=spaa](https://CRAN.R-project.org/package=spaa), 2016.

1120 Zhang, Z., Chang, J., Xu, C., Zhou, Y., Wu, Y., Chen, X., Jiang, S. and Duan, Z.: The
1121 response of lake area and vegetation cover variations to climate change over the
1122 Qinghai-Tibetan Plateau during the past 30 years, *The Science of the total environment*,
1123 635, 443-451, doi:10.1016/j.scitotenv.2018.04.113, 2018.

1124 Zhou, C. P.: The spatial dataset of climate on the Tibetan Plateau (1961-2020). National
1125 Tibetan Plateau Data Center, doi:10.11888/AtmosphericPhysics.tpe.49.file, 2018.

1126 Zhou, J. and Ning, D.: Stochastic Community Assembly: Does It Matter in Microbial
1127 Ecology? *Microbiol. Mol. Biol. Rev.*, 81, e00002-17, doi:10.1128/MMBR.00002-17,
1128 2017.

1129 Zhou, J., Deng, Y., Zhang, P., Xue, K., Liang, Y., Van Nostrand, J. D., Yang, Y., He, Z.,
1130 Wu, L., Stahl, D. A., Hazen, T. C., Tiedje, J. M. and Arkin, A. P.: Stochasticity,
1131 succession, and environmental perturbations in a fluidic ecosystem, *Proceedings of the*
1132 *National Academy of Sciences*, 111, E836-E845, doi:10.1073/pnas.1324044111, 2014.

1133 Zhou, J., Liu, W., Deng, Y., Jiang, Y., Xue, K., He, Z., Van Nostrand, J. D., Wu, L., Yang,
1134 Y., Wang, A., Lawrence Berkeley National Lab. Lbnl, B. C. U. S. and Handelsman, J.:
1135 Stochastic assembly leads to alternative communities with distinct functions in a
1136 bioreactor microbial community, *mBio*, 4, e00584-12, doi:10.1128/mBio.00584-12,
1137 2013.

1138 Zhou, L., Zhou, Y., Yao, X., Cai, J., Liu, X., Tang, X., Zhang, Y., Jang, K. and Jeppesen,
1139 E.: Decreasing diversity of rare bacterial subcommunities relates to dissolved organic
1140 matter along permafrost thawing gradients, *Environ. Int.*, 134, 105330,
1141 doi:10.1016/j.envint.2019.105330, 2020.

1142 Zou, D., Zhao, L., Sheng, Y., Chen, J., Hu, G., Wu, T., Wu, J., Xie, C., Wu, X., Pang, Q.,
1143 Wang, W., Du, E., Li, W., Liu, G., Li, J., Qin, Y., Qiao, Y., Wang, Z., Shi, J. and Cheng,
1144 G.: A new map of permafrost distribution on the Tibetan Plateau, *Cryosphere*, 11, 2527-

1145 2542, doi:10.5194/tc-11-2527-2017, 2017.
1146