1 **Homogeneous environmental selection overturns distance-decay relationship of soil**

2 **prokaryotic community**

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23   **Abstract**

24   Though being fundamental to global diversity distribution, little is known about the geographic

25   pattern of soil microorganisms across different biomes on a large scale. Here, we investigated soil

26   prokaryotic communities from Chinese northern grasslands on a scale up to 4,000 km in both alpine

27   and temperate biomes. Surprisingly, prokaryotic similarities increased with geographic distance

28   after tipping points of 1,760 - 1,920 km, overturning the well-accepted distance-decay relationship

29   and generating a significant U-shape pattern. Such U-shape pattern was likely due to decreased

30   disparities in environmental heterogeneity along with geographic distance when across biomes, as

31   homogeneous environmental selection dominated prokaryotic assembly based on βNTI analysis.

32   Consistently, short-term environmental heterogeneity also followed the U-shape pattern spatially,

33   mainly attributed to dissolved nutrients. In sum, these results demonstrate that homogeneous

34   environmental selection via dissolved nutrients overwhelmed the "distance" effect when across

35   biomes, subverting the previously well-accepted geographic pattern for microbes on a large scale.

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Introduction

To clarify the spatial pattern of biodiversity is one of primary aims in ecology and biogeography (1, 2). In past decades, intensive biogeographic studies have been conducted for macro-organisms, including plants (3-8), insects (9, 10) and animals (11, 12). With the emergence and development of next-generation sequencing, increasing attention has been paid recently to the spatial pattern of microorganisms. The similarity of microbial communities has been observed to decrease as that of macro-organisms does with geographic distance, so-called distance-decay relationship, in different habits (e.g., forests (13, 14), grasslands (15), deserts (16) and agriculture soils (17-19)) for bacteria (15, 20, 21), archaea (18, 22), fungi (23-26) and speicific micorbial functional groups (e.g. ammonia-oxidizing archaea, ammonia-oxidizing and sulfate-reducing bacteria (27-30)). The reported distance-decay relationship has been regarded as a principal generalization in nature, which generally rejects the hypothesis of "everything is everywhere, but environment selects" (31).

However, no consensus has been reached so far on underlying mechanisms of the distance-decay relationship for soil microbial communities. A few mechanisms for biodiversity maintenance (20) were proposed to be responsible for such relationship as well, including dispersal limitation, environmental heterogeneity and stochastic processes (4, 32, 33). Microorganisms have been observed to have the dispersal limitation as macro-organisms do (2, 34, 35), crucial in biodiversity maintenance and evolution (33, 36). Spatial configurations and the nature of landscapes influence the dispersal rate of organisms among sites (32), and communities tend to be more similar in open and topographically homogeneous settings than in heterogeneous landscapes. Moreover, environmental heterogeneity tends to increase with geographic distance, responsible for the

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67   distance-decay relationship as well (2, 18). Communities are expected to become increasingly

68   different along with geographic distance as their species are sorted according to their niche

69   requirements (34). Under this scenario, dissimilarities among communities parallel to increasing

70   disparities in environmental heterogeneity along with geographic distance. Furthermore, stochastic

71   processes in birth, death, migration, disperse and drift may also contribute to the distance-decay

72   relationship for soil microbial communities (37-39).

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74   The relative importance of various mechanisms is still unclear (4, 32, 33), likely being scale- and

75   biome-dependent. Environment heterogeneity has been reported to be more important in influencing

76   the spatial distribution of microorganisms at local scales up to hundreds of kilometers (19, 40, 41),

77   while dispersal limitation dominated the distance-decay relationship on larger scales (42, 43).

78   Moreover, different biomes under distinct climate conditions/latitudes may have different turnover

79   rates for community similarity over geographic distance. High temperature in forest soils was

80   reported to lead to a lower turnover rate (30), likely due to accelerated biochemical reactions and

81   increased ecological niche breadth (44). Community similarity was observed to decline faster at

82   high than low latitudes on large scales, while the turnover rate was higher at low latitudes on small

83   scales (32). However, most previous studies were conducted locally or regionally within a single

84   biome or climate type. Thus, surveys for the geographic pattern of soil microorganisms across

85   different biomes is essential to understand the spatial pattern of microbial communities beyond these

86   scales.

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88   Here, we collected grassland soil samples from two biomes with distinct hydrothermal conditions

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89   to investigate the spatial pattern of prokaryotic communities and underlying mechanisms. A total of

90   258 samples were collected from the top- and subsoils in alpine and temperate biomes on a scale up

91   to 4,000 km, on Tibet Plateau and Inner Mongolia Plateau, respectively. Our objectives were to test

92   the following hypotheses: (I) soil prokaryotic community similarity would decrease over geographic

93   distance within and across biomes; (II) the turnover rate of soil prokaryotic community similarity

94   over geographic distance would be higher in the temperate than alpine biome, as temperate biome

95   is with a wider temperature range; (III) the turnover rate of soil prokaryotic community similarity

96   over geographic distance would be lower in top- than subsoil, as the subsoil may be less dynamic

97   and affected by environmental factors like UV and wind.

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99   **Results**

100   **Prokaryotic and plant community similarity over geographic distance**

101   A total of 11,063 OTUs were detected from all 258 grassland soil samples in both alpine and

102   temperate biomes. A significant ($P < 0.001$) binomial relationship (U shape) was observed for the

103   prokaryotic community over geographic distance in top- ($R^2 = 0.161$) or subsoil ($R^2 = 0.114$) from

104   all sites on a scale up to 4,000 km. Specifically, the prokaryotic community similarity in topsoil

105   decreased over geographic distance on a scale of < 1,920 km mostly within either temperate or

106   alpine biome, but increased after this tipping point when across biomes (in pairwise sites between

107   alpine and temperate biomes). Similarly, the prokaryotic community similarity in subsoil decreased

108   over geographic distance on a scale of < 1,760 km mostly within either temperate or alpine biome,

109   but increased after this tipping point when across biomes (Figure 1). When across biomes, the

110   prokaryotic community similarity increased significantly over geographic distance with similar

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slopes (turnover rates) in top- (slope = 0.007, R^2 = 0.199) and subsoil (slope = 0.006, R^2 = 0.134).
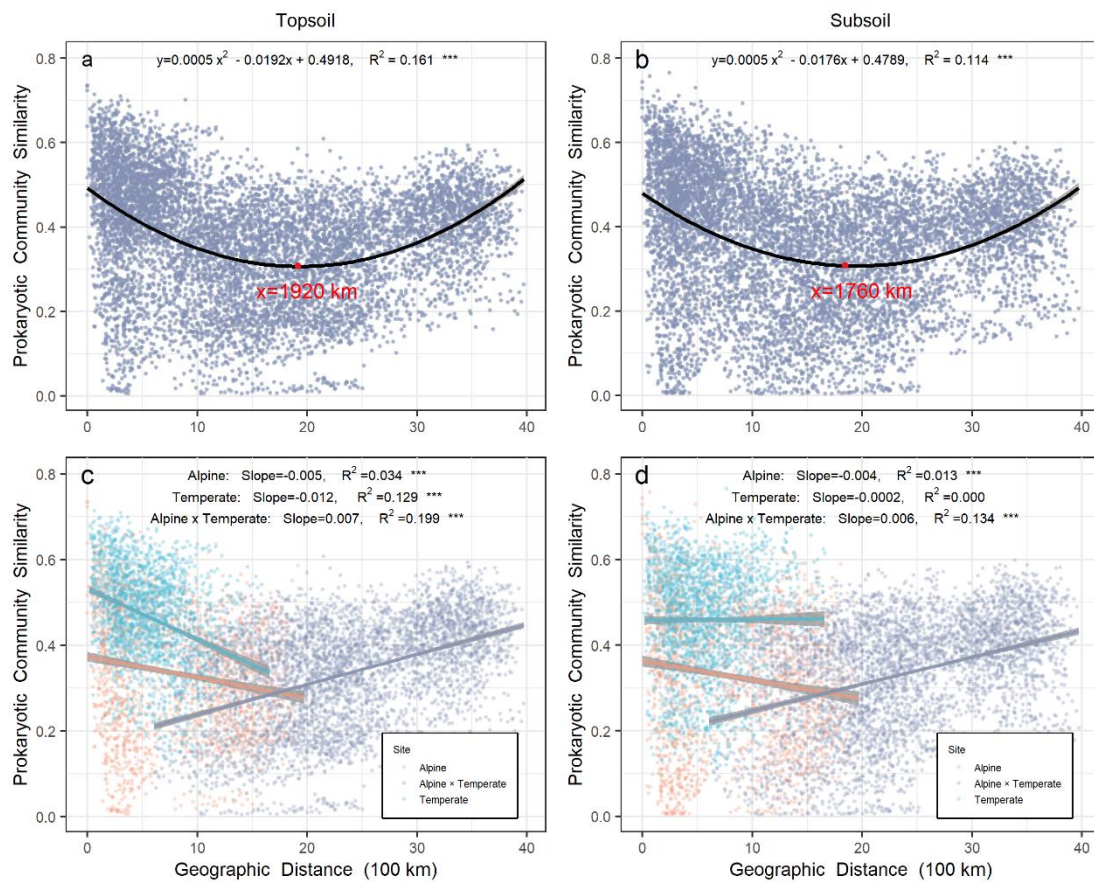
Figure 1 Relationship between prokaryotic community similarity over geographic distance in Chinese northern grassland. Panel **a** and **c** represent the prokaryotic community in topsoil, while panel **b** and **d** represent the prokaryotic community in subsoil. Orange and light blue points represent pairwise sites within the alpine and temperate biome, respectively. Grey points represent pairwise sites between the alpine biome cross temperate biome. Grey shades stand for 95% confidence interval.

Within the alpine biome, a valid ($P < 0.001$) distance-decay relationship was observed for the prokaryotic community over geographic distance in top- (R^2 = 0.034) or subsoil (R^2 = 0.013). However, within the temperate biome, the distance-decay relationship for the prokaryotic

123 community occurred only in topsoil ($R^2 = 0.129$, $P < 0.001$), while no relationship was observed in

124 subsoil. In topsoil, prokaryotic community similarity had a higher turnover rate in the temperate (-

125 0.012) than alpine biome (- 0.005).

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127 Similar to the prokaryotic community, the plant community also exhibited a significant U-shape

128 relationship ($R^2 = 0.071$, $P < 0.001$) for its similarity over geographic distance in all sites on a scale

129 up to 4,000 km, with a tipping point of 1,858 km (Figure S2a). A significant ($P < 0.001$) distance-

130 decay relationship for plant community was observed within the alpine (Figure S2b, $R^2 = 0.015$, P

131 < 0.001) or temperate biome (Figure S2b, $R^2 = 0.005$, $P < 0.01$).

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133 **Prokaryotic community similarity over environmental distance**
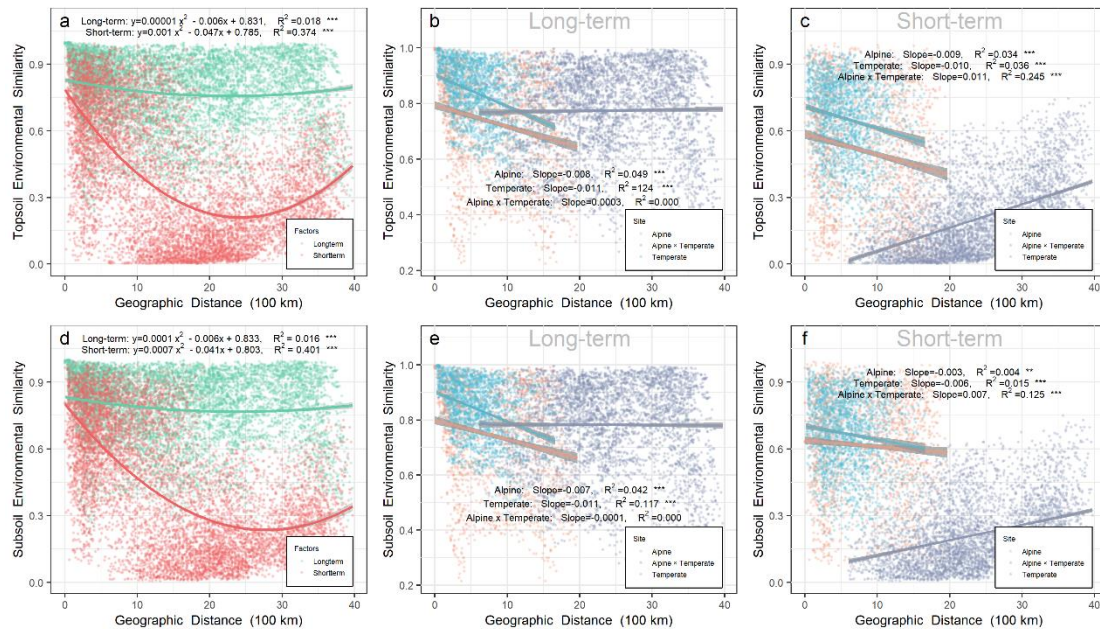
135 **Figure 2 Relationship between environmental similarity over geographic distance in Chinese**

136 **northern grassland.** Panel a, b and c represent the environmental similarity in topsoil, while panel

137 d, e and f represent the environmental similarity in subsoil. The environmental similarity was

138   calculated by Bray-Curtis index based on relatively long-term (b, e & green points in a and d) or

139   short-term variables (c, f & red points in a and d). Relatively long-term environmental variables

140   included mean annual precipitation (MAP), mean annual temperature (MAT), pH, soil organic

141   carbon (SOC), soil total nitrogen (TN) and soil total phosphorus (TP). Relatively short-term

142   environmental variables included soil water content (SWC), soil available phosphorus (AP),

143   dissolved organic carbon (DOC), dissolved organic nitrogen (DON), NH_4^+, and NO_3^-. Orange, grey,

144   and light blue points represent pairwise sites within the alpine biome, within temperate biome, and

145   alpine biome cross temperate biome, respectively. Grey shades stand for 95% confidence interval.

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147   Relatively short-term environmental similarity also exhibited a U-shape pattern over geographic

148   distance in either top- or subsoil from all sites on a scale up to 4,000 km (Figure 2 a and d). In

149   contrast, relatively long-term environmental similarity, much higher than short-term environmental

150   similarity on the same scale, did not change greatly over geographical distance in either top- or

151   subsoil.

152

153   Soil prokaryotic community similarity decreased significantly ($P < 0.001$) in all sites over the

154   relatively long-term (turnover rate = - 0.291 or - 0.278 in top- or subsoil, respectively) or short-term

155   (turnover rate = - 0.193 or - 0.159 in top- or subsoil, respectively) environmental distance (Figure

156   S3 a and e). In the topsoil, the prokaryotic community similarity decreased significantly ($P < 0.001$)

157   over the relatively long-term (turnover rate = - 0.277, $R^2 = 0.131$) or short-term (turnover rate = -

158   0.194, $R^2 = 0.135$) environmental distance within the alpine biome (Figure S3 b), as well as

159   decreased over long-term （turnover rate=-0.339, $R^2 = 0.108$）or short-term（turnover rate=-0.063,

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160     $R^2 = 0.011$）environmental distance within the temperate biome (Figure S3 c). In the subsoil, the

161   prokaryotic community similarity decreased significantly over the relatively long-term (turnover

162   rate = - 0.279, $R^2 = 0.104$) or short-term (turnover rate = - 0.215, $R^2 = 0.093$) environmental distance

163   within the alpine biome (Figure S3 f), while there was no relationship within the temperate biome

164   (Figure S3 g). In pairwise sites between the alpine cross temperate biome (Figure S3 d and h), the

165   prokaryotic community similarity decreased over relatively long-term (turnover rate = - 0.161 or -

166   0.130 in top- or subsoil, respectively) or short-term (turnover rate = - 0.191 or - 0.175 in top- or

167   subsoil, respectively) environmental distance.

168

169   **Table 1** Partial Mantel test for relationship between prokaryotic community similarity and relatively

170   short-term environmental variables across biomes.

| | Alpine × Temperate | | | |
| | Topsoil | | Subsoil | |
	r	p	r	p
SWC	0.167	**0.002**	0.225	**<0.001**
AP	0.163	**<0.001**	0.038	0.231
DOC	0.221	**<0.001**	0.117	**0.027**
DON	0.225	**<0.001**	0.200	**0.002**
NH_4^+	0.026	0.331	0.065	0.093
NO_3^-	0.068	0.140	0.024	0.323
Short-term environment factors[*]	0.273	**0.001**	0.219	**0.001**

171   *by Mantel test

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173   As revealed by Partial Mantel test (Table 1), the significant decay relationship between topsoil

174   prokaryotic community similarity and relatively short-term environmental distance across biomes

175   was mainly driven by soil water content (SWC, r = 0.167, p = 0.002), available phosphorus (AP, r

176 = 0.163, p = 0.004), dissolved organic carbon (DOC, r = 0.221, p < 0.001) and dissolved organic

177 nitrogen (DON, r = 0.225, p < 0.001). Similar short-term environmental variables (except AP) were

178 responsible for the significant decay relationship in the subsoil.

179

180 Within the alpine biome, the significant distance-decay relationship between topsoil prokaryotic

181 community similarity and relatively long-term environmental distance was mainly driven by mean

182 annual precipitation (MAP, r = 0.249, p < 0.001), soil organic carbon (SOC, r = 0.269, p < 0.001),

183 and soil total nitrogen (TN, r = 0.239, p < 0.001), while SWC (r = 0.534, p < 0.001), AP (r = 0.297,

184 p < 0.001), DOC (r = 0.285, p < 0.001), DON (r = 0.278, p < 0.001), NH_4^+ (r = 0.223, p < 0.001),

185 and NO_3^- (r = 0.133, p = 0.031) were responsible for the significant distance-decay relationship

186 between topsoil prokaryotic community similarity and relatively short-term environmental distance

187 (Table S1 and S2, Figure S4). Similar relatively long-term and short-term environmental variables

188 (except AP, NH_4^+, and NO_3^-) were responsible for the significant distance-decay relationship of

189 prokaryotic community similarity in the subsoil within the alpine biome. Within the temperate

190 biome, the significant distance-decay relationship between topsoil prokaryotic community

191 similarity and relatively long-term environmental distance was driven by MAP (r = 0.334, p < 0.001),

192 SOC (r = 0.128, p = 0.022), and TN (r = 0.117, p = 0.021), while DOC (r = 0.059, p = 0.034) was

193 responsible for the significant distance-decay relationship between topsoil prokaryotic community

194 similarity and relatively short-term environmental distance.

195

196 **Deterministic and stochastic processes in prokaryotic community assembly**

197 βNTI (β-nearest taxon index) analysis was used to distinguish different processes in prokaryotic

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198   community assembly. As shown in Figure 3, the range of $|\beta\text{NTI}| > 2$ indicated that deterministic

199   processes played a dominant role (>85%) in prokaryotic community assembly. The contribution of

200   deterministic processes was relatively lower in the alpine (Figure 3j; 92.91% and 87.47% in top-

201   and sub-soil, respectively) than temperate (96.63% and 94.04% in top- and sub-soil, respectively)

202   biome, and higher in the topsoil than subsoil in all sites. Moreover, most βNTI values were less than

203   -2 either in the top- or subsoil from all sites, indicating that prokaryotic communities were assembled

204   mainly by homogeneous selection in deterministic processes.

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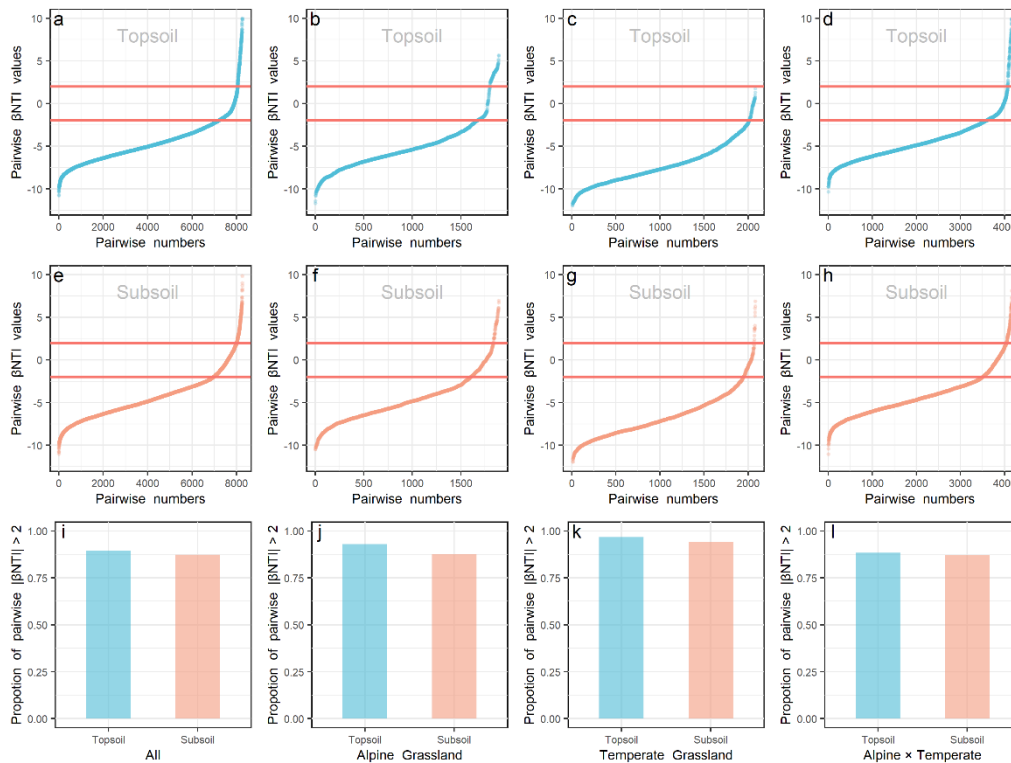
Figure 3 βNTI values. βNTI values of prokaryote in the top- (a, b, c and d) and subsoil (e, f, g and

208   h) from all sites (a and e), within alpine (b and f) or temperate biome (c and g), and pairwise sites

209   in the alpine cross temperate biomes (d and h) were presented. The proportion of $|\beta\text{NTI}| > 2$

210   (deterministic processes) in the top- and subsoil from all sites (i), within alpine biome (j), within

211   temperate biome (k) and pairwise sites in the alpine cross temperate biomes (l) were presented.

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212   Light blue and orange colors stand for βNTI values and their proportions in the top- and subsoil,

213   respectively.

214

215   We further compared the immigration rates (*m*) of prokaryotes (Figure S7) based on the algorithm

216   developed by Hubbell for the neutral theory (49). Prokaryotic immigration rates were significantly

217   lower in the alpine (0.159 ± 0.008 and 0.146 ± 0.008 in top- and subsoil, respectively) than temperate

218   biome (0.261 ± 0.010 and 0.246 ± 0.009 in top- and subsoil, respectively) in the same soil layer.

219   Moreover, immigration rates were higher in the top- (0.159 ± 0.008 and 0.261 ± 0.010 in the alpine

220   and temperate biomes, respectively) than subsoil (0.146 ± 0.008 and 0.246 ± 0.009 in the alpine and

221   temperate biomes, respectively) in the same biome.

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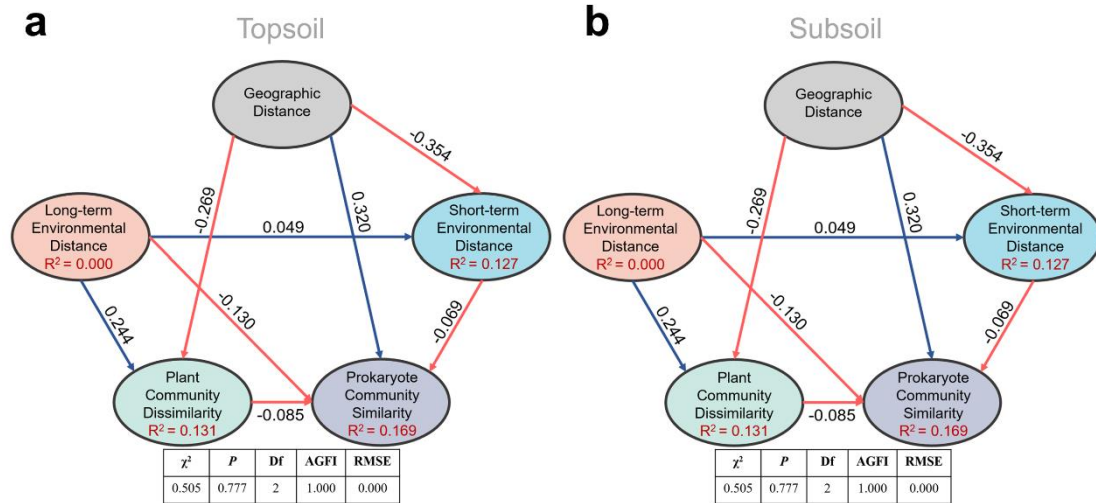
223

224   **Figure 4 Structure equation model to quantify effects of geographic distance, relatively long-**

225   **term and short-term environmental distance, and plant community dissimilarity on soil**

226   **prokaryotic community similarity in the pairwise sites between alpine cross temperate biomes,**

227   **either in** top-**(a)** or subsoil **(b)**. Red and blue lines stand for negative and positive correlations,

228   respectively; bold lines stand for significance at p = 0.05 level.

229

230   In the pairwise sites between alpine cross temperate biomes, structural equation model (SEM; Figure

231   4) showed prokaryotic community similarity was mainly affected by geographic distance (r = 0.388

232   and 0.320 in top- and subsoil, respectively), relatively long-term environmental distance (r = -0.171

233   and -0.130 in top- and subsoil, respectively), plant community dissimilarity (r = -0.124 and -0.065

234   in top- and subsoil, respectively), and relatively short-term environmental distance (r = -0.046 and

235   -0.069 in top- and subsoil, respectively).

236

237   Within each biome, geographic distance only had a direct effect on topsoil prokaryotic community

238   similarity (r = -0.275) in the temperate grassland, while its effect in topsoil of the alpine biome was

239   only indirect through relatively short-term (r = 0.140) and long-term environmental distances (r =

240   0.222), as well as plant community dissimilarity (r = 0.232). In the alpine biome, increases in plant

241   community dissimilarity, relatively long-term and short-term environmental distances directly

242   decreased the similarity of soil prokaryotic community. The explained variances of soil prokaryotic

243   community similarity were 26.8% and 21.3% in the top- and subsoil, respectively (Figure S6). In

244   the temperate biome, other than the direct effect of geographic distance, plant community

245   dissimilarity, relatively long-term and short-term environmental distances also affected topsoil

246   prokaryotic community similarity directly. In the subsoil of the temperate biome, prokaryotic

247   community similarity was not significantly correlated with any factors and the explained variance

248   was merely 0.2%.

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251 **Discussion**

252 "Everything is related to everything else, but near things are more related to each other" is termed

253 as "the first law of geography" (45). Ecologists and biogeographers refer it to the negative

254 relationship between community similarity and distance as a geographical distance-decay

255 relationship (46, 47). Though being regarded as a principal generalization, distance-decay

256 relationship was denied and overturned in this study as prokaryotic community similarity increased

257 with the geographical distance after tipping points of 1,920 - 1,760 km when across biomes. This

258 finding is contradictory with most previous studies, including a report that was conducted even at

259 the similar scale of 4,000 km to ours but within a single biome (temperate biome) (15). Consistently,

260 when within a single biome of alpine or temperate grassland, the distance-decay relationship was

261 also valid in this study.

262

263 However, over environmental distance, prokaryotic community similarity showed significant decay

264 relationships (Figure S5) in all sites on a scale up to 4,000 km in this study, consistent with previous

265 reports (48, 49). Thus, the U-shape pattern of prokaryotic community similarity over geographic

266 distance in all sites may be attributed to disparities in environmental heterogeneity over geographic

267 distance. This is also supported by the βNTI analysis showing that the prokaryotic community

268 assembly was dominantly determined by homogeneous selection in deterministic processes,

269 referring similar habitats (environment) harbor similar prokaryotic communities (50, 51). The role

270 of environment filtering, including biotic and abiotic factors, on microbial community assembly has

271 been widely reported at the scale of hundreds to thousands of kilometers (19, 40, 41).

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273   Environmental variables measured in this study were separated into relatively long-term and short-

274   term environmental variables, judged by dynamic time. Interestingly, the similarity of relatively

275   short-term environmental variables exhibited a U shape pattern over geographic distance in all sites

276   on a scale up to 4,000 km, while that of relatively long-term environmental variables did not,

277   indicating that relatively short-term environmental variables may be responsible for the

278   homogeneous selection shaping the U shape pattern of prokaryotic community over geographic

279   distance. Consistently, SEM analyses also revealed a significant direct effect of relatively short-term

280   environmental distance on prokaryotic community similarity when across biomes. The Partial

281   Mantel test further demonstrated that water (MAP, SWC) and dissolved nutrients (DOC, DON) were

282   the primary short-term environmental factors in the significant relationship between prokaryotic

283   community similarity and geographic distance. The effects of water on the microbial community

284   have been widely reported (52, 53), as soil water content could determine soil texture, bulk density,

285   oxygen availability and connectivity within soils (54-56), which can vitally influence soil microbial

286   community composition (57) and microbial basal respiration in the semi-arid area (58). Soil water

287   content also influences microbial communities through changing nutrient availability. Plenty of

288   studies found that DOC and DON affected the distribution pattern of soil microbes (41, 59-62).

289   Compared to other nutrients, DOC and DON can be utilized by microbes more directly and easily

290   to provide energy and nutrients for supporting their growth (63, 64).

291

292   In addition to abiotic factors, plant community attributes (65-69), especially plant species identity

293   (48, 70, 71), may also be important for the U-shape pattern of prokaryotic community similarity.

294   Our results demonstrated that soil prokaryotic community similarity decreased over the plant

295   community dissimilarity in all sites on a scale up to 4,000 km. Moreover, SEM also revealed the

296   significantly direct effect of plant community dissimilarity on prokaryotic community similarity in

297   pairwise sites between alpine cross temperate biomes. Plant community composition and diversity

298   can affect soil prokaryotic communities through altering the quality and quantity of organic matter

299   input to soils by the forms of litterfall and root exudates (72). Plants exude a substantial proposition

300   (11 - 40%) of photosynthesis-derived carbon (73), including sugars, amino acids, organic acids, fatty

301   acids and secondary metabolites (73-75). Their compounds in exudation can attract beneficial

302   microorganisms deliberately and influence the assembly of soil microbiomes to promote plants'

303   adaptation to the surrounding environment (76-81).

304

305   In the topsoil of temperate biome, MAP rather than MAT was responsible for the significant decay

306   relationship between prokaryotic community similarity and long-term environmental distance,

307   together with SOC and TN. In the alpine biome, both MAP and MAT were responsible for the

308   significant distance-decay relationship between the prokaryotic community similarity and long-term

309   environmental distance in either top- or subsoil, together with pH, SOC, and TN. These phenomena

310   indicated that the spatial pattern of soil prokaryotic community was driven by both temperature and

311   water in the alpine biome with a low temperature range (-0.8 to 5.9 °C) and a low precipitation range

312   (84 to 528 mm) (82). However, the spatial pattern of soil prokaryotic community in the temperate

313   biome was driven by precipitation (159 to 460 mm) only in the topsoil (15), where temperatue is a

314   limting factor (1.23 to 4.4 °C). Consistently, our previous study demonstrated that soil microbial

315   diversity in the alpine biome was mainly affected by temperature especially under the condition of

316   limited precipitation (83).

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317

318   In the topsoil, the turnover rate of prokaryote was higher in temperate than alpine biome (Figure 1).

319   We found that the immigration rate (m) of topsoil prokaryotes was higher in the temperate biome

320   than that of the alpine biome (Figure S7), indicating a weakened dispersal limitation that may be

321   responsible for the higher similarity of prokaryotic community in the temperate biome (2, 50). The

322   effects of dispersal limitation on microbial communities (43, 84, 85) were dependent on ecosystems

323   or environmental habitats (86-88). Harsh environments (50, 51, 89) with low temperature, high UV

324   and complex mountain terrain in the alpine biome on the Tibet Plateau would not be conducive for

325   soil prokaryote to disperse. In contrast, the temperate biome has benign temperature, low UV and

326   better landscape connectivity to promote the spatial dispersal of microorganisms.

327

328   Notably, within the temperate biome across 1,661 km, prokaryotic community similarity did not

329   change with the geographic distance in the subsoil. Moreover, subsoil prokaryotic community

330   similarity was not linked with plant community dissimilarity (Figure S5) and long-term

331   environmental distance (Figure S3), or correlated weakly with short-term environmental distance

332   based on the correlation test ($R^2 = 0.011$) and SEM (r = - 0.002), denying the possibility of plant

333   dependent and environment heterogeneity driven. Cases of no distance-decay relationship for

334   microorganism have also been reported previously (29, 90, 91), explained by the absence of

335   dispersal limitation (29), paleogeographic history (90, 91) that may also be applied in our case and

336   plant dependent (91).

337

338   **Conclusion**

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339   This study provides a systematical analysis of the spatial pattern of soil prokaryotic communities in

340   the northern grassland of China. Soil prokaryotic similarity exhibited a U-shape distribution pattern

341   over geographic distance at a scale of up to 4,000 km. This finding overturns the well-accepted

342   distance-decay relationship, which was only valid in the top- or subsoils within the alpine biome

343   and topsoil only within the temperate biome. Despite different climate and ecosystem types in the

344   alpine and temperate biomes, habitats far more apart when across biomes were more similarly as

345   revealed by the U-shape pattern for short-term environmental factors over geographic distance.

346   Consistently, deterministic processes were found to dominate the soil prokaryotic community

347   assembly by βNTI analysis, and further partial Mantel analysis revealed that water (MAP and SWC)

348   and dissolved nutrients (DOC and DON) together may be responsible for the U pattern of

349   prokaryotic community similarity over geographic distance, overturning the distance-decay

350   relationship.

351

352   **Materials and Methods**

353   **Study sites and field sampling**

354

355   A total of 129 sites and 258 samples were collected from the northern grassland of China. Among

356   them, 128 samples from 64 sites (red dots in Figure S1) were collected from July 29 to August 14,

357   2014 in the Qinghai-Tibet Plateau alpine biome, China. Alpine biome sample sites covered a variety

358   of alpine ecosystems, including alpine meadow, alpine steppe, alpine desert, and shrub. One hundred

359   and twenty-eight samples from 65 sites (blue dots in Figure S1) were collected from September 10

360   to 24, 2015 in the Inner Mongolia temperate biome, China. Temperate biome sample sites covered

361 three types of temperate biome ecosystem, namely temperate meadow, temperate steppe, and

362 temperate desert. The distance between each two adjacent sample sites was no less than 60 km and

363 removed from potential human interference such as towns, villages, and roads.

364 A GPS (global positioning system) was used to record the geographic coordinates and altitudes of

365 each sample site. Five plots were selected randomly at each site and the distance between the

366 adjacent two plots was no less than 10 m. After removing plant aboveground biomass and litter,

367 three topsoil (0-5 cm in depth) and subsoil (5-20 cm in depth) cores (7 cm in diameter) were

368 randomly sampled within each plot.

369

370 Topsoil or subsoil samples from each plot were pooled and then sieved through a 2 mm mesh, and

371 the roots were selected as belowground biomass (BGB). Sieved topsoil or subsoil samples were

372 divided into two subsamples. One part was stored at room temperature and dried in the shade for

373 measuring physical and chemical properties. The other part was stored at approximately 4 °C in the

374 field by a mobile refrigerator, delivered with dry ice to the laboratory in Beijing, and finally frozen

375 at -80 °C in a freezer before DNA extraction.

376

377 **Soil properties**

378 Soil pH was measured by pH meter (STARTER3100, Ohaus Instruments Co., Ltd., Shanghai, China)

379 with a 1:5 of soil water ratio (5 g soil: 25 mL ddH$_2$O). SWC was measured by ovening fresh soil

380 samples at 105°C for 24 h. SOC was measured by a TOC analyzer (Liqui TOC II; Elementar

381 Analysensysteme GmbH, Hanau, Germany). Soil TN was measured on an auto-analyzer (SEAL

382 Analytical GmbH, Norderstedt, Germany). Soil TP and AP were measured by a UV-VIS

383   spectrophotometer (UV2700, SHIMADZU, Japan). Nitrate-N (NO_3^-) and ammonium-N (NH_4^+)

384   were extracted with 2 M KCl (soil mass to solution ratio of 1:5) and then analyzed on a continuous-

385   flow ion auto-analyzer (SEAL Analytical GmbH, Norderstedt, Germany). Soil DOC and DON were

386   measured on a TOC Analyser (Liqui TOC II; Elementar Analysensysteme GmbH, Hanau, Germany).

387   Plant aboveground biomass (AGB) and belowground biomass (BGB) were measured after oven

388   drying at 65 °C for 72 h. MAT and MAP of each study site were obtained from "China

389   Meteorological Data Service Center" (CMDC: https://data.cma.cn/) by latitude and longitude.

390

391   **Microbial analysis**

392   Soil genomic DNA was extracted from 0.25 g frozen soil three times at each soil layer at each site

393   and then mixed into one DNA sample using PowerSoil™ DNA Isolation Kits (MO BIO Laboratories,

394   Carlsbad, CA, USA). The quality of extracted DNA was assessed based on OD 260/280 nm and

395   260/230 nm absorbance ratios by NanoDrop (2000) spectrophotometer (NanoDrop Technologies

396   inc., Wilmington, DE, USA).

397   Primer pair 515F (5'-GTGYCAGCMGCCGCGGTA-3') and 909R (5'-

398   CCCCGYCAATTCMTTTRAGT-3') was selected to amplify the V4-V5 region of 16S rRNA and

399   the target fragment length was 374 bp, and the 12bp barcode was added at the end of 5' of 515F. A

400   50 μL PCR reaction system was configured in 0.2 mL tube, including 2 μL template DNA diluent,

401   4 μL dNTP, 4 μL Mg_2^+, 5 μL Buffer, 0.5 L Ex Taq™ enzyme, 1 μL forward primer, 1 μL reverse

402   primer, 32.5 L ddH$_2$O. The PCR procedure was performed as follows: predenaturation at 95°C for

403   10 min, 30 PCR cycles (deformation at 94°C for 30 s, annealing at 53°C for 25 s, extension at 68°C

404   for 45 s), and a final extension at 72°C for 10 min.

405   The PCR products were purified by 1% agarose gel using GeneJET Gel Extraction Kit (Thermo,

406   USA). The purified DNA was tested by NanoDrop (2000) spectrophotometer (NanoDrop

407   Technologies inc., Wilmington, DE, USA). All purified DNA samples were mixed in 100 ng before

408   database construction and sequencing, which was performed by Illumina Miseq in Chengdu Biology

409   Institute.

410   The MiSeq raw data was analyzed by UPARSE pipeline with USEARCH 8 software to obtain an

411   operational taxon units (OTU) table. Each OTU was annotated by Mothur (v1.27) (92) with

412   classify.seqs command, and sliva.nr_v128.align was selected as the reference database. The OTU

413   table was resampled to the same sequence before further analysis by R 3.5.0 with the resample

414   package.

415

416   **Statistical analysis**

417   To compare the soil bacterial samples from different climate regions, we divided the soil samples

418   into alpine samples and temperate samples according to collection sites. The altitude of the alpine

419   biome sampling sites ranged from 2,796 to 4,891 m, and that of temperate biome was from 10 to

420   1,796 m. According to the sampling position in the soil layers, samples were divided into topsoil (0-

421   5 cm) samples and subsoil (5-20 cm) samples. The geographic distance between sites was calculated

422   based on geographic coordinates by the Euclidean distance method using the vegan package of R.

423   Plant communities were classified into four functional groups (grasses, sedges, legumes, and forbs)

424   and plant communities' similarity and dissimilarity were calculated based on Bray-Curtis distance

425   by the vegan package of R. Environmental factors were divided into relatively long-term

426   environmental variables that remain relatively stable at least within a year, representing historical

21

427   contingencies, and relatively short-term environmental variables that are dynamic within a year,

428   reflecting contemporary disturbances. Relatively long-term environmental variables included MAP,

429   MAT, pH, SOC, TN, and TP, while relatively short-term environmental variables included SWC,

430   AP, DOC, DON, NH_4^+, and NO_3^-.

431

432   The Bray-Curtis similarity and dissimilarity of the prokaryotic community were calculated using

433   OTU tables resampled to a minimum number of sequences from each sample (7500 in this study).

434   The Mantel test and Partial Mantel test based on a Pearson correlation were used to test the

435   relationship of soil prokaryotic similarity, geographic distance, and long-term multiple

436   environmental factors or short-term multiple environmental factors. The turnover rate was estimated

437   by the slope of the linear regression model based on the least square method. The tipping point was

438   calculated by the function of $d(Y)/d(x) = 0$ in binomial function. Pearson correlation was used

439   to test the relationship of soil prokaryotic diversity with environmental variables.

440

441   The βNTI was used to distinguish different ecological processes, including deterministic processes

442   (homogenous selection and heterogeneous selection), random dispersal (homogenous dispersal,

443   dispersal limitation), drift, and diversification (50). The $|\beta$NTI$| > 2$ means community was

444   constructed by deterministic processes, and βNTI < -2 means homogenous selection plays a major

445   role, while βNTI $> +2$ means heterogenous is more important. The $-2 < \beta$NTI $< +2$ means stochastic

446   processes determined community succession (93). A βNTI analysis was performed by R 3.5.0 with

447   the ape package. The estimation of immigration rate (m) was calculated by TeTame 2.0 (94) based

448   on Hubbell's neutral theory of biodiversity (95). Parameter estimation was rigorously performed by

449   maximum-likelihood using the sampling formula developed by Etienne (96-99). This model is seen

450   as a potentially useful null model in ecology; in this model, the species relative abundances in a

451   guild are determined by two parameters, namely θ and m. The θ governs the appearance of a new

452   species in the regional species pool, and m governs immigration into local communities of

453   individuals from the regional species pool. We further used SEM to disentangle the causal pathways

454   through which geographic distance, short-term environmental distance, long-term environmental

455   distance, and plants' community dissimilarity influence soil prokaryotic similarity. SEM in this

456   study is implemented by AMOS software.

457

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463

464   **Additional information**

465   Supplementary information is available for this paper. Reprints and permissions information is

466   available at www.nature.com/reprints.

467

468   **Competing interests**

469   The authors declare no competing financial interests.

470

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1   **Supplementary**
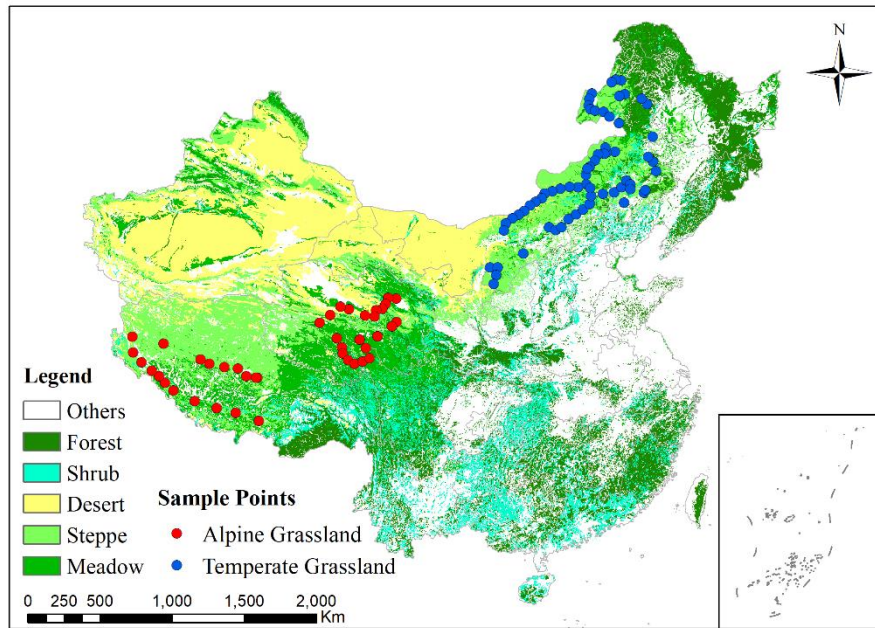
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3   **Figure S1 Sampling sites across 1,921 km of the alpine grassland in Qinghai-Tibet Plateau**

4   **(in red) and 1,661 km of the temperate grassland in Inner Mongolia (in blue).**

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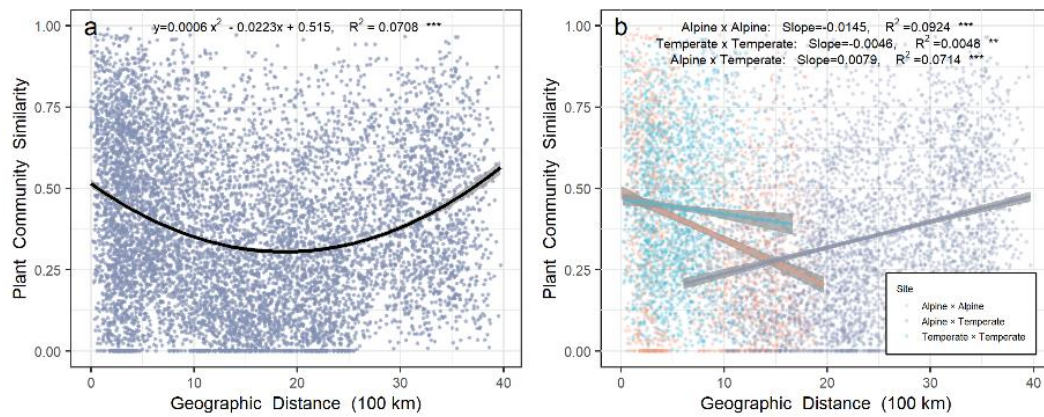
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Figure S2 Relationship between plant community similarity over geographic distance in northern grassland of China. Light blue points are for pairwise sites in the alpine grassland. Orange points are for pairwise sites in the temperate grassland. Grey points are for pairwise sites between the alpine grassland cross temperate grassland. Grey shades stand for 95% confidence interval.
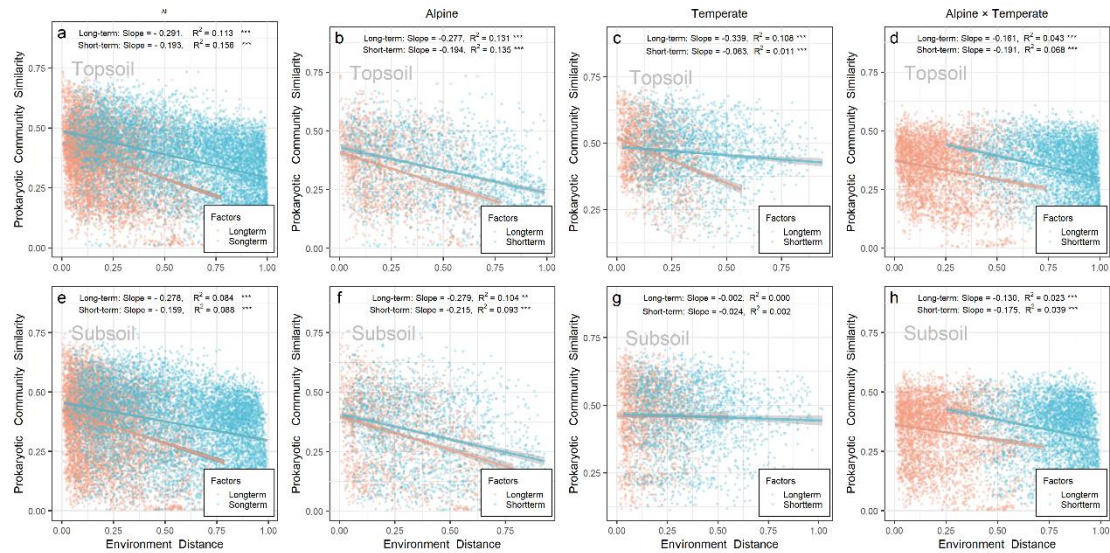
Figure S3 Distance decay relationship for prokaryotic community similarity over relative long-term (orange points) and short-term (light blue points) environmental factors. (a-d) topsoil; (e-h) subsoil. Shades stand for 95% confidence interval.
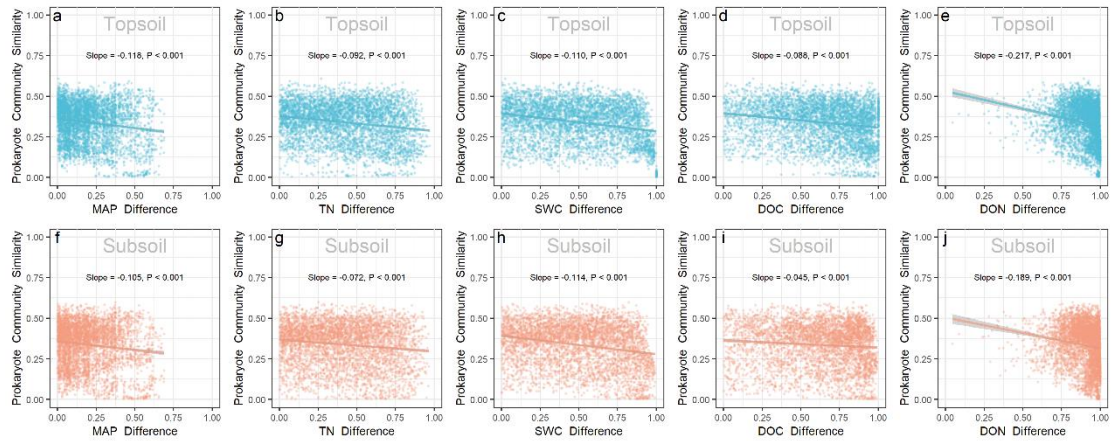
Figure S4 The scatter plots of the mantel correlation for prokaryotic community similarity with individual environment factor (MAP, TN, SWC, DOC, and DON) Bray-Curtis distance. Panels **a-e** were for topsoil, and panel **f-j** for subsoil.
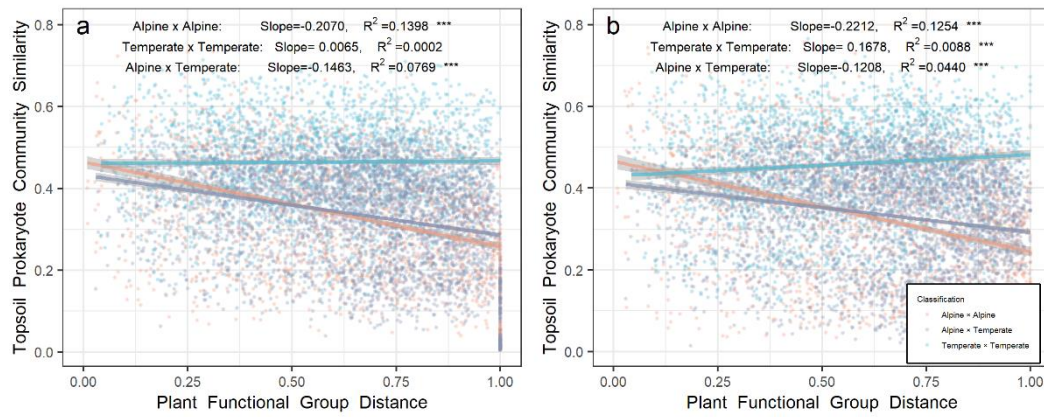
Figure S5 Relationship between prokaryote community over plant community dissimilarity.
(a) topsoil; (b)subsoil. Light blue points are for pairwise sites in the alpine grassland. Orange points are for pairwise sites in the temperate grassland. Grey points are for pairwise sites between the alpine grassland cross temperate grassland. Grey shades stand for 95% confidence interval.
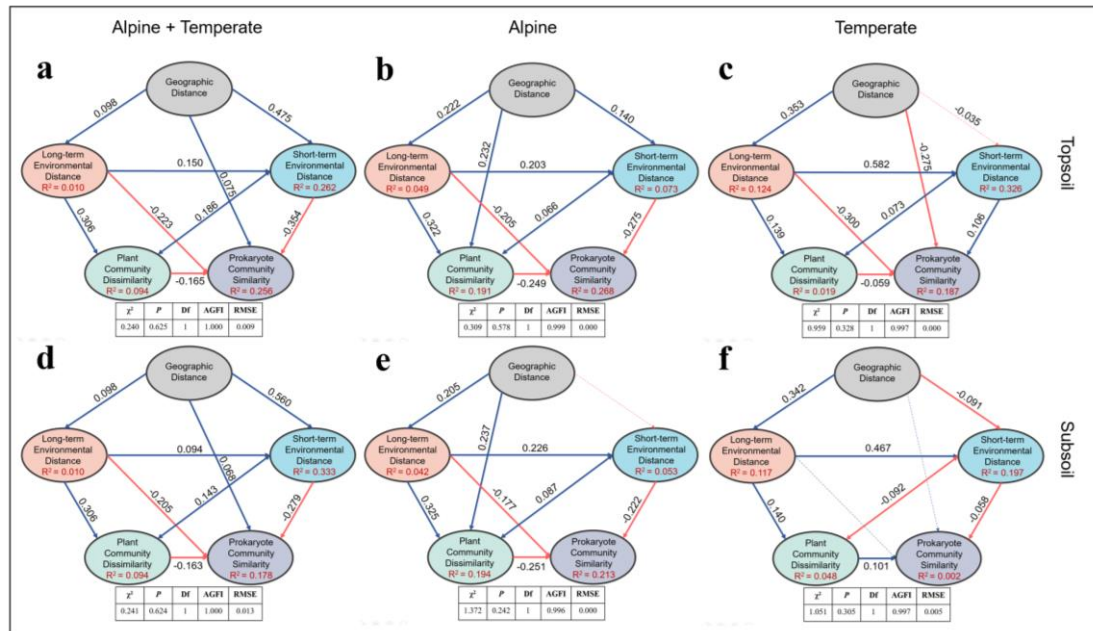
Figure S6 Structure equation model for geographic distance (based on Euclidean index), long-term environmental distance, short-term environmental distance, and plant community dissimilarity based on Bray-Curtis distance in affecting soil prokaryote community similarity. (a) topsoil of the northern grassland; (b) topsoil of the alpine grassland; (c) topsoil of the temperate grassland; (d) subsoil of the northern grassland; (e) subsoil of the alpine grassland; (f) subsoil of the temperate grassland. Red lines stand for negative correlation and blue lines stand for positive correlation; Bold lines stand for significance at 0.05 level.

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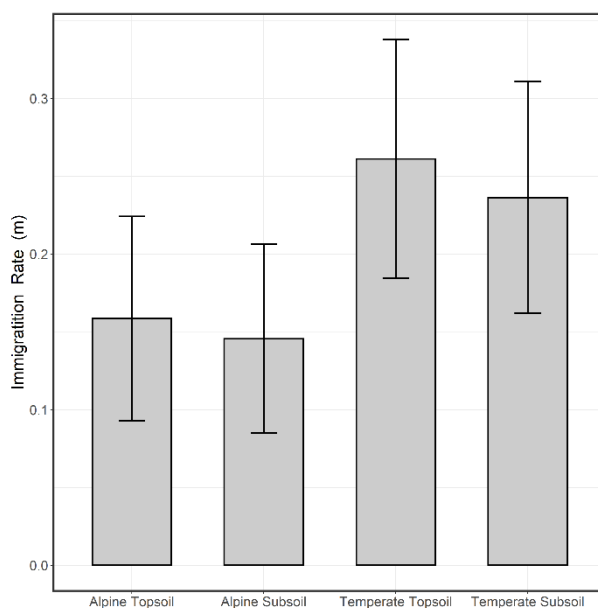
Figure S7 The immigration rate (m) of soil prokaryote community in topsoil and subsoil of the alpine and temperate grassland biomes

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Table S1 The mantel correlation of single environment factor with prokaryotic community similarity based on Bray-Curtis distance

| | All | | | | Alpine | | | | Temperate | | | | Alpine × Temperate | | | |
| | Topsoil | | Subsoil | | Topsoil | | Subsoil | | Topsoil | | Subsoil | | Topsoil | | Subsoil | |
	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p
MAP	0.276	<0.001	0.259	<0.001	0.274	<0.001	0.280	<0.001	0.331	<0.001	-0.006	0.797	0.157	<0.001	0.128	<0.001
MAT	-0.027	0.013	-0.008	0.441	0.022	0.343	-0.099	<0.001	-0.063	0.004	-0.035	0.109	-0.039	0.013	-0.006	0.694
pH	0.137	<0.001	0.094	<0.001	0.253	<0.001	0.204	<0.001	0.053	0.016	0.023	0.289	-0.042	0.006	-0.027	0.084
SOC	0.274	<0.001	0.191	<0.001	0.420	<0.001	0.277	<0.001	0.124	<0.001	0.067	0.002	0.187	<0.001	0.148	<0.001
TN	0.265	<0.001	0.170	<0.001	0.387	<0.001	0.254	<0.001	0.115	<0.001	0.060	0.006	0.193	<0.001	0.137	<0.001
TP	0.028	<0.001	0.030	0.006	-0.000	0.985	0.027	0.240	0.118	<0.001	0.094	<0.001	-0.146	<0.001	-0.124	<0.001
Long-term environment factors	0.337	0.001	0.291	0.001	0.363	0.001	0.322	0.001	0.328	0.001	0.002	0.447	0.181	0.002	0.154	0.006
SWC	0.353	<0.001	0.332	<0.001	0.602	<0.001	0.553	<0.001	0.093	<0.001	0.003	0.909	0.264	<0.001	0.241	<0.001
AP	0.185	<0.001	0.074	<0.001	0.355	<0.001	0.145	<0.001	0.002	0.912	0.080	<0.001	0.197	<0.001	0.080	<0.001
DOC	0.230	<0.001	0.145	<0.001	0.350	<0.001	0.279	<0.001	0.052	0.017	0.002	0.913	0.195	<0.001	0.088	<0.001
DON	0.397	<0.001	0.330	<0.001	0.334	<0.001	0.260	<0.001	0.073	<0.001	0.035	0.116	0.199	<0.001	0.159	<0.001
NH_4^+	0.217	<0.001	0.109	<0.001	0.284	<0.001	0.077	<0.001	0.018	0.408	0.026	0.236	0.109	<0.001	0.092	<0.001
NO_3^-	0.222	<0.001	0.111	<0.001	0.121	<0.001	0.078	<0.001	0.048	0.027	0.053	0.016	0.008	0.611	-0.002	0.920
Short-term environment factors	0.391	0.001	0.294	0.001	0.367	0.001	0.305	0.001	0.104	0.091	0.039	0.251	0.258	0.001	0.216	0.001

191 **Table S2** Partial Mantel test for correlations between prokaryotic community similarity based on Euclidean distance and each variable within long-term or short-term
192 environmental factors in topsoil or subsoil.

	All				Alpine				Temperate				Alpine × Temperate			
	Topsoil		Subsoil		Topsoil		Subsoil		Topsoil		Subsoil		Topsoil		Subsoil	
	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p
MAP	0.251	**<0.001**	0.241	**<0.001**	0.249	**0.002**	0.253	**0.003**	0.334	**<0.001**	-0.010	0.546	0.176	**<0.001**	0.149	**<0.001**
MAT	-0.039	0.984	-0.016	0.740	-0.003	0.518	-0.131	0.991	-0.064	0.983	-0.043	0.876	-0.065	0.980	0.003	0.439
pH	0.072	0.092	0.067	0.115	0.129	0.070	0.194	**0.025**	0.052	0.215	0.011	0.377	0.064	0.139	0.028	0.302
SOC	0.193	**<0.001**	0.144	**0.004**	0.269	**<0.001**	0.152	**0.009**	0.128	**0.022**	0.049	0.210	0.089	**0.021**	0.064	0.057
TN	0.197	**<0.001**	0.131	**0.007**	0.239	**<0.001**	0.150	**0.003**	0.117	**0.021**	0.042	0.237	0.116	**0.007**	0.101	**0.016**
TP	0.017	0.358	0.030	0.278	-0.022	0.576	0.035	0.265	0.119	0.056	0.080	0.123	-0.232	1.000	-0.181	0.996
Long-term environment factors	0.290	**0.001**	0.262	**0.001**	0.306	**<0.001**	0.272	**0.003**	0.329	**0.001**	-0.017	0.562	0.203	**0.001**	0.159	**0.003**
SWC	0.265	**<0.001**	0.266	**<0.001**	0.534	**<0.001**	0.488	**<0.001**	0.008	0.407	0.002	0.435	0.167	**0.002**	0.225	**<0.001**
AP	0.143	**0.004**	0.065	0.093	0.297	**<0.001**	0.114	0.056	-0.010	0.515	0.080	0.129	0.163	**<0.001**	0.038	0.231
DOC	0.187	**<0.001**	0.114	**0.007**	0.285	**<0.001**	0.207	**0.010**	0.059	**0.034**	0.002	0.407	0.221	**<0.001**	0.117	**0.027**
DON	0.365	**<0.001**	0.286	**<0.001**	0.278	**<0.001**	0.211	**0.002**	-0.089	0.919	0.037	0.247	0.225	**<0.001**	0.200	**0.002**
NH_4^+	0.143	**0.006**	0.083	0.078	0.223	**<0.001**	0.053	0.241	-0.071	0.908	0.026	0.315	0.026	0.331	0.065	0.093
NO_3^-	0.202	**<0.001**	0.094	**0.032**	0.133	**0.031**	0.091	0.073	0.018	0.368	0.053	0.224	0.068	0.140	0.024	0.323
Short-term environment factors	0.353	**0.001**	0.266	**0.001**	0.311	**<0.001**	0.250	**0.002**	-0.107	0.968	0.042	0.276	0.273	**0.001**	0.219	**0.001**

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194   **Table S3** Partial correlation of single environment factor with prokaryotic community similarity based on Euclidean distance at the scale of 0-1,920 km and 1,920 -
195   4,000 km in top-soil, and at the scale of 0-1,760 km and 1,760 - 4,000 km in sub-soil.

	Topsoil				Subsoil			
	0 - 1,920		1,920 - 4,000		0 - 1,760		1,760 - 4,000	
	r	p	r	p	r	p	r	p
MAP	0.194	**<0.001**	0.177	**<0.001**	0.207	**<0.001**	0.130	**<0.001**
MAT	-0.125	**<0.001**	0.059	**0.001**	-0.152	**<0.001**	0.027	0.119
pH	0.101	**<0.001**	0.065	**<0.001**	0.100	**<0.001**	0.068	**<0.001**
SOC	0.199	**<0.001**	0.288	**<0.001**	0.091	**<0.001**	0.204	**<0.001**
TN	0.159	**<0.001**	0.244	**<0.001**	0.023	0.114	0.156	**<0.001**
TP	0.065	**<0.001**	0.047	**0.011**	0.096	**<0.001**	0.071	**<0.001**
Long-term Environment Factors	0.272	**<0.001**	0.274	**<0.001**	0.268	**<0.001**	0.223	**<0.001**
SWC	0.187	**<0.001**	0.306	**<0.001**	0.095	**<0.001**	0.193	**<0.001**
AP	0.154	**<0.001**	0.214	**<0.001**	0.132	**<0.001**	0.118	**<0.001**
DOC	0.320	**<0.001**	0.318	**<0.001**	0.167	**<0.001**	0.221	**<0.001**
DON	0.311	**<0.001**	0.257	**<0.001**	0.241	**<0.001**	0.219	**<0.001**
NH_4^+	0.269	**<0.001**	0.219	**<0.001**	0.065	**<0.001**	0.065	**<0.001**
NO_3^-	0.104	**<0.001**	0.013	0.479	0.036	**0.013**	0.079	**<0.001**
Short-term Environment Factors	0.377	**<0.001**	0.267	**<0.001**	0.275	**<0.001**	0.180	**<0.001**

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