

1 **Differentiation of cognate bacterial communities in thermokarst**
2 **landscapes: implications for ecological consequences of permafrost**
3 **degradation**

4 Running title: Bacterial communities in thermokarst landscape

5 **Ze Ren^{1,2*}, Shudan Ye³, Hongxuan Li³, Xilei Huang³, Luyao Chen³**

6 1 State Key Laboratory of Lake Science and Environment, Nanjing Institute of Geography and Limnology,
7 Chinese Academy of Sciences, Nanjing 210008, China

8 2 Advanced Institute of Natural Sciences, Beijing Normal University, Zhuhai, 519087, China

9 3 Faculty of Arts and Sciences, Beijing Normal University, Zhuhai 519087, China

10 ***Corresponding Authors:**

11 Ze Ren: renzedyk@gmail.com

12 **Emails:**

13 Shudan Ye: yeshudan@mail.bnu.edu.cn; Hongxuan Li: lihongxuan@mail.bnu.edu.cn; Xilei Huang:

14 202111079031@mail.bnu.edu.cn; Luyao Chen: 202011059371@mail.bnu.edu.cn

15

16 **Abstract**

17 Thermokarst processes likely result in new habitats harboring novel bacterial communities
18 in degraded permafrost soil (PBCs), thermokarst lake sediments (SBCs), and lake water
19 (WBCs). Our study aimed to investigate the paired PBCs, SBCs, and WBCs 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 WBCs than in SBCs
23 and PBCs. However, up to 41% of the OTUs were shared by PBCs, SBCs, and WBCs,
24 suggesting that many taxa originate from the same sources via dispersal. SBCs and WBCs
25 had reciprocal dispersal effects and both were correlated with PBCs. Dispersal limitation
26 was the most dominant assembly process shaping PBCs and SBCs while homogeneous
27 selection was the most dominant for WBCs. 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 1). It is well known that thermokarst processes substantially influence regional
52 hydrological, ecological, and biogeochemical processes (Chin et al., 2016; In'T Zandt et
53 al., 2020; Manasypov et al., 2021) and initiate a strong positive climate feedback to global
54 warming (Walter et al., 2006; Schuur et al., 2008; Schaefer et al., 2011; Anthony et al.,
55 2018). However, the microbial differences and relationships among these distinct habitats
56 in thermokarst landscapes are largely unknown.

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

62 the assembly of microbial communities, both deterministic and stochastic processes occur
63 simultaneously but with contributions that can vary (Chase, 2010; Zhou et al., 2013;
64 Vellend et al., 2014; Makoto et al., 2019). Typically, deterministic processes place a strong
65 emphasis on niche-based mechanisms, including ecological selection, environmental
66 filtering, and biotic interactions (Zhou and Ning, 2017). Conversely, stochastic processes
67 involve neutral mechanisms like random birth and death, unforeseen disturbance,
68 probability-based dispersal, and ecological drift (Chave, 2004; Chase, 2010; Zhou et al.,
69 2014). In various ecosystems or habitats, the significance of deterministic and stochastic
70 processes can differ greatly and be shaped by a multitude of environmental factors (Tripathi
71 et al., 2018; Aguilar and Sommaruga, 2020; Jiao and Lu, 2020; She et al., 2021). During
72 thermokarst formation, vast areas of permafrost have been transformed to thermokarst
73 lakes, leading to major changes in physicochemical environments as well as in biological
74 communities of these regions. Thus, it is also expected that the microbial communities
75 experience major changes in occupying degraded permafrost soil, thermokarst lake
76 sediments, and lake water, and in doing so, display different assembly mechanisms (Figure
77 1).

78 Better understanding community assembly in these systems is important because thawing
79 permafrost and thermokarst lakes are greenhouse gas emission hotspots (In'T Zandt et al.,
80 2020; Mu et al., 2020; Elder et al., 2021). Close relationships between biogeochemical
81 processes and microbial community assembly have been generally demonstrated (Bier et
82 al., 2015; Graham et al., 2016; Le Moigne et al., 2020; Ren et al., 2022a). Assembly
83 processes inevitably influence biogeochemical functions by shaping community diversity
84 and composition (Graham et al., 2016; Leibold et al., 2017; Mori et al., 2018). For example,

85 dispersal (a stochastic process) can suppress biogeochemical functioning by increasing the
86 proportion of maladapted taxa (Strickland et al., 2009; Nemergut et al., 2013; Graham and
87 Stegen, 2017), while selection (a deterministic process) may have positive influence on
88 biogeochemical function by facilitating locally adapted taxa (Graham et al., 2016). In
89 particular, stochastic dispersal has been suggested to suppress the mineralization of organic
90 carbon in soil and water (Le Moigne et al., 2020; Luan et al., 2020). Therefore, it is
91 hypothesized that the relative influence of deterministic and stochastic processes on
92 community assembly could impact the biogeochemical functions of microbial
93 communities (Strickland et al., 2009; Nemergut et al., 2013; Pholchan et al., 2013; Graham
94 and Stegen, 2017). Given the importance to understand how microbial community
95 variations affect the biogeochemical cycles in permafrost and thermokarst landscapes, it is
96 necessary to have a deeper understanding of the assembly mechanisms in shaping
97 microbial communities that form following permafrost degradation.

98 In this paper we evaluated these ideas on the Qinghai-Tibet Plateau (QTP), which is known
99 as the “Third Pole” of the Earth and is therefore uniquely positioned as an indicator of
100 global change (Yao et al., 2012). Pronounced environmental changes in response to climate
101 warming on the QTP have been observed and documented, especially in the past half
102 century (Piao et al., 2012; Zhang et al., 2018; Ren et al., 2019a). Major changes are
103 predicted to continue on the QTP and permafrost thawing is among the most prominent but
104 little is known about the microbial communities in these rapidly emerging ecosystems. To
105 fill this gap, we investigated water and sediment in thermokarst lakes across the QTP as
106 well as permafrost soil around the lakes (Figure 1). Our aims were to (1) assess the spatial
107 pattern of alpha and beta diversity of bacterial communities, and (2) evaluate the

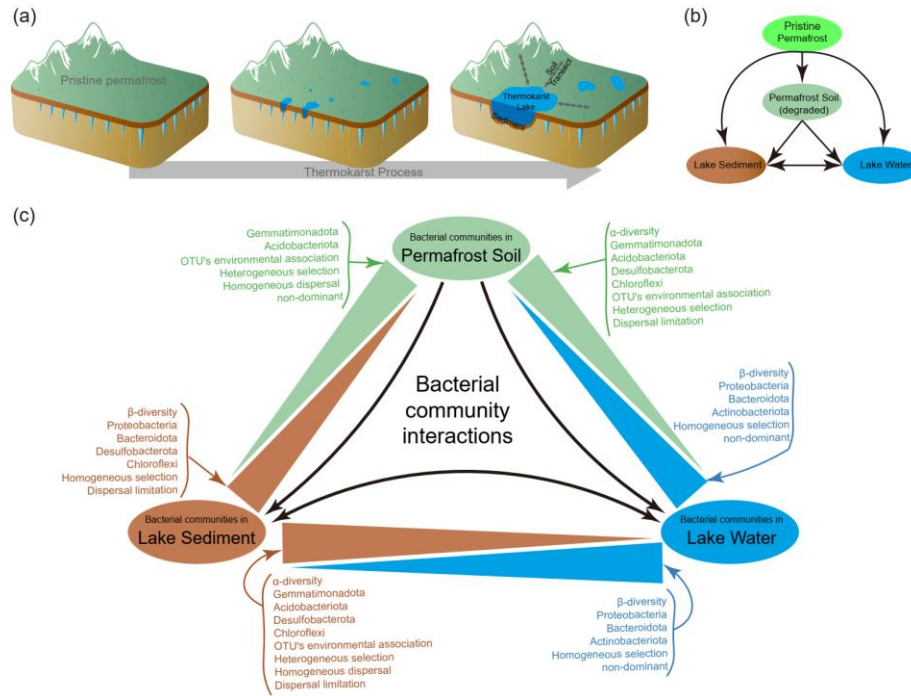
108 community assembly mechanisms and environmental responses of the bacterial
109 communities in degraded permafrost soil, as well as in the sediment and water of
110 thermokarst lakes.

111 **2 Methods**

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

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

131 climate dataset (Zhou, 2018) was obtained from the National Tibetan Plateau Data Center
 132 (<https://data.tpdc.ac.cn/en/>), and was utilized to extract the mean annual temperature (MAT)
 133 and mean annual precipitation (MAP) for each of the study sites.



134
 135 Figure 1 (a) The process of thermokarst lake formation in ice-rich permafrost (modified
 136 from Ren et al, 2022a). (b) A schematic view of the relationships between permafrost soil,
 137 lake sediment, and lake water. (c) Summary of the differences between distinct habitats of
 138 the bacterial communities in permafrost soil, lake sediment, and lake water.

139 **2.2 DNA extraction, PCR, and sequencing**

140 ~~The 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, sediment, and water samples according
 143 to the manufacturer's protocols. Extraction blanks were routinely performed in parallel.
 144 The prokaryotic 16S rRNA gene's V3-V4 hypervariable regions were amplified using

145 universal primers 343F-TACGGRAGGCAGCAG and 798R-AGGGTATCTAATCCT
146 (Nossa et al., 2010). PCRs were conducted in 25 µl reaction mixture containing 2.5 µl of
147 TransStart buffer, 2 µl of dNTPs, 1 µl of each primer, 0.5 µl of TransStart Taq DNA
148 polymerase, and 20 ng template DNA. The PCR reactions were conducted on a thermal
149 cycler (ABI GeneAmp® 9700, USA) using the followed procedure: initial denaturation at
150 94 °C for 5 min, 24 cycles of denaturation at 94 °C for 30 s followed by annealing at 56 °C
151 for 30 s and extension at 72 °C for 20 s, and a final extension at 72 °C for 5 min. To reduce
152 amplification bias, three individual PCR amplifications were performed for each sample
153 and the triplicate PCR products were combined and, purified. DNA libraries were verified
154 on 2% agarose gels and quantified using a Qubit 4 Fluorometer (Thermo Fisher Scientific,
155 Waltham, USA)., ~~and quantified.~~ Sequencing of the amplicon products was done on ~~the an~~
156 Illumina MiSeq platform (Illumina, San Diego, CA, USA) following the manufacturer's
157 instructions. The forward and reverse reads were joined and assigned to samples based on
158 barcode and truncated by cutting off the barcode and primer sequence. Raw sequences were
159 trimmed of ambiguous bases and low-quality sequences (quality score lower than 20).
160 After trimming, and the paired-end reads were joined and de-noised using QIIME1.9.1
161 (Caporaso et al., 2010). The sequences were subjected to the following denoising criteria:
162 sequences with ambiguous or homologous regions, as well as those below 200 bp in length,
163 were excluded; sequences with at least 75% of bases having a quality score above Q20
164 were retained; and chimeric sequences were identified and eliminated. All sequences from
165 extraction blanks were removed. The effective sequences were grouped into Operational
166 Taxonomic Units (OTUs) using a 97% sequence similarity threshold and annotated the
167 taxonomic classifications against the SILVA 138 database (released on 02-Nov-2020)

168 (Quast et al., 2013). The singletons were removed, and the sequences were rarefied to the
169 lowest number of sequences per sample were normalized to (24,251 sequences) per sample
170 to eliminate the bias from the sampling effort.

171 **2.3 Analyses**

172 Three α -diversity indices, including observed number of OTUs (OTU richness), Shannon
173 diversity, and phylogenetic diversity (PD), were calculated using QIIME 1.9.1 (Caporaso
174 et al., 2010). The “ses.mntd” function in the picante 1.8.2 package was used to calculate
175 the standardized effect size measure of the mean nearest taxon distance (SES.MNTD) for
176 assessing the phylogenetic clustering of bacterial communities (Kembel et al., 2010). The
177 β -diversity was calculated as the Bray-Curtis distance based on the relative abundance of
178 OTUs. Differences in α -diversity and β -diversity among bacterial communities in different
179 habitats, including permafrost soil bacterial communities (PBCs), lake sediment bacterial
180 communities (SBCs), and lake water bacterial communities (WBCs), were assessed using
181 Wilcoxon rank-sum test. The relationships between taxonomic and environmental
182 variables were assessed using Spearman correlation, and the P-values were corrected using
183 the FDR method (Benjamini and Hochberg, 1995). Mantel tests were performed to
184 examine the correlation between environmental variables and β -diversity. A Non-metric
185 Multidimensional Scaling (NMDS) analysis was conducted to examine the distribution of
186 PBCs, SBCs, and WBCs using the “metaMDS” function in the vegan 2.5-7 package
187 (Oksanen et al., 2020). The distinctiveness of these communities was confirmed through a
188 non-parametric statistical test (ANOSIM) using the “anosim” function in the vegan
189 package. The habitat niche occupied by each species was estimated by calculating Levin’s
190 niche breadth (Levins, 1968) with the use of the spa 0.2.2 package (Zhang, 2016). Species

191 with a broader niche breadth were distributed more evenly across a wider range of habitats,
192 compared to those with a narrower niche breadth.

193 Structural equation modeling (SEM) was conducted to assess the relationships among
194 location (including latitude, longitude, and elevation), climate (including mean annual
195 temperature and mean annual precipitation), and physicochemical ~~parameters~~variables
196 (including pH, conductivity, nutrients concentrations and stoichiometric ratios) of each
197 habitat (permafrost soil, lake sediment, and lake water), as well as their bacterial
198 communities (PBCs, SBCs, and WBCs). In model building, the SEM incorporated prior
199 knowledges: (a) location and climate factors potentially influence all the studied bacterial
200 communities, (b) physicochemical factor of each habitat potentially influences the
201 corresponding bacterial communities, and (c) permafrost soil potentially influences
202 thermokarst lake sediment and water, while lake sediment and water interact with each
203 other. In the SEM, location, climate, and physicochemical environments were reduced in
204 dimensions by principal component analysis (PCA), respectively, using the “prcomp”
205 function of the vegan package, and the first axis (PCA1) was used in SEM. For community
206 structure, the first axis of NMDS was used. SEM was constructed using the lavaan package
207 (Rosseel, 2012). The fit of SEM was assessed using standard indices, including chi-square
208 (χ^2), goodness-of-fit index (GFI), comparative fit index (CFI), root mean square residual
209 (RMR), and root mean squared error of approximation (RMSEA) (Hu and Bentler, 1999;
210 Barrett, 2007).

211 Phylogenetic trees of bacteria were constructed in the R package ggtree 3.2.1 (Yu et al.,
212 2017) using the top 1000 abundant OTUs in PBCs, SBCs, and WBCs, respectively. For
213 each phylogenetic tree, a heatmap was built in the inner ring represents Spearman’s

214 correlation between OTUs and environmental variables. The middle ring was built to
215 represent the frequency of the OTUs in our studied sites. The outer ring was built to
216 represent the relative abundance of the OTUs.

217 A null model analysis was performed to investigate the processes shaping the assembly of
218 bacterial communities in permafrost soil, lake sediment, and lake water using the R
219 package picante 1.8.2 (Kembel et al., 2010). This analysis based on the calculation of the
220 beta nearest taxon index (β NTI) to measure the extent of deterministic processes in shaping
221 the phylogenetic composition of the communities, as well as a Bray–Curtis-based Raup-
222 Crick matrix (RC_{Bray}) to assess the relative influences of stochastic processes (Stegen et al.,
223 2013; Zhou and Ning, 2017). β NTI values <-2 or $>+2$ indicate signals for heterogeneous
224 selection and homogenous selection, respectively. The values with $-2 < \beta$ NTI < 2 and RC_{Bray}
225 < -0.95 indicate homogeneous dispersal, while $-2 < \beta$ NTI < 2 and $RC_{Bray} > 0.95$ indicate
226 dispersal limitation. The values with $-2 < \beta$ NTI < 2 and $-0.95 < RC_{Bray} < 0.95$ indicate
227 “undominated”. Mantel tests were conducted to test the relationships between
228 environmental variables and β NTI.

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

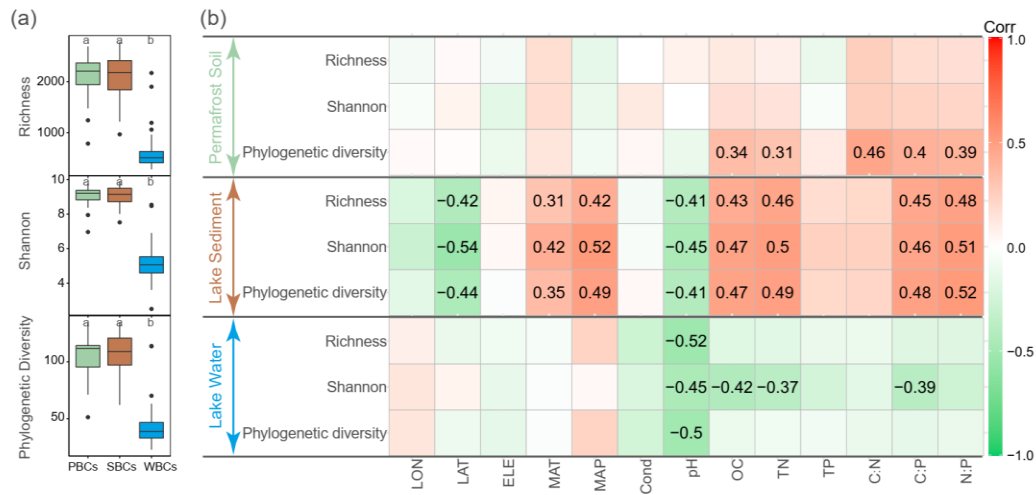
230 **3 Results**

231 *3.1 General distribution patterns of α -diversity*

232 After quality filtering, 3,201,132 high quality sequences were obtained and clustered into
233 9,361 OTUs, of which, 3870 OTUs were core OTUs shared by bacterial communities in
234 permafrost soil, lake sediment, and lake water (Figure S2). Moreover, a large number of
235 OTUs were shared by PBCs and SBCs ($n=7053$), of which, 16.4% were enriched in lake

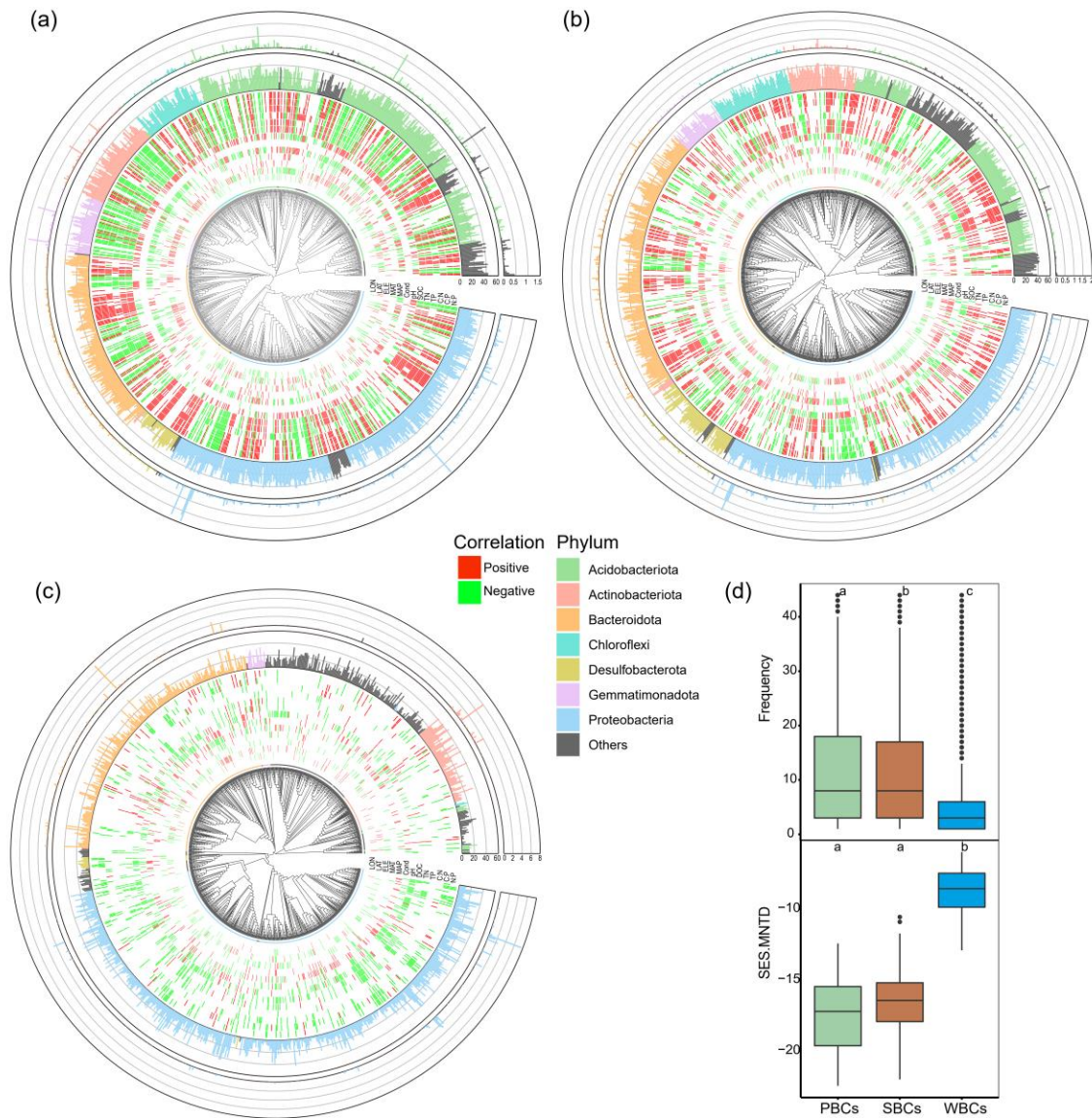
236 sediment and 19.3% were enriched in permafrost soil (Figure S2). However, a relatively
237 small number of OTUs were shared by PBCs and WBCs (n=4007) and by SBCs and WBCs
238 (n=4431), and only a very small proportion of OTUs were enriched in lake water (Figure
239 S2). Bacterial communities had a significantly lower α -diversity in lake water than in lake
240 sediment and permafrost soil (Figure 2a). α -diversity was not significantly different
241 between PBCs and SBCs (Figure 2a). Correlation analyses showed that phylogenetic
242 diversity of PBCs was positively correlated with SOC, TN, and C:N:P ratios (Figure 2b).
243 For SBCs, α -diversity indices were positively correlated with MAT, MAP, SOC, TN, C:P
244 and N:P, while negatively correlated with latitude and pH (Figure 2b). For WBCs, α -
245 diversity indices were negatively correlated with pH, and Shannon diversity was negatively
246 correlated with DOC, TN, and C:P (Figure 2b).

247 PBCs and SBCs had a significantly greater phylogenetic diversity than WBCs (Figure 2a
248 and Figure 3). The OTUs in PBCs had significantly higher frequency than that of SBCs
249 and WBCs (Figure 3). The top 1000 abundant OTUs in PBCs were highly correlated with
250 environmental variables, particularly with latitude, MAP, SOC, TN, TP, and C:N:P ratios
251 (Figure 3a). The top 1000 abundant OTUs in SBCs were more commonly positively
252 correlated with MAP, SOC, TN, and C:N:P ratios, but more commonly negatively
253 correlated with latitude and pH (Figure 3b). The top 1000 abundant OTUs in WBCs had
254 relatively fewer significant relationships with environmental variables in general, but were
255 negatively correlated with latitude, conductivity, pH, DOC, TN, and C:N:P ratios, while
256 more positively correlated with MAP (Figure 3c). In addition, WBCs had significantly
257 higher SES.MNTD than PBCs and SBCs (Figure 3d), suggesting higher phylogenetic
258 clustering of bacterial taxa in WBCs.



259

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



266

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

273 SES.MNTD values among bacterial communities in permafrost soil (PBCs), lake sediment
274 (SBCs), and lake water (WBCs). The different lower-case letters represent significant
275 differences assessed using Wilcoxon rank-sum test.

276 ***3.2 Community composition and β -diversity patterns***

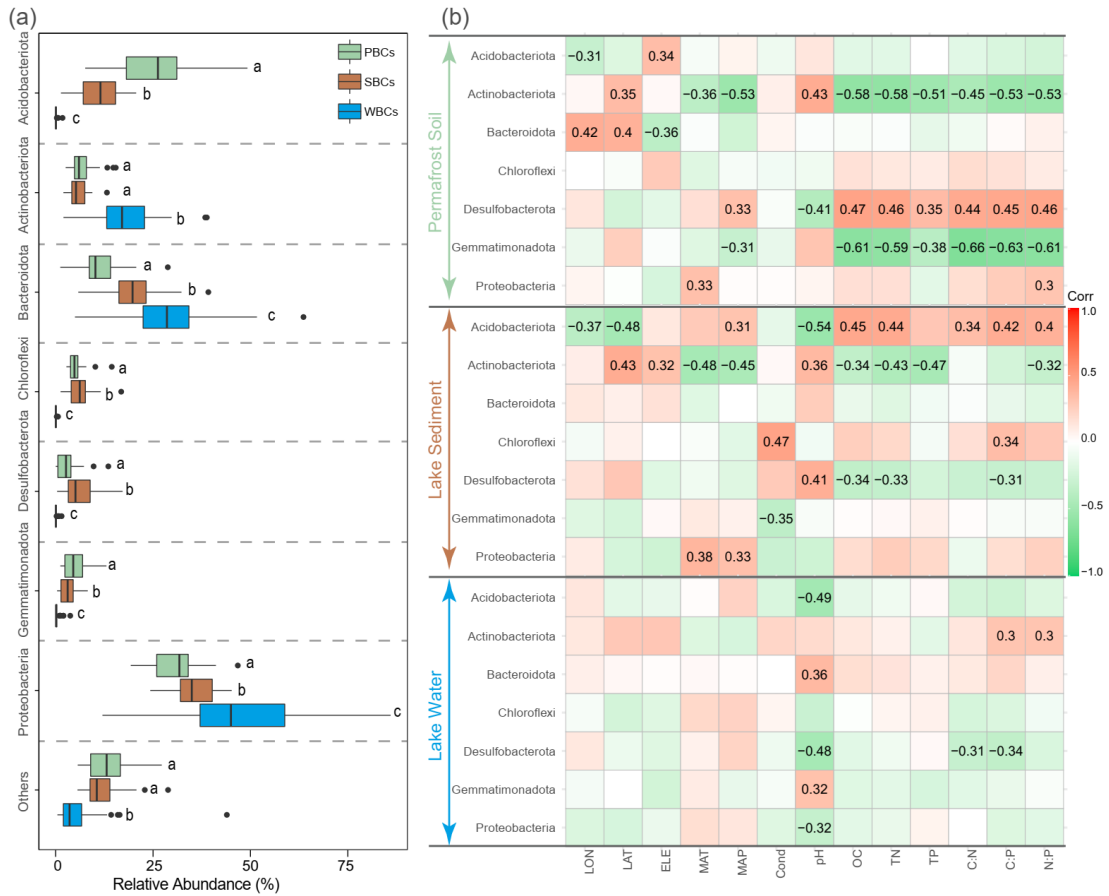
277 PBCs were dominated by Proteobacteria (30.4%), Acidobacteriota (25.3%), Bacteroidota
278 (11.4%), Actinobacteriota (6.8%), Chloroflexi (5.2%), and Gemmatimonadota (5.2%)
279 (Figure 4a). SBCs were dominated by Proteobacteria (35.2%), Bacteroidota (20.0%),
280 Acidobacteriota (11.3%), Desulfobacterota (6.4%), Chloroflexi (6.3%), and
281 Actinobacteriota (5.8%) (Figure 4a). WBCs were dominated by Proteobacteria (46.9%),
282 Bacteroidota (29.2%), and Actinobacteriota (17.4%) (Figure 4a). While Proteobacteria
283 were predominant in all three habitat types, these dominant phyla had significantly
284 different relative abundances among these habitats. Proteobacteria and Bacteroidota had a
285 significantly higher relative abundance in WBCs than in SBCs and PBCs (Figure 4a). The
286 relative abundance of Actinobacteriota was the highest in WBCs but was not significantly
287 different between PBCs and SBCs (Figure 4a). Gemmatimonadota and Acidobacteriota
288 were significantly enriched in PBCs than in SBCs and WBCs. Desulfobacterota and
289 Chloroflexi were significantly enriched in SBCs than in PBCs and WBCs (Figure 4a).

290 These phyla responded differently to environmental variables (Figure 4b). For example,
291 Actinobacteriota and Gemmatimonadota in PBCs and Actinobacteriota and
292 Desulfobacterota in SBCs were negatively correlated with nutrient concentrations and
293 ratios, while Desulfobacterota in PBCs and Acidobacteriota in SBCs were positively
294 correlated with nutrient concentrations and ratios (Figure 4b). pH was a frequent correlate
295 for taxa in various taxonomic groups across all three habitats (Figure 5b)

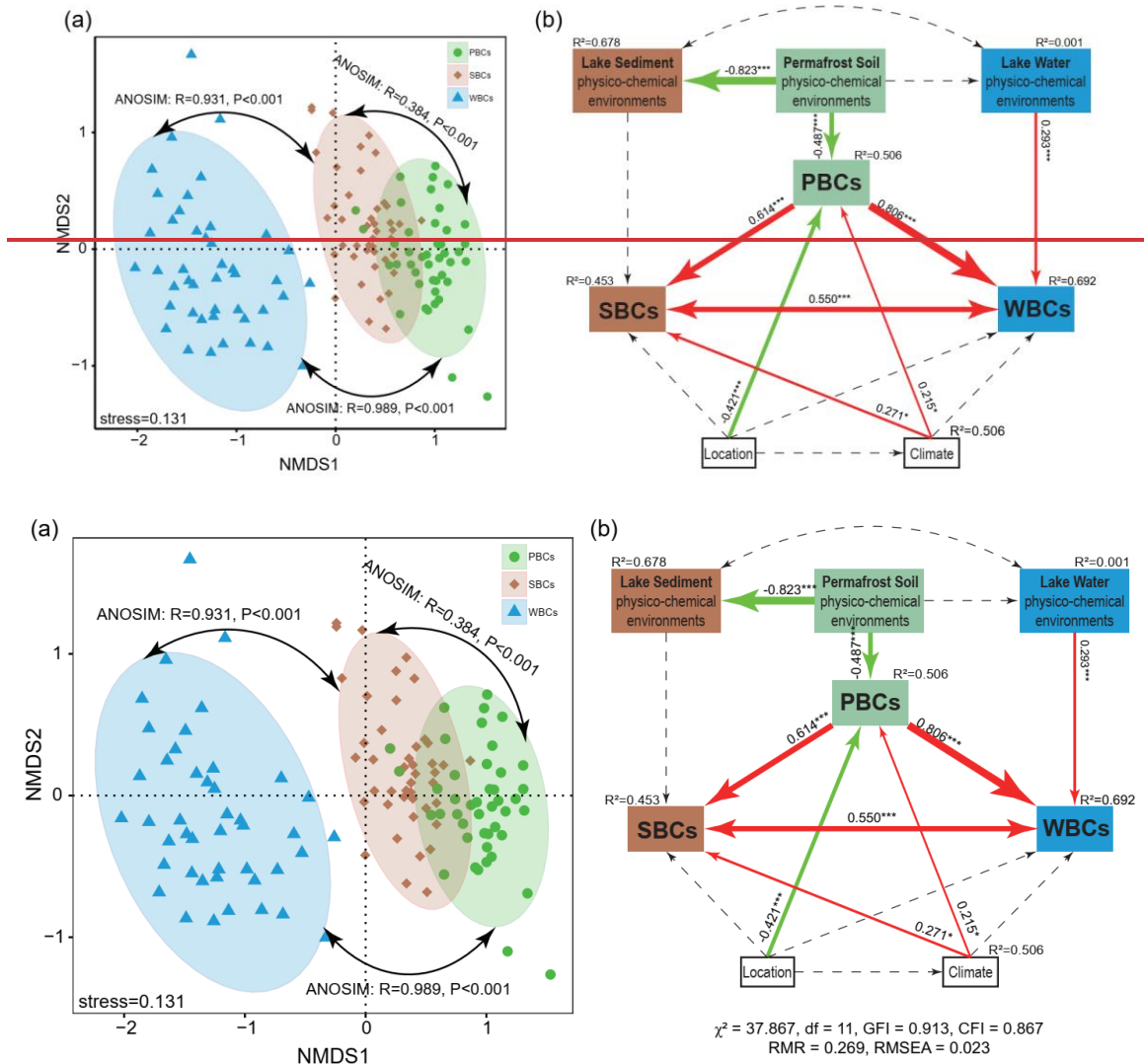
296 Nonmetric multidimensional scaling (NMDS) analysis along with non-parametric
297 statistical tests showed that bacteria in different habitats formed distinct communities
298 (Figure 5a). The extent of difference was larger for WBCs vs PBCs ($\beta=0.98$; $R_{ANOSIM} =$
299 0.989 , $P<0.001$) than the differences for WBCs vs SBCs ($\beta=0.96$; $R_{ANOSIM} = 0.967$,
300 $P<0.001$). There was the least dissimilarity between PBCs and SBCs ($\beta=0.81$; $R_{ANOSIM} =$
301 0.384 , $P<0.001$). The fitted SEM model showed that PBCs had direct effects on SBCs and
302 WBCs, and the latter two had reciprocal effects on each other (Figure 5b). In addition,
303 location, climate, and permafrost soil physicochemical environments had direct effects on
304 PBCs. Climate had direct effects on SBCs while lake water physicochemical environments
305 had direct effects on WBCs (Figure 5b).

306 WBCs had a higher β -diversity than SBCs and PBCs, suggesting that bacterial
307 communities were more spatially heterogeneous in lake water than in lake sediment and
308 permafrost soil (Figure 6a). Taxa in PBCs had higher habitat niche breadths than taxa in
309 SBCs and WBCs (Figure 6b). We estimated the distance decay relationship of bacterial
310 community similarity. Significant distance-decay relationships were observed for all
311 communities but the fitness values were relatively low (Figure S3), indicating weak decay
312 of community similarity with geographic distance in thermokarst landscape. We also
313 explored the main environmental variables that influence the variations of the bacterial
314 communities (Figure 7). β -diversities of PBCs, SBCs, and WBCs were all significantly
315 correlated with latitude, MAP, and pH (Figure 7). In addition, β -diversity of PBCs was also
316 significantly correlated with all the other environmental variables except MAT and
317 conductivity. β -diversity of SBCs was also significantly correlated with conductivity and
318 C:N (Figure 7). β -diversity of WBCs was also significantly correlated with elevation, MAT,

319 conductivity, DOC, TN, and TP (Figure 7). The results suggested that the compositional
 320 variation among PBCs, SBCs, and WBCs was differentially structured by spatial, climatic,
 321 and physicochemical variables.



322
 323 Figure 4 (a) Relative abundances of major phyla in bacterial communities in permafrost
 324 soil (PBCs), lake sediment (SBCs), and lake water (WBCs). The different low-case letters
 325 represent significant differences assessed using Wilcoxon rank-sum test. (b) Spearman
 326 correlations show the relationships between the relative abundance of major phyla and
 327 environmental factors. The color represents the correlation coefficient, which shown in
 328 number when the result is significant ($p < 0.05$).

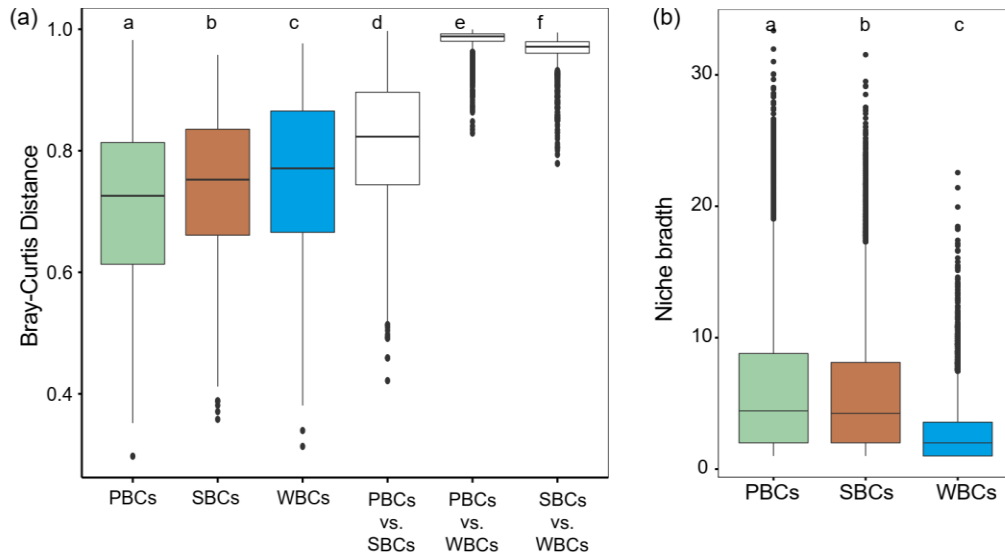


329

330

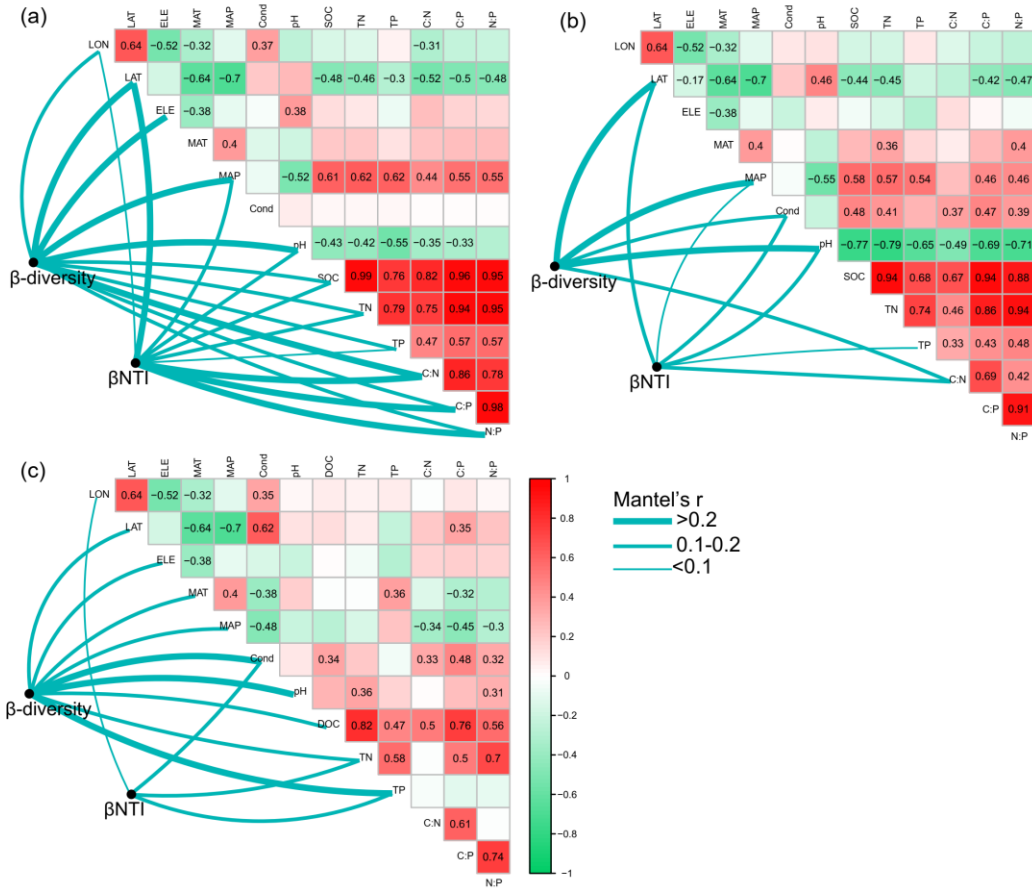
331 Figure 5 (a) Non-metric multidimensional scaling (NMDS) ordination showing the
 332 distribution of bacterial communities in permafrost soil (PBCs), lake sediment (SBCs), and
 333 lake water (WBCs). The differences between these communities are confirmed by the non-
 334 parametric statistical test (ANOSIM). (b) Structural equation modeling analysis depicting
 335 the relationships between location (including latitude, longitude, and elevation), climate
 336 (including mean annual temperature and mean annual precipitation), physicochemical
 337 environments (pH, conductivity, nutrients concentrations and stoichiometric ratios) of each

338 habitat. Solid and dashed arrows represent the significant and nonsignificant relationships,
 339 respectively. Red and green arrows represent positive and negative relationships,
 340 respectively. Significant path coefficients are shown adjacent to the path with *, **, and
 341 *** denoting the significant level of $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.



342

343 Figure 6 (a) β -diversities within and between PBCs, SBCs and WBCs. (b) Habitat niche
 344 breadth of the bacterial communities.



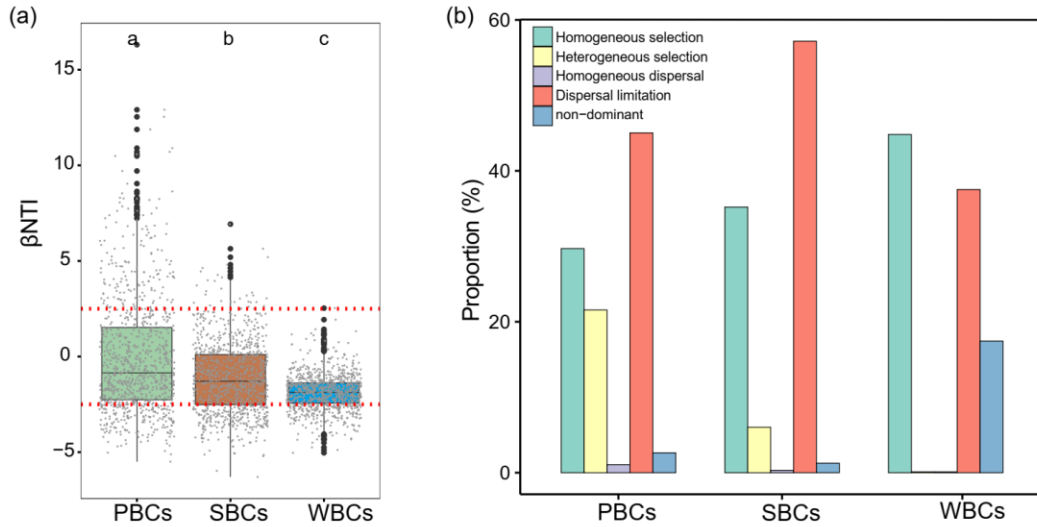
345

346 Figure 7 Pairwise correlations between environmental variables as well as the Mantel tests
 347 between environmental variables and β -diversity and beta nearest taxon index (β NTI) for
 348 (a) bacterial communities in permafrost soil, (b) bacterial communities in lake sediment,
 349 and (c) bacterial communities in lake water. β -diversity was calculated as Bray-Curtis
 350 distance. The lines denote significant relationships while the line width represents the
 351 Mantel's r statistic. Pairwise correlations between environmental variables are shown in
 352 color gradient matrix. The color represents Pearson's correlation coefficient, which shown
 353 in number when the result is significant (p < 0.05). The abbreviations of the environmental
 354 variables are explained in the Methods section.

355 **3.3 Assembly processes**

356 To explore the mechanisms underlining the observed distribution patterns, a null-model-
357 based framework was employed to quantify the deviation of phylogenetic turnover. PBCs
358 had significantly higher β NTI than SBCs and WBCs (Figure 8a). Deterministic processes
359 contributed 51.3%, 41.2%, and 44.9% to community variations for the bacterial
360 communities in permafrost soil, lake sediment, and lake water, respectively (Figure 8b). In
361 particular, the results showed that homogeneous selection contributed a larger fraction to
362 the assembly of the WBCs (44.8%), followed by SBCs (35.2%) and PBCs (29.7%) (Figure
363 8b). Heterogeneous selection influenced PBCs (21.6%) more than SBCs (6.0%) and WBCs
364 (0.1%). Dispersal limitation contributed a larger fraction to SBCs (57.2%) than to PBCs
365 (45%) and WBCs (37.5%).

366 The relationships between β NTI and major environmental variables were used to estimate
367 changes in the relative influences of deterministic and stochastic assembly processes.
368 Mantel tests showed that the assembly processes of bacterial communities in permafrost
369 soil, lake sediment, and lake water had similarities and differences in the responses to
370 environmental variables (Figure 7). Particularly, differences of TP were significantly
371 associated with β NTI of PBCs, SBCs, and WBCs, implying that an increasing divergence
372 of TP could contribute to a shift from homogeneous selection to heterogeneous selection
373 in the assembly of bacterial communities in the QTP thermokarst landscape. Moreover,
374 β NTI of PBCs was also significantly associated with other environmental variables, except
375 elevation, MAT, and conductivity. β NTI of SBCs was also significantly associated with
376 latitude, MAP, conductivity, pH, and C:N, while β NTI of WBCs was significantly
377 associated with longitude, conductivity, and TN.



378

379 Figure 8 (a) The values of β NTI with horizontal dashed red lines indicate upper and lower
 380 significance thresholds at β NTI = +2 and -2, respectively in the three habitat types. (b) The
 381 contribution of deterministic (homogeneous and heterogeneous selection) and stochastic
 382 (dispersal limitations and homogenizing dispersal) processes to turnover in the assembly
 383 of PBCs, SBCs and WBCs. “Non-dominant” indicates that the fraction was not dominated
 384 by any single process.

385 4 Discussion

386 Thermokarst lakes and degraded permafrost are distinct habitats derived from original
 387 permafrost during the process of thermokarst formation. Degraded permafrost can be
 388 further converted to thermokarst lake sediment during the continuous process of
 389 thermokarst formation (Figure 1). In our studied thermokarst landscapes across the QTP,
 390 bacterial communities in degraded permafrost soil (PBCs), thermokarst lake sediment
 391 (SBCs), and thermokarst lake water (WBCs) differed in multiple aspects, such as α -
 392 diversity, β -diversity, community composition, community assembly rules, and
 393 environmental responses (Figure 1c), supporting a view in which thermokarst formation

394 generates novel habitat conditions and microbial communities in landscapes formerly
395 occupied by permafrost.

396 ***4.1 Alpha diversity and community composition***

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

405 Thermokarst lakes are known to have sediments that derive from the permafrost soil and
406 are constantly replenished by the collapse of nearby permafrost (Payette et al., 2004; West
407 and Plug, 2008; Veremeeva et al., 2021). This suggests that permafrost soil and lake
408 sediments are likely to have high levels of similarity in bacterial diversity and community
409 composition. Additionally, our prior research has shown that there are close correlations
410 between the abiotic features of the two environments (Ren et al., 2022b). However, despite
411 these similarities and connections, we found substantial differences in the bacterial
412 communities of permafrost soil and lake sediments. As proposed by the Baas-Becking
413 hypothesis (Baas-Becking, 1934), environmental selection is partially responsible for
414 variation in microbial communities, which are also shaped by other ecological processes,
415 such as diversification and dispersal limitation. Indeed, in our study, alpha diversity and

416 the dominant phyla found in PBCs and SBCs responded differently to various
417 environmental variables.

418 Bacterial communities in lake water had significantly lower alpha diversity as well as
419 distinct community composition and structure in comparison to bacterial communities in
420 permafrost soil and lake sediment. However, PBCs and SBCs had direct influence on
421 WBCs. For thermokarst lakes, the water first originates from the thawing of the ice-rich
422 permafrost and the lake is then fed by precipitation-derived and permafrost-derived water
423 (Yang et al., 2016a; Narancic et al., 2017; Wan et al., 2019). Microorganisms present in
424 lake water have a diverse range of sources, including terrestrial inputs and other sources
425 such as bacteria distributed with the atmosphere, associated with plants and animals, and
426 carried by migratory birds and animals (Ruiz-Gonzalez et al., 2015). Thus, there was a
427 relatively small proportion of OTUs shared between permafrost soil and lake water, as well
428 as between lake sediment and water, and only a few shared OTUs were enriched in lake
429 water. It is a well-established fact that different habitats often support distinct microbial
430 communities (Fierer et al., 2012; Hugerth et al., 2015; Louca et al., 2016). The contrast in
431 bacterial community composition between lake sediments and water has been extensively
432 documented (Briee et al., 2007; Gough and Stahl, 2011; Yang et al., 2016b; Ren et al.,
433 2017). In addition, sediment generally harbor a higher species-level diversity of bacteria
434 compared to lake water (Lozupone and Knight, 2007; Ren et al., 2019b). For example, in
435 a permafrost thaw pond of Andes, it was also found that water samples had lower alpha
436 diversity than lake sediment and permafrost samples (Aszalós et al., 2020). Permafrost soil
437 and lake sediment may provide more habitat heterogeneity for bacterial taxa than the water
438 column, supported by our observation that the bacterial taxa had higher niche breadth in

439 permafrost soil and lake sediment than in lake water. Moreover, in hydrologically
440 connected terrestrial-aquatic ecosystems, bacterial communities can present distinct but
441 directional spatial structure driven by terrestrial recruited taxa (Ruiz-Gonzalez et al., 2015).
442 Thus, these community similarities between distinct bacterial habitats might be the result
443 of common bacterial source (original permafrost) and the differences are likely caused by
444 subsequent environmental selection, colonization from multiple other bacterial sources,
445 and distinct assembly mechanisms.

446 Despite connections driven by dispersal, distinct thermokarst habitats had ~~distinct~~ different
447 bacterial community composition, as seen in previous work (Ottoni et al., 2022). All the
448 dominant phyla were significantly different in relative abundance among permafrost soil
449 (PBCs), lake sediment (SBCs), and lake water (WBCs). In this study, Proteobacteria,
450 Bacteroidota, Actinobacteriota, Gemmatimonadota, Acidobacteriota, Desulfobacterota,
451 and Chloroflexi dominated bacterial communities in permafrost soil and/or thermokarst
452 lakes despite high variability. Similar dominance of these taxa has also been found in
453 permafrost and thermokarst landscapes in other areas (Aszalós et al., 2020; Belov et al.,
454 2020; Wu et al., 2022). The most commonly reported bacterial groups in permafrost
455 environments include members of Proteobacteria, Acidobacteria, Actinobacteria,
456 Bacteroidetes, Firmicutes, and Chloroflexi (Steven et al., 2009; Altshuler et al., 2017;
457 Ottoni et al., 2022), as observed in our samples.

458 ***4.2 Beta diversity and assembly processes***

459 In our studied regions across the QTP, PBCs, SBCs, and WBCs all had a high beta diversity
460 (average values > 0.7), with WBCs showing the highest, suggesting that bacterial
461 communities shifted substantially across the large spatial scale of our sampling. Moreover,

462 beta diversities of PBCs, SBCs, and WBCs were significantly correlated with each other,
463 further suggesting that the bacteria in different habitats had a considerable proportion of
464 members from the same source, the original pristine permafrost soil. The significantly
465 lower mean SES.MNTD for PBCs indicate that bacterial communities in permafrost soil
466 were more closely phylogenetically clustered and suffered stronger environmental filtering
467 than those in lake sediment and water (Langenheder et al., 2017), consistent with the
468 observation that PBCs had lower beta diversity than SBCs and WBCs. SES.MNTD is
469 sensitive to changes in lineage close to the phylogenetic tips (Kembel et al., 2010). The
470 higher SES.MNTD observed for SBCs and WBCs suggest the possibility that the bacteria
471 in lake sediment and water exhibit a substantial divergence in the co-occurring species, and
472 thermokarst lakes have experienced colonization by bacterial species originating from
473 distinct clades or lineages from external sources following permafrost thaw (Webb et al.,
474 2002; Stegen et al., 2013).

475 The structure of bacterial communities can vary across spatiotemporal scales and different
476 habitats (Ren et al., 2017; Aguilar and Sommaruga, 2020; Pearman et al., 2020). A key
477 objective in the field of microbial ecology is to determine the relative influence of
478 stochastic and deterministic processes in shaping the assembly of communities (Stegen et
479 al., 2013; Zhou and Ning, 2017). In this study, ~~deterministic processes contributed 51.3%,~~
480 ~~41.2%, and 44.9% to community variation for the bacterial communities in permafrost soil,~~
481 ~~lake sediment, and lake water, respectively. Homogeneous selection contributed a larger~~
482 ~~fraction to the assembly of the WBCs (44.8%), followed by SBCs (35.2%) and PBCs~~
483 ~~(29.7%). Heterogeneous selection influenced PBCs (21.6%) more strongly than SBCs~~
484 ~~(6.0%) and WBCs (0.1%).~~ In addition, bacterial communities in lake water displayed a

485 higher influence of homogeneous selection but lower influence of heterogeneous selection
486 compared to those in lake sediments and permafrost soil in our study. The reason for this
487 might be that Long-term changes in thermokarst lakes result in homogenized habitats and
488 consequently strong homogenous selection on bacterial communities (Ning et al., 2019),
489 In contrast, permafrost soil is a highly heterogeneous environment across spatial scales
490 (Etzelmüller, 2013; Nitzbon et al., 2021), creating a wide range of habitats which can
491 impose strong heterogeneous selection pressures on bacterial communities. Furthermore,
492 permafrost soil is characterized by limited nutrient availability due to the frozen state of
493 organic matters (Beermann et al., 2017; Zhang et al., 2023), while lake water offers a more
494 diverse and abundant array of dissolved organic compounds and nutrients. As a result,
495 bacterial communities in permafrost soil might be more sensitive to variations in resource
496 availability, rendering them more strongly influenced by heterogeneous selection.
497 ~~Deterministic processes could also cause the communities to be more dissimilar through~~
498 ~~heterogeneous selection, which also imposed strong control on PBCs.~~In addition,
499 ~~D~~ispersal limitation contributed a larger fraction to SBCs (57.2%) than to PBCs (45%)
500 and WBCs (37.5%). The dispersal of microorganisms is often considered as a passive
501 process that results in community variation and turnover coupled with the function of
502 environmental filtering (Cline and Zak, 2014; Stegen et al., 2015; Custer et al., 2022). The
503 high dispersal limitation of microbial communities in thermokarst lakes could be
504 potentially explained that their ir isolated nature ~~of thermokarst lakes~~ being endorheic results
505 in limited connectivity and strong restriction of microbial dispersal, as well as strong
506 environmental filtering. Additionally, the prolonged frozen phase of thermokarst lakes and
507 permafrost soil restrict the movement of microorganisms (Vargas Medrano, 2019;

508 Vigneron et al., 2019). Furthermore, geographical barriers, exemplified by prominent
509 mountain ranges like the Tanggula Mountains, Kunlun Mountains, Nyenchen Tanglha
510 Mountains, and Bayan Har Mountains, serve as impediments to the dispersal of both
511 macro- and microorganisms (Wan et al., 2016; Yu et al., 2019; Ren et al., 2022c).
512 Particularly in lake sediment, where bacterial communities are more isolated over distances
513 and will not disperse as far as those in lake water and permafrost soil, resulting in stronger
514 influence of dispersal limitation (Martiny et al., 2006; Xiong et al., 2012). Although the
515 “everything is everywhere” hypothesis suggests that many microorganisms have a
516 cosmopolitan distribution, their slow mobility allows for the development of regional
517 phylogenetic differences and the emergence of specialized, endemic taxa in isolated
518 habitats, resulting in a low likelihood of microorganisms dispersing to suitable distant sites
519 (Telford et al., 2006). Therefore, dispersal processes in this thermokarst landscape may be
520 restricted by the lack of hydrological connection, limited movement of water, short
521 duration since thawing, and strong environmental filtering, contributing to the observed
522 high dispersal limitation in the studied permafrost soil and thermokarst lakes. This
523 inference is supported by many previous studies showing that dispersal limitation plays a
524 major role in structuring microbial communities in lakes (Lindstrom and Langenheder,
525 2012; Yang et al., 2019; Liu et al., 2021; Ren et al., 2022d). Strong dispersal limitation for
526 bacterial communities in permafrost has also been documented across an Alaskan boreal
527 forest landscape (Bottos et al., 2018). ~~In addition, bacterial communities in lake water~~
528 ~~displayed a higher influence of homogeneous selection compared to those in lake~~
529 ~~sediments and permafrost soil in our study. The reason for this might be that long-term~~
530 ~~changes in thermokarst lakes result in homogenized habitats and consequently strong~~

531 ~~homogenous selection on bacterial communities. Deterministic processes could also cause~~
532 ~~the communities to be more dissimilar through heterogeneous selection, which also~~
533 ~~imposed strong control on PBCs.~~

534 ***4.3 Environmental influences***

535 Understanding how environmental factors shape bacterial communities is a crucial aspect
536 in the field of microbial ecology (Fierer and Jackson, 2006; Pla-Rabes et al., 2011). With
537 global warming, climatic and physicochemical environments will be strongly altered in
538 permafrost areas. On the QTP in particular, air temperature and precipitation are increasing
539 in most regions (Xu et al., 2008; Lu et al., 2018). Moreover, organic carbon and nutrient
540 stocks in permafrost are decreasing (Turetsky et al., 2020; Wu et al., 2021) and thermokarst
541 lakes are developing, driving dynamic environmental change (Luo et al., 2015; Vucic et
542 al., 2020). These environmental disruptions likely impose strong influences on bacterial
543 communities in thermokarst landscapes. In our study, bacterial communities were
544 differentially correlated with various measured environmental variables. In our data, pH
545 was consistently identified as a strong correlate of microbial community structure and
546 diversity, as is often observed in terrestrial and aquatic ecosystems worldwide (Fierer and
547 Jackson, 2006; Xiong et al., 2012). While such correlations between pH and bacterial
548 communities have been widely found, the regulation mechanisms still remain unknown
549 (Malard and Pearce, 2018). Moreover, the influences of pH are often species- and location-
550 specific (Malard and Pearce, 2018; Egelberg et al., 2021). In this study, pH had
551 significantly negative relationships with alpha diversity of bacterial communities in lake
552 sediment and water, and had negative or positive correlations with some phyla. Moreover,
553 differences in pH might drive community variation observed between PBCs, SBCs, and

554 WBCs, and shift community assembly processes for PBCs and SBCs. Our study also
555 showed that nutrient (C, N, and P) concentrations and stoichiometric ratios were strongly
556 related to alpha diversity (particularly for SBCs) and community variation and assembly
557 (particularly for PBCs). The role of nutrient availability in shaping bacterial communities
558 has been well established (Torsvik et al., 2002; Lee et al., 2017; Zhou et al., 2020). High
559 organic matter content, for instance, has been shown to support diverse and complex
560 microbial communities (Garrido-Benavent et al., 2020; Ren and Gao, 2022). Due to their
561 ecological strategies, metabolic features, and environmental preferences, bacteria respond
562 differentially to nutrient status (Carbonero et al., 2014). Compared to permafrost soil and
563 lake water, lake sediment can exhibit more stable physicochemical conditions. However,
564 permafrost soil and lake water experience more dynamic and extreme environmental
565 changes, which drive the bacterial communities. The results of SEM also in line with
566 bacterial community assembly that deterministic processes had stronger influences on
567 PBCs and WBCs than on SBCs. In addition, Thermokarst lakes have sediment directly
568 formed from permafrost soil, and thus, permafrost soil environments and bacterial
569 communities had strong associations with that of lake sediment.

570 In addition to physicochemical environments, location and climate were also suggested to
571 influence bacterial communities in distinct habitats. Warming and altered precipitation
572 regimes under climate change have been demonstrated to affect alpha diversity and
573 composition of stream microbial communities at continental scales (Picazo et al., 2020).
574 Our study indicates that location (particularly latitude) and climate (particularly MAP)
575 factors are important in shifting bacterial communities in thermokarst landscapes.
576 Particularly for bacterial communities in permafrost soil, location and climate have been

577 evidenced as strong factors in shaping microbial communities (Taş et al., 2018; Barbato et
578 al., 2022). Understanding large-scale pattern of bacterial communities is increasingly
579 important to offer insights into the impacts of climate change (Picazo et al., 2020; Ren et
580 al., 2021). As global climate changes, QTP is getting warmer and more humid (Xu et al.,
581 2008; Lu et al., 2018). Therefore, significant alterations to the physical, chemical, and
582 biological properties of thermokarst lakes on the QTP can be expected in the coming
583 decades. Based on “space-for-time” substitution, our study serves as a foundation for
584 predicting the potential impact of climate change on bacterial communities in thermokarst
585 landscapes.

586 **5 Conclusion**

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

597 **Acknowledgements**

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

600 **Data availability statement:**

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

603 **Funding statement:**

604 This study was supported by the open funding of the State Key Laboratory of Lake Science
605 and Environment (2022SKL010).

606 **Conflict of interest disclosure:**

607 The authors declare no competing interests.

608 **Author's contributions:**

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

611 **Ethics approval statement:**

612 Not applicable

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

614 Not applicable

615 **Originality-Significance Statement:**

616 This is our original study and not submitted to elsewhere

617 **Supplementary Information**

618 Figure S1 Map of the 44 sampling sites of permafrost soil and thermokarst lakes across the
619 Qinghai-Tibet Plateau. The distribution of the permafrost was cited from Zou et al., 2017.
620 This map was cited from Ren et al, 2022a.

621 Figure S2 (a) Venn diagram showing the unique and shared OTUs among distinct bacterial
622 communities. (b) The volcano plot showing the shared OTUs that significantly (t-test, $P <$
623 0.05) enriched in a certain habitat. The volcano plot was constructed using \log_2 (fold
624 change) on x-axis and $-\log_{10}$ (p-values of t-test) on y-axis.

625 Figure S3 Distance-decay curves showing community similarity against geographic
626 distances between sampling sites. Solid lines denote the ordinary least-squares linear
627 regressions.

628

629 **References**

- 630 Aguilar, P. and Sommaruga, R.: The balance between deterministic and stochastic
631 processes in structuring lake bacterioplankton community over time, *Mol. Ecol.*, 29,
632 3117-3130, doi:10.1111/mec.15538, 2020.
- 633 Altshuler, I., Goordial, J. and Whyte, L. G.: Microbial life in permafrost. In *Psychrophiles:*
634 *from biodiversity to biotechnology* (pp. 153-179). Springer, Cham, 2017.
- 635 Anthony, K. M., von Deimling, T. S., Nitze, I., Frolking, S., Emond, A., Daanen, R.,
636 Anthony, P., Lindgren, P., Jones, B. and Grosse, G.: 21st-century modeled permafrost
637 carbon emissions accelerated by abrupt thaw beneath lakes, *Nat. Commun.*, 9, 3262,
638 doi:10.1038/s41467-018-05738-9, 2018.
- 639 Aszalós, J. M., Szabó, A., Megyes, M., Anda, D., Nagy, B. and Borsodi, A. K.: Bacterial
640 Diversity of a High-Altitude Permafrost Thaw Pond Located on Ojos del Salado (Dry
641 Andes, Altiplano-Atacama Region), *Astrobiology*, 20, 754-765,
642 doi:10.1089/ast.2018.2012, 2020.
- 643 Baas-Becking, L. G. M.: *Geobiologie of Inleiding Tot de Milieukunde*, Van Stockkum &
644 Zoon, The Hague, 1934.
- 645 Barbato, R. A., Jones, R. M., Douglas, T. A., Doherty, S. J., Messan, K., Foley, K. L.,
646 Perkins, E. J., Thurston, A. K. and Garcia-Reyero, N.: Not all permafrost microbiomes
647 are created equal: Influence of permafrost thaw on the soil microbiome in a laboratory
648 incubation study, *Soil Biology and Biochemistry*, 167, 108605,

649 doi:<https://doi.org/10.1016/j.soilbio.2022.108605>, 2022.

650 Barrett, P.: Structural equation modelling: Adjudging model fit, *Pers. Individ. Differ.*, 42,
651 815-824, doi:[10.1016/j.paid.2006.09.018](https://doi.org/10.1016/j.paid.2006.09.018), 2007.

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

655 Belov, A. A., Cheptsov, V. S., Manucharova, N. A. and Ezhelev, Z. S.: Bacterial
656 Communities of Novaya Zemlya Archipelago Ice and Permafrost, *Geosciences*, 10, 67,
657 doi:[10.3390/geosciences10020067](https://doi.org/10.3390/geosciences10020067), 2020.

658 Benjamini, Y. and Hochberg, Y.: Controlling the false discovery rate: a practical and
659 powerful approach to multiple testing, *Journal of the Royal Statistical Society: Series*
660 *B (Methodological)*, 57, 289-300, doi:[10.2307/2346101](https://doi.org/10.2307/2346101), 1995.

661 Bier, R. L., Bernhardt, E. S., Boot, C. M., Graham, E. B., Hall, E. K., Lennon, J. T.,
662 Nemergut, D. R., Osborne, B. B., Ruiz-Gonzalez, C., Schimel, J. P., Waldrop, M. P.
663 and Wallenstein, M. D.: Linking microbial community structure and microbial
664 processes: an empirical and conceptual overview, *FEMS Microbiol. Ecol.*, 91,
665 doi:[10.1093/femsec/fiv113](https://doi.org/10.1093/femsec/fiv113), 2015.

666 Biskaborn, B. K., Smith, S. L., Noetzi, J., Matthes, H., Vieira, G., Streletskiy, D. A.,
667 Schoeneich, P., Romanovsky, V. E., Lewkowicz, A. G., Abramov, A., Allard, M.,
668 Boike, J., Cable, W. L., Christiansen, H. H., Delaloye, R., Diekmann, B., Drozdov, D.,
669 Etzelmuller, B., Grosse, G., Guglielmin, M., Ingeman-Nielsen, T., Isaksen, K.,
670 Ishikawa, M., Johansson, M., Johannsson, H., Joo, A., Kaverin, D., Kholodov, A.,
671 Konstantinov, P., Kroger, T., Lambiel, C., Lanckman, J. P., Luo, D., Malkova, G.,
672 Meiklejohn, I., Moskalenko, N., Oliva, M., Phillips, M., Ramos, M., Sannel, A.,
673 Sergeev, D., Seybold, C., Skryabin, P., Vasiliev, A., Wu, Q., Yoshikawa, K.,
674 Zheleznyak, M. and Lantuit, H.: Permafrost is warming at a global scale, *Nat. Commun.*,
675 10, 264, doi:[10.1038/s41467-018-08240-4](https://doi.org/10.1038/s41467-018-08240-4), 2019.

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

680 Brie, C., Moreira, D. and Lopez-Garcia, P.: Archaeal and bacterial community
681 composition of sediment and plankton from a suboxic freshwater pond, *Res. Microbiol.*,
682 158, 213-227, doi:[10.1016/j.resmic.2006.12.012](https://doi.org/10.1016/j.resmic.2006.12.012), 2007.

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

689 Carbonero, F., Oakley, B. B. and Purdy, K. J.: Metabolic flexibility as a major predictor of
690 spatial distribution in microbial communities, *PLoS One*, 9, e85105,
691 doi:[10.1371/journal.pone.0085105](https://doi.org/10.1371/journal.pone.0085105), 2014.

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

695 Chase, J. M.: Stochastic community assembly causes higher biodiversity in more
696 productive environments, *Science*, 328, 1388-1391, doi:10.1126/science.1187820,
697 2010.

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

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

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

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

708 de Jong, A., In, T. Z. M., Meisel, O. H., Jetten, M., Dean, J. F., Rasigraf, O. and Welte, C.
709 U.: Increases in temperature and nutrient availability positively affect methane-cycling
710 microorganisms in Arctic thermokarst lake sediments, *Environ. Microbiol.*, 20, 4314-
711 4327, doi:10.1111/1462-2920.14345, 2018.

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

715 Elder, C. D., Thompson, D. R., Thorpe, A. K., Chandanpurkar, H. A., Hanke, P. J., Hasson,
716 N., James, S. R., Minsley, B. J., Pastick, N. J., Olefeldt, D., Walter Anthony, K. M. and
717 Miller, C. E.: Characterizing Methane Emission Hotspots From Thawing Permafrost,
718 *Glob. Biogeochem. Cycle*, 35, e2020GB006922, doi:10.1029/2020GB006922, 2021.

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

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

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

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

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

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

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

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

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

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

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

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

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

761 Jiao, S. and Lu, Y.: Abundant fungi adapt to broader environmental gradients than rare
762 fungi in agricultural fields, *Glob. Change Biol.*, 26, 4506-4520, doi:10.1111/gcb.15130,
763 2020.

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

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

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

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

775 Lawrence, D. M., Slater, A. G. and Swenson, S. C.: Simulation of Present-Day and Future
776 Permafrost and Seasonally Frozen Ground Conditions in CCSM4, *J. Clim.*, 25, 2207-
777 2225, doi:10.1175/JCLI-D-11-00334.1, 2012.

778 Le Moigne, A., Bartosiewicz, M., Schaeppman Strub, G., Abiven, S. and Pernthaler, J.: The
779 biogeochemical variability of Arctic thermokarst ponds is reflected by stochastic and
780 niche - driven microbial community assembly processes, *Environ. Microbiol.*, 22,
781 4847-4862, doi:10.1111/1462-2920.15260, 2020.

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

786 Leibold, M. A., Chase, J. M. and Ernest, S. K.: Community assembly and the functioning

787 of ecosystems: how metacommunity processes alter ecosystems attributes, *Ecology*, 98,
788 909-919, doi:10.1002/ecy.1697, 2017.

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

791 Lindstrom, E. S. and Langenheder, S.: Local and regional factors influencing bacterial
792 community assembly, *Environ. Microbiol. Rep.*, 4, 1-9, doi:10.1111/j.1758-
793 2229.2011.00257.x, 2012.

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

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

799 Lozupone, C. A. and Knight, R.: Global patterns in bacterial diversity, *Proceedings of the*
800 *National Academy of Sciences*, 104, 11436-11440, doi:10.1073/pnas.0611525104,
801 2007.

802 Lu, W., Wang, W., Shao, Q., Yu, Z., Hao, Z., Xing, W., Yong, B. and Li, J.: Hydrological
803 projections of future climate change over the source region of Yellow River and
804 Yangtze River in the Tibetan Plateau; a comprehensive assessment by coupling
805 RegCM4 and VIC model, *Hydrol. Process.*, 32, 2096-2117, doi:10.1002/hyp.13145,
806 2018.

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

810 Luo, D., Jin, H., Du, H., Li, C., Ma, Q., Duan, S. and Li, G.: Variation of alpine lakes from
811 1986 to 2019 in the Headwater Area of the Yellow River, Tibetan Plateau using Google
812 Earth Engine, *Adv. Clim. Chang. Res.*, 11, 11-21, doi:10.1016/j.accre.2020.05.007,
813 2020.

814 Luo, J., Niu, F., Lin, Z., Liu, M. and Yin, G.: Thermokarst lake changes between 1969 and
815 2010 in the Beilu River Basin, Qinghai-Tibet Plateau, China, *Sci. Bull.*, 60, 556-564,
816 doi:10.1007/s11434-015-0730-2, 2015.

817 Makoto, K., Wilson, S. D., Turner, B. and Turner, B.: When and where does dispersal
818 limitation matter in primary succession? *J. Ecol.*, 107, 559-565, doi:10.1111/1365-
819 2745.12988, 2019.

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

822 Manasypov, R. M., Pokrovsky, O. S., Shirokova, L. S., Auda, Y., Zinner, N. S., Vorobyev,
823 S. N. and Kirpotin, S. N.: Biogeochemistry of macrophytes, sediments and porewaters
824 in thermokarst lakes of permafrost peatlands, western Siberia, *Sci. Total Environ.*, 763,
825 144201, doi:10.1016/j.scitotenv.2020.144201, 2021.

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

831 Mishra, U., Hugelius, G., Shelef, E., Yang, Y., Strauss, J., Lupachev, A., Harden, J. W.,
832 Jastrow, J. D., Ping, C. L., Riley, W. J., Schuur, E., Matamala, R., Siewert, M., Nave,

833 L. E., Koven, C. D., Fuchs, M., Palmtag, J., Kuhry, P., Treat, C. C., Zubrzycki, S.,
834 Hoffman, F. M., Elberling, B., Camill, P., Veremeeva, A. and Orr, A.: Spatial
835 heterogeneity and environmental predictors of permafrost region soil organic carbon
836 stocks, *Sci. Adv.*, 7, 5236, doi:10.1126/sciadv.aaz5236, 2021.

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

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

843 Narancic, B., Wolfe, B. B., Pienitz, R., Meyer, H. and Lamhonwah, D.: Landscape-gradient
844 assessment of thermokarst lake hydrology using water isotope tracers, *J. Hydrol.*, 545,
845 327-338, 2017.

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

850 Ning, D., Deng, Y., Tiedje, J. M. and Zhou, J.: A general framework for quantitatively
851 assessing ecological stochasticity, *Proceedings of the National Academy of Sciences*,
852 116, 16892-16898, doi:10.1073/pnas.1904623116, 2019.

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

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

860 Nossa, C. W., Oberdorf, W. E., Yang, L., Aas, J. A., Paster, B. J., Desantis, T. Z., Brodie,
861 E. L., Malamud, D., Poles, M. A. and Pei, Z.: Design of 16S rRNA gene primers for
862 454 pyrosequencing of the human foregut microbiome, *World J. Gastroenterol.*, 16,
863 4135-4144, doi:10.3748/wjg.v16.i33.4135, 2010.

864 Obu, J.: How much of the Earth's surface is underlain by permafrost? *Journal of*
865 *Geophysical Research: Earth Surface*, 126, 2021JF006123, doi:10.1029/2021JF006123,
866 2021.

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

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

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

877 Pearman, J. K., Biessy, L., Thomson-Laing, G., Waters, S., Vandergoes, M. J., Howarth,
878 J. D., Rees, A., Moy, C., Pochon, X. and Wood, S. A.: Local factors drive bacterial and

879 microeukaryotic community composition in lake surface sediment collected across an
880 altitudinal gradient, *FEMS Microbiol. Ecol.*, 96, doi:10.1093/femsec/fiaa070, 2020.

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

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

888 Picazo, F., Vilmi, A., Aalto, J., Soininen, J., Casamayor, E. O., Liu, Y., Wu, Q., Ren, L.,
889 Zhou, J., Shen, J. and Wang, J.: Climate mediates continental scale patterns of stream
890 microbial functional diversity, *Microbiome*, 8, e92, doi:10.1186/s40168-020-00873-2,
891 2020.

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

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

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

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

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

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

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

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

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

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

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

928 Ren, Z., Wang, Z. M., Wang, Y., Ma, P. P., Niu, D. C., Fu, H. and Elser, J. J.: Soil bacterial
929 communities vary with grassland degradation in the Qinghai Lake watershed, *Plant Soil*,
930 460, 541-557, doi:10.1007/s11104-020-04823-7, 2021.

931 Rosseel, Y.: lavaan: An R Package for Structural Equation Modeling, *J. Stat. Softw.*, 48,
932 1-36, doi:10.18637/jss.v048.i02, 2012.

933 Ruiz-Gonzalez, C., Pablo Nino-Garcia, J. and Del Giorgio, P. A.: Terrestrial origin of
934 bacterial communities in complex boreal freshwater networks, *Ecol. Lett.*, 18, 1198-
935 1206, doi:10.1111/ele.12499, 2015.

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

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

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

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

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

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

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

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

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

967 Taş, N., Prestat, E., Wang, S., Wu, Y., Ulrich, C., Kneafsey, T., Tringe, S. G., Torn, M. S.,
968 Hubbard, S. S., Jansson, J. K., Pacific Northwest National Laboratory Pnnl, R. W. U.
969 S. and Lawrence Berkeley National Laboratory Lbnl, B. C. U. S.: Landscape
970 topography structures the soil microbiome in arctic polygonal tundra, *Nat. Commun.*,

971 9, 777-13, doi:10.1038/s41467-018-03089-z, 2018.

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

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

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

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

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

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

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

995 Vigneron, A., Lovejoy, C., Cruaud, P., Kalenitchenko, D., Culley, A. and Vincent, W. F.:
996 Contrasting Winter Versus Summer Microbial Communities and Metabolic Functions
997 in a Permafrost Thaw Lake, *Front. Microbiol.*, 10, 1656, doi:10.3389/fmicb.2019.01656,
998 2019.

999 Vucic, J. M., Gray, D. K., Cohen, R. S., Syed, M., Murdoch, A. D. and Sharma, S.: Changes
1000 in water quality related to permafrost thaw may significantly impact zooplankton in
1001 small Arctic lakes, *Ecol. Appl.*, 30, e02186, doi:10.1002/eap.2186, 2020.

1002 Walter, K. M., Zimov, S. A., Chanton, J. P., Verbyla, D. and Chapin, F. S.: Methane
1003 bubbling from Siberian thaw lakes as a positive feedback to climate warming, *Nature*,
1004 443, 71-75, doi:10.1038/nature05040, 2006.

1005 Wan, C., Gibson, J. J., Shen, S., Yi, Y., Yi, P. and Yu, Z.: Using stable isotopes paired with
1006 tritium analysis to assess thermokarst lake water balances in the Source Area of the
1007 Yellow River, northeastern Qinghai-Tibet Plateau, China, *Sci. Total Environ.*, 689,
1008 1276-1292, doi:10.1016/j.scitotenv.2019.06.427, 2019.

1009 Wan, D. S., Feng, J. J., Jiang, D. C., Mao, K. S., Duan, Y. W., Miede, G. and Opgenoorth,
1010 L.: The Quaternary evolutionary history, potential distribution dynamics, and
1011 conservation implications for a Qinghai-Tibet Plateau endemic herbaceous perennial,
1012 *Anisodus tanguticus* (Solanaceae), *Ecol. Evol.*, 6, 1977-95, doi:10.1002/ece3.2019,
1013 2016.

1014 Webb, C. O., Ackerly, D. D., Mcpeek, M. A. and Donoghue, M. J.: Phylogenies and
1015 community ecology, *Annual review of ecology and systematics*, 33, 475-505, 2002.

1016 West, J. J. and Plug, L. J.: Time-dependent morphology of thaw lakes and taliks in deep

1017 and shallow ground ice, *Journal of Geophysical Research*, 113, 1009,
1018 doi:10.1029/2006JF000696, 2008.

1019 Wu, L., Yang, F., Feng, J., Tao, X., Qi, Q., Wang, C., Schuur, E. A. G., Bracho, R., Huang,
1020 Y., Cole, J. R., Tiedje, J. M. and Zhou, J.: Permafrost thaw with warming reduces
1021 microbial metabolic capacities in subsurface soils, *Mol. Ecol.*, 31, 1403-1415,
1022 doi:10.1111/mec.16319, 2022.

1023 Wu, M., Chen, S., Chen, J., Xue, K., Chen, S., Wang, X., Chen, T., Kang, S., Rui, J., Thies,
1024 J. E., Bardgett, R. D. and Wang, Y.: Reduced microbial stability in the active layer is
1025 associated with carbon loss under alpine permafrost degradation, *Proceedings of the*
1026 *National Academy of Sciences*, 118, e2025321118, doi:10.1073/pnas.2025321118,
1027 2021.

1028 Wu, Q., Dong, X., Liu, Y., Jin, H. and Zhang, T.: Responses of permafrost on the Qinghai-
1029 Tibet Plateau, China, to climate change and engineering construction, *Arctic, Antarctic,*
1030 *and Alpine Research*, 39, 682-687, doi:10.1657/1523-0430(07-508)[WU]2.0.CO;2,
1031 2007.

1032 Xiong, J., Liu, Y., Lin, X., Zhang, H., Zeng, J., Hou, J., Yang, Y., Yao, T., Knight, R. and
1033 Chu, H.: Geographic distance and pH drive bacterial distribution in alkaline lake
1034 sediments across Tibetan Plateau, *Environ. Microbiol.*, 14, 2457-2466,
1035 doi:10.1111/j.1462-2920.2012.02799.x, 2012.

1036 Xu, Z. X., Gong, T. L. and Li, J. Y.: Decadal trend of climate in the Tibetan Plateau-
1037 regional temperature and precipitation, *Hydrol. Process.*, 22, 3056-3065,
1038 doi:10.1002/hyp.6892, 2008.

1039 Yang, J., Jiang, H., Dong, H. and Liu, Y.: A comprehensive census of lake microbial
1040 diversity on a global scale, *Science China Life Sciences*, 62, 1320-1331,
1041 doi:10.1007/s11427-018-9525-9, 2019.

1042 Yang, J., Ma, L., Jiang, H., Wu, G. and Dong, H.: Salinity shapes microbial diversity and
1043 community structure in surface sediments of the Qinghai-Tibetan Lakes, *Sci. Rep.*, 6,
1044 e25078, doi:10.1038/srep25078, 2016b.

1045 Yang, Y., Wu, Q., Yun, H., Jin, H. and Zhang, Z.: Evaluation of the hydrological
1046 contributions of permafrost to the thermokarst lakes on the Qinghai-Tibet Plateau using
1047 stable isotopes, *Glob. Planet. Change*, 140, 1-8, doi:10.1016/j.gloplacha.2016.03.006,
1048 2016a.

1049 Yao, T., Thompson, L. G., Mosbrugger, V., Zhang, F., Ma, Y., Luo, T., Xu, B., Yang, X.,
1050 Joswiak, D. R., Wang, W., Joswiak, M. E., Devkota, L. P., Tayal, S., Jilani, R. and
1051 Fayziev, R.: Third Pole Environment (TPE), *Environ. Dev.*, 3, 52-64,
1052 doi:10.1016/j.envdev.2012.04.002, 2012.

1053 Yu, G., Smith, D. K., Zhu, H., Guan, Y. and Lam, T. T. Y.: ggtree: an R package for
1054 visualization and annotation of phylogenetic trees with their covariates and other
1055 associated data, *Methods Ecol. Evol.*, 8, 28-36, 2017.

1056 Yu, H., Favre, A., Sui, X., Chen, Z., Qi, W., Xie, G., Kleunen, M. and van Kleunen, M.:
1057 Mapping the genetic patterns of plants in the region of the Qinghai - Tibet Plateau:
1058 Implications for conservation strategies, *Diversity & distributions*, 25, 310-324,
1059 doi:10.1111/ddi.12847, 2019.

1060 Zhang, D., Wang, L., Qin, S., Kou, D., Wang, S., Zheng, Z., Peñuelas, J. and Yang, Y.:
1061 Microbial nitrogen and phosphorus co - limitation across permafrost region, *Glob.*
1062 *Change Biol.*, 29, 3910-3923, doi:10.1111/gcb.16743, 2023.

1063 Zhang, J. L.: Species association analysis, Version 0.2.2. [https://CRAN.R-](https://CRAN.R-project.org/package=spaa)
1064 [project.org/package=spaa](https://CRAN.R-project.org/package=spaa),2016.

1065 Zhang, Z., Chang, J., Xu, C., Zhou, Y., Wu, Y., Chen, X., Jiang, S. and Duan, Z.: The
1066 response of lake area and vegetation cover variations to climate change over the
1067 Qinghai-Tibetan Plateau during the past 30 years, *The Science of the total environment*,
1068 635, 443-451, doi:10.1016/j.scitotenv.2018.04.113, 2018.

1069 Zhou, C. P.: The spatial dataset of climate on the Tibetan Plateau (1961-2020). National
1070 Tibetan Plateau Data Center, doi:10.11888/AtmosphericPhysics.tpe.49.file, 2018.

1071 Zhou, J. and Ning, D.: Stochastic Community Assembly: Does It Matter in Microbial
1072 Ecology? *Microbiol. Mol. Biol. Rev.*, 81, e00002-17, doi:10.1128/MMBR.00002-17,
1073 2017.

1074 Zhou, J., Deng, Y., Zhang, P., Xue, K., Liang, Y., Van Nostrand, J. D., Yang, Y., He, Z.,
1075 Wu, L., Stahl, D. A., Hazen, T. C., Tiedje, J. M. and Arkin, A. P.: Stochasticity,
1076 succession, and environmental perturbations in a fluidic ecosystem, *Proceedings of the*
1077 *National Academy of Sciences*, 111, E836-E845, doi:10.1073/pnas.1324044111, 2014.

1078 Zhou, J., Liu, W., Deng, Y., Jiang, Y., Xue, K., He, Z., Van Nostrand, J. D., Wu, L., Yang,
1079 Y., Wang, A., Lawrence Berkeley National Lab. Lbnl, B. C. U. S. and Handelsman, J.:
1080 Stochastic assembly leads to alternative communities with distinct functions in a
1081 bioreactor microbial community, *mBio*, 4, e00584-12, doi:10.1128/mBio.00584-12,
1082 2013.

1083 Zhou, L., Zhou, Y., Yao, X., Cai, J., Liu, X., Tang, X., Zhang, Y., Jang, K. and Jeppesen,
1084 E.: Decreasing diversity of rare bacterial subcommunities relates to dissolved organic
1085 matter along permafrost thawing gradients, *Environ. Int.*, 134, 105330,
1086 doi:10.1016/j.envint.2019.105330, 2020.

1087 Zou, D., Zhao, L., Sheng, Y., Chen, J., Hu, G., Wu, T., Wu, J., Xie, C., Wu, X., Pang, Q.,
1088 Wang, W., Du, E., Li, W., Liu, G., Li, J., Qin, Y., Qiao, Y., Wang, Z., Shi, J. and Cheng,
1089 G.: A new map of permafrost distribution on the Tibetan Plateau, *Cryosphere*, 11, 2527-
1090 2542, doi:10.5194/tc-11-2527-2017, 2017.

1091