

Parental material and climate jointly determine the biomass and diversity of soil microbial communities along an elevational gradient on a subtropical karst mountain

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1	Parental material and climate jointly determine the biomass and diversity of soil		
2	microbial communities along an elevational gradient on a subtropical karst		
3	mountain		
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5	Xianjin He ^{1, 2, 3} , Lian Zeng ^{1, 2} , Guangyu Zhu ^{1, 2} , M. D. Farnon Ellwood ⁴ , Lihua Zhou ¹ ,		
6	² , Junlong Huang ^{1, 2} , Chenchen Wang ^{1, 2} , Wei Li ^{1, 2} , Dunmei Lin ^{1, 2} , Pei Wei ^{1, 2} , Shijun		
7	Liu ^{1, 2} , Min Luo ^{1, 2} , Yong-Hua Zhang ⁵ , Yongchuan Yang ^{1, 2, #}		
8			
9	¹ Key Laboratory of the Three Gorges Reservoir Region's Eco-Environment, Ministry of		
10	Education, Chongqing University, Chongqing, 400045, China.		
11	² College of Environment & Ecology, Chongqing University, Chongqing, 400045, China.		
12	³ CEA-CNRS-UVSQ, LSCE/IPSL, Université Paris Saclay, 91190 Gif sur Yvette, France.		
13	⁴ School of Environmental and Natural Sciences, Bangor University, Bangor, Gwynedd, LL57		
14	2DG, United Kingdom.		
15	⁵ College of Life and Environmental Science, Wenzhou University, Wenzhou, 325035, China.		
16	#Corresponding author: Yongchuan Yang (ycyang@cqu.edu.cn).		
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18			
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33 Abstract:

34	Aim: Climate is widely understood to determine elevational patterns of soil microbial			
35	communities, whereas the effects of parental material are uncertain. Changes in the			
36	composition of parental materials along elevational transects could also affect soil			
37	microbial communities by influencing soil pH and nutrient availability. Here, we aim			
38	to illustrate the combined effects of climate and parental material on the biomass and			
39	composition of soil microbial communities along an elevational transect.			
40	Location: A subtropical forest on a karst mountain (Mt. Jinfo), China.			
41	Taxon: Bacteria and Fungi.			
42	Methods: We use phospholipid fatty acid analysis (PLFA) and DNA amplicon high-			
43	throughput sequencing to determine biomass and diversity patterns of soil microbial			
44	communities along a subtropical elevational gradient with contrasting parental			
45	materials (limestone and clasolite).			
46	Results : We observed that the microbial communities were more diverse (α -diversity)			
47	and productive (biomass) on limestone than on clasolite. Additionally, we found that			
48	parental material played a role in shaping the composition (β -diversity) of soil			
49	microbial communities along the elevational gradient. The impact of climate on soil			
50	microbial communities was found to be significant, albeit relatively weak. Structural			
51	equation models provided evidence for both direct and indirect effects of climate and			
52	parental material on microbial biomass and α -diversity along the elevational gradient.			
53	Notably, the changes in soil pH, influenced by both parental material and climate,			
54	were identified as a key factor driving these effects.			
55	Main Conclusions: Our results underline the importance of both climate and parental			
56	material variations in space-for-time studies investigating soil microbial communities			
57	along elevational gradients.			
58				
59	Keywords: altitude; bedrock; clasolite; climate; karst mountains; limestone; soil			

60 biogeography.

62 63

64 1. Introduction

A central goal of modern ecology is a mechanistic understanding of global 65 biodiversity (Fierer and Jackson, 2006; Martiny et al., 2006; Fierer et al., 2009; 66 Bahram et al., 2018; Delgado-Baquerizo et al., 2018). Much of this biodiversity is 67 68 made up of soil microorganisms, which are linked directly to soil properties such as pH (Griffiths et al., 2011; Tripathi et al., 2018), organic matter content (Smith et al., 69 2021), nutrient content (Delgado-Baquerizo et al., 2017), and texture (Seaton et al., 70 2020). More generally, soil properties are determined by multiple factors (Jenny, 71 72 1941) such as climate, parental material, topography, and plants, and together these factors influence the spatial distribution of soil microbial organisms. Parental 73 materials affect the soil formation process and the resulting soil physicochemical 74 properties (Jenny, 1941; Wardle et al., 2004; Doetterl et al., 2015; Gu et al., 2020). 75 76 Previous biogeographical studies of soil microbial communities have focused on the effects of climatic and biotic factors (Tedersoo et al., 2014; Zhou et al., 2016; Zheng 77 et al., 2020; Ma et al., 2022), with the underlying mechanisms and indirect effects of 78 parental material on microbial communities being relatively understudied (Hu et al., 79 2020; Weemstra et al., 2020). Clarifying the mechanisms by which parental material 80 can influence the generation and maintenance of soil microbial communities through 81 82 changes in soil conditions is fundamental for predicting the distribution of soil 83 microbial communities in terrestrial ecosystems.

84 Several studies conducted at regional spatial scales have provided evidence for 85 the significant influence of parental material on soil microbial communities. For 86 example, an incubation experiment in tropical montane forests found that, despite the 87 advanced weathering degree of soils and similar stand age of vegetation, microbial 88 biomass was higher in soils developed from the mafic parent material than from 89 mixed sediment (Kidinda et al., 2022). A study in the Antarctic revealed that bacterial

communities were distinguished by the parent material type; soils derived from gneiss 90 were dominated by Acidobacteria and Actinobacteria, whereas granite derived soils 91 were dominated by Proteobacteria and Cyanobacteria (Tytgat et al., 2016). Another 92 study in subtropical agricultural soils found that, after 30 years of artificial 93 management, a significant effect of parental material on soil microbial diversity 94 persisted (Sun et al., 2016). However, these studies have concentrated primarily on 95 regional scales, overlooking the effects of parental material at local scales. For 96 97 instance, when examining the spatial pattern of soil microbial communities along an elevational transect, researchers often fail to consider the potential impact of changes 98 in the parental material within the transect. 99

Elevational gradients in mountainous regions are invaluable as a natural laboratory for the empirical testing of biodiversity patterns (Sanders, 2002; Sundqvist et al., 2013; Mayor et al., 2017; Steinbauer et al., 2018; McCain et al., 2021).

103 Elevational gradients usually cover small horizontal distances, and researchers

104 generally assume that the parental material underlying all plots along the elevational

105 gradient are homogeneous (Frindte et al., 2019; Ma et al., 2022; Zhu et al., 2022).

106 Consequently, elevational patterns are usually attributed to climatic effects, and

107 thenceforth used to predict the consequences of climate change for microbial

108 biodiversity (Körner, 2007; Fierer et al., 2011; Shen et al., 2014). However, mountain

109 ecosystems are usually characterized by complex geological conditions (Hahm et al.,

110 2014; He et al., 2021), and parental materials can change dramatically along an

elevational gradient (Lanzén et al., 2016; Hu et al., 2020). Neglecting the effects of

112 parental materials may therefore mislead attempts to explain the distribution of soil

113 microbial organisms (Hu et al., 2020), or even lead to incorrect conclusions that the

114 variation is driven solely by climatic gradients (Lanzén et al., 2016).

115 Many studies have examined the elevational patterns of soil microbial

116 communities (Hendershot et al., 2017; Wang et al., 2017; He et al., 2020; Chen et al.,

117 2023; Huang et al., 2023), but few have considered the importance of underlying

118 parental materials (Lanzén et al., 2016; Hu et al., 2021). The α-diversity of soil

microbial communities on two parent materials in the Pyrenees was significantly 119 correlated with parental material but not elevation (Lanzén et al., 2016). Further 120 121 evidence of the importance of parental material on the elevational pattern of soil microbial diversity can be found in two recent studies which revealed elevational 122 breakpoints of bacterial α -diversity coinciding with geological faults (Li et al., 2018; 123 Hu et al., 2020). Elsewhere, the inclusion of geological parental material substantially 124 increased the explained variation of bacterial α -diversity (Hu et al., 2020), suggesting 125 126 that parent material and climatic gradients jointly controlled soil bacterial α-diversity. However, these limited studies confirm that different types of parental material can 127 affect the alpha-diversity of soil microbial communities. The mechanisms underlying 128 the influence of parental material on soil microbial communities remain poorly 129 understood, and there is a notable absence of systematic investigations examining 130 multiple indicators of microbial communities, such as biomass, alpha diversity, and 131 beta diversity. 132

Here, we address the question of how the biomass and diversity of soil microbial 133 134 communities vary along a subtropical elevational gradient, and the mechanism by which parental material and climate jointly influence the elevational patterns of soil 135 microbial communities. The selected elevational gradient includes two different 136 parental materials, i.e. limestone and clasolite (Fig. 1). It is well-known that climate 137 significantly affects soil physical and chemical properties (Sanders, 2002; Sundqvist 138 et al., 2013; Mayor et al., 2017; Zeng et al., 2023). However, despite the changes in 139 elevation along the transect, the average climate did not differ significantly, allowing 140 us to effectively control for climate whilst isolating the effects of parental material. 141 142 Soils derived from limestone and clasolite usually differ (Jiang et al., 2020; Zhong et al., 2022; Zeng et al., 2023), including soil pH, SOC, and nutrient content, which are 143 known to influence soil microbial communities greatly (Fierer and Jackson, 2006; 144 Delgado-Baquerizo et al., 2016; Smith et al., 2021). We therefore hypothesize that: 1) 145 the elevational patterns of soil microbial communities are controlled jointly by 146 parental material and climate; and 2) parental materials and climate indirectly affect 147

soil microbial communities by influencing soil properties such as soil pH and SOCconcentration.

150

151 **2. Materials and methods**

152 **2.1 Study sites**

We worked in Jinfo Mountain (28°50'-29°20'N, 107°00'-107°20'E) which is 153 known as a karst mountain located in the Nanchuan District of Chongqing city, 154 155 southwestern China. This area experiences a subtropical humid monsoon climate with a mean annual air temperature of 8.2°C and a mean annual precipitation of 1395.5 156 mm. The vegetation type is subtropical evergreen broad-leaved forest, and is mainly 157 composed of Quercus glauca, Castanopsis fargesii, Sorbus folgneri, Cyclobalanopsis 158 159 sessilifolia, Carpinus turczaninowii, Polyspora speciosa, Cinnamomum wilsonii, and Albizia julibrissin (Zhu et al., 2022). Our transect covered an elevation range of 800 160 to 2100 m.a.s.l. A total of 14 sites were established along the transect, with five sites 161 on the limestone and nine sites on the clasolite parental materials (Figure 1). Sites 162 163 were situated at different heights (approximately 100 m) along the transect (determined by GPS). To reduce the influence of aspect, sites were located on the 164 sunny side of any topographical features. 165

166 **2.2. Sampling and analytical methods**

All sites were sampled in May 2021. We created 20 m \times 20 m plots at each site, 167 in which we surveyed all trees with a diameter at breast height (DBH) above 1 cm and 168 calculated their Shannon index as a measure of plant diversity. We estimated the forest 169 above-ground biomass (AGB) using the DBH of each tree (Réjou-Méchain et al., 170 2017). Each plot was divided into four 10 m × 10 m subplots. We used a stainless-171 steel soil corer (inner diameter 3.5 cm; depth 0 - 15 cm) to collect six soil cores at 172 random from each subplot, and homogenized the cores into composite sample for 173 each subplot. A total of 56 soil samples were collected, which were transported on ice 174 directly to the laboratory. We sampled fresh parental materials using sledgehammers 175 or drills (Hahm et al., 2014). We calculated mean annual soil temperature (MAT) and 176

177 mean annual soil moisture (MAM) from measurements of soil temperature and soil

moisture taken throughout the year from May 7th 2020 to May 7th 2021 at each site

179 using 14 HOBO dataloggers (HOBO Pro v2 Temp/RH Logger onset computer

180 corporation, Pocasset, USA).

All soil samples were sieved (2 mm) to remove visible roots and other plant 181 material. Each of the 56 soil samples was divided into two subsamples: one was 182 stored at -80°C for the PLFA and HTL analysis, and one was air-dried at room 183 184 temperature for the measurement of soil physicochemical properties in the laboratory. Soil pH was determined using a PHS-3C pH acidometer (soil-water ratio of 1:5). 185 Soil total organic C and total N concentrations were determined by dry combustion 186 with an elemental analyser (Perkin Elmer 2400 Series II); Total P concentration was 187 measured using a nitric acid-perchloric acid digestion, followed by a colourimetric 188 analysis (Murphy and Riley, 1962) using a UV-Vis spectrophotometer (UV1800; 189 Shimadzu, Kyoto, Japan). Particle size distribution was measured using a laser 190 particle analyzer based on the laser diffraction technique operating over a range of 191 192 0.02-2000 µm (Mastersizer 2000 particle size analyzer, Malvern Instruments, Ltd., UK). 193

Bacterial and fungal biomass was determined using a modified PLFA analysis 194 (Frostegård and Bååth, 1996). The abundance of individual fatty acids was 195 determined as µg per g of dry soil. Concentrations of each PLFA were calculated 196 based on the 19:0 internal standard concentrations. We chose a set of fatty acids to 197 198 represent bacterial PLFAs. Microbial biomass was expressed as the sum of identifiable PLFAs. Bacterial PLFAs were obtained by summing the phospholipid 199 200 fatty acid 14:00, 15:00, 16:00, 18:00, 13:0 anteiso, 13:0 iso, 14:0 iso, 14:1 w5c, 15:0 anteiso, 15:0 iso, 15:1 w6c, 16:0 iso, 16:1 w5c, 16:1 w7c, 17:0 anteiso, 17:0 cyclo 201 w7c, 17:0 iso, 18:1 w7c, 18:1 w9c, 19:0 cyclo w7c, and 19:0 cyclo w9c contents. The 202 sum of 18:206c and 18:3 w6c represented fungal PLFAs. 203 204 Soil DNA was extracted from composite soil samples using the FastDNA SPIN Kit for Soil (MP Biomedicals, Heidelberg, Germany) and purified by agarose gel 205

206 electrophoresis. The quality of the DNA samples was checked on a spectrophotometer

207 (NanoDrop, ND2000, ThermoScientific, USA). Total DNA was used for high-

- 208 throughput sequencing on an Illumina MiSeq platform (San Diego, CA, USA). The
- 209 bacterial V4 hypervariable region of the 16S rRNA gene and fungal internal
- transcribed spacer (ITS) region was amplified using the primer pair 505F/816R (5'-
- 211 GTGCCAGCMGCCGCGG-3'/5'-GGACTACHVGGGTWTCTA AT-3') (Caporaso et
- al., 2011) and ITS1F/ITS2 (5'-GGAAGTAAAAGTCGTAACAAGG-3'/5'-
- 213 GCTGCGTTCTTCATCGATGC-3') (Shen et al., 2020) along with the Illumina
- adaptor sequence and barcode sequences, respectively.

The raw sequence data were processed and analyzed using QIIME Pipeline 215 (Caporaso et al., 2011). Briefly, sequencing reads with an average quality value ≤ 20 , 216 with ambiguous nucleotides in barcodes, homopolymer reads longer than 8 bp and 217 shorter than 150 bp were removed to improve sequence quality and paired ends were 218 joined with FLASH (Magoc and Salzberg, 2011). Chimeric sequences were detected 219 and eliminated using the Uchime algorithm (Edgar, 2013). All sequences were 220 221 clustered into operational taxonomic units (OTUs) at a 97% identity threshold. Finally, the representative sequences of each OTU were classified against the RDP 222 16S rRNA database for bacteria and UNITE Fungal ITS database for fungi with an 223 80% confidence threshold. The resultant OTU abundance tables from these analyses 224 were rarefied to an even number of sequences per sample to ensure equal sampling 225 depth (40,851 and 64,923 for 16S rDNA and ITS, respectively). We calculated 226 Shannon diversity index and Bray-Curtis dissimilarities based on rarefied OTU 227 abundance matrices to analyse microbial diversity. The raw reads have been deposited 228 229 into the National Centre for Biotechnology Information (NCBI) Sequence Read Archive database (Accession Number: PRJNA936849). 230

231 **2.3 Statistical analyses**

We calculated the Shannon diversity index as α -diversity indices of soil microbial and plant communities. We used a Wilcoxon test to compare the climatic, plant, and soil measures between the two parental materials. To examine possible trends along

the environmental gradient (elevation, temperature, or soil pH gradient), we regressed
these response variables on the environmental gradient using univariate linear
regression models.

We identified the most important predictors of soil microbial biomass and α -238 diversity using a random forest regression analysis (Breiman, 2001). The predictors 239 include soil mean annual temperature (MAT), soil mean annual moisture (MAM), soil 240 pH, SOC, TN, TP, Clay, Sand, above-ground biomass (AGB), and Plant Shannon 241 242 diversity index. The importance of each predictor variable is determined by the percentage increase in the mean square error (%IncMSE) between observations and 243 predictions, and the decrease is averaged over all the trees to produce the final 244 estimation for importance (Liaw and Wiener, 2002). Greater values of %IncMSE 245 denote higher variable importance. In this study, the importance measure was 246 calculated for each tree and averaged over the forest (500 trees). These variable 247 importance analyses were conducted using the randomForest R package (Liaw and 248 Wiener, 2002). 249

250 We used the Bray-Curtis-dissimilarities-based Principal Coordinates Analysis (PCoA) to assess differences (β -diversity) in microbial communities in different sites 251 and parental materials. We transformed the data of relative abundances of OTUs by 252 square root before the PCoA. We used the Bray-Curt distance metric to compute a 253 254 dissimilarity matrix between the parental materials, followed by, a PERMANOVA (Permutational Multivariate Analysis of Variance) to test for significant differences 255 256 between the different parental materials. We performed redundancy analysis (RDA) of the correlation between predictor variables and microbial composition. These 257 258 ordination analyses were conducted using the vegan R package (Oksanen et al., 259 2020).

We used a structural equation modelling (SEM) framework to explore the direct and indirect effects of climate and parental material type on soil microbial biomass and α -diversity. We allowed climate and parental material to affect soil microbial community parameters both directly, and indirectly through various soil physical-

264 chemical properties. Temperature was the only climate variable which correlated

significantly with soil microbial community parameters, and we thus used

temperature to represent climate in the SEM analysis. As for the soil physical-

chemical properties, we selected the top two soil predictor variables affecting soil

268 microbial communities in the random forest regression model results. Parental

269 material was a categorial variable and thus treated as a regular numeric covariate (e.g.

270 1, 2) in the SEM analysis (Wang et al., 2019).

All statistical analyses were performed using R (R Core Team, 2018) and graphs were generated with the ggplot2 package (Wickham, 2016).

273

274 **3. Results**

275 **3.1 Temperature, vegetation, and soil on different parental materials**

Mean annual soil temperature (MAT), mean annual soil moisture (MAM) and vegetation (both Shannon diversity and above-ground biomass) were not significantly different between clasolite and limestone sites (Table 1). However, soil pH, SOC, TN, TP, and clay content in the surface soil (0–10 cm) were significantly higher on limestone sites than on the clasolite sites (Table 1). Parent material exerted significant effects on soil physical and chemical properties, whereas temperature, soil moisture, and vegetation were not significantly affected.

Elevation caused significant linear increases in soil pH and AGB on both 283 parental materials (Fig. S1 & 2). Significant linear correlations also existed between 284 elevation and temperature, some edaphic properties, and plant community 285 composition, regardless of parental material, and MAT, SOC, and silt content (Fig. S1 286 & 2). Elevation correlated significantly with some variables on one parent material 287 288 only. For example, soil clay and total nitrogen content decreased with elevation on Clasolite, while no significant trend was observed on Limestone (Fig. S1 & 2). 289 Elevation caused a significant decrease in the Shannon diversity of plant communities 290 on Limestone, while no significant elevational trend was observed on Clasolite (Fig. 291 292 S1). Soil total P concentration showed no significant elevational pattern on either of

293 the parent material types (Fig. S2).

294 **3.2** Soil microbial biomass and α-diversity on different parental materials

295 Microbial biomass and Shannon diversity of both bacteria and fungi were significantly higher on limestone sites than on clasolite sites (Table 1). The biomass 296 ratios of bacteria to fungi were significantly higher on limestone sites than on the 297 clasolite sites (Table 1). Across all samples, the dominant soil bacterial phyla were 298 Proteobacteria, Acidobacteria, Chloroflexi, Rokubacteria, Actinobacteria, 299 300 Verrucomicrobia, Bacteroidetes, and Gemmatimonadetes, which collectively accounted for 91.69% of all taxon sequences (Fig. S3). The relative abundance of 301 Acidobacteria and Actinobacteria was significantly higher on the clasolite than on the 302 limestone, while there was significantly less Chloroflexi on the clasolite than on the 303 limestone (Fig. S3). Soil fungi mainly belonged to three phyla: Ascomycota, 304 Basidiomycota, and Mortierellomycota, which altogether account for 86.67% of all 305 taxon sequences. We found significantly more Mortierellomycota on the clasolite than 306 on the limestone, and significantly less Ascomycota on the clasolite than on the 307 308 limestone (Fig. S3).

We found significant albeit relatively weak effects of MAT on soil microbial community parameters. MAT exerted no significant impact on soil bacterial biomass or fungal Shannon diversity on either parental material. (Fig. 2a & d). MAT caused a significant linear reduction in soil fungal biomass on clasolite, whereas we observed no significant trend with MAT on limestone (Fig. 2b). Shannon diversity of soil bacteria increased with MAT on clasolite, but there was no significant trend on limestone (Fig. 2c).

Random forest regression models revealed that ten selected variables could explain 77.82%, 55.64%, 81.64%, and 13.25% variation of soil bacterial biomass, fungal biomass, bacterial Shannon diversity, and fungal Shannon diversity along the subtropical elevational gradient. These models indicated that soil pH was the most important predictor to explain the variation of these four soil microbial community parameters (Fig. 3).

We found significantly positive correlations between soil pH and soil microbial 322 community parameters, including bacterial biomass, fungal biomass, bacterial 323 Shannon diversity, and fungal Shannon diversity, along the subtropical elevational 324 gradient (Fig. 4). However, the positive correlations were found only in fungal 325 biomass and Shannon diversity on the limestone, and bacterial Shannon diversity on 326 the clasolite. We even found a significantly negative correlation between soil pH and 327 bacterial Shannon diversity on the limestone (Fig. 4). These results suggest that it was 328 329 the significant difference in soil pH on the two parental materials that led to the positive correlations between soil pH and microbial communities. 330

The SEM results supported the notion that parental material and climate jointly controlled the soil microbial biomass and Shannon diversity (Fig. 5 & 6). Soil pH and SOC directly explained the variation of soil microbial biomass and diversity. And the variance in soil pH and SOC were jointly influenced by climate and parental material. Soil bacterial community biomass and α -diversity were more susceptible to parental material and climate variation than the soil fungal communities, with soil fungal Shannon diversity explaining just 15% of the total variation in the SEM.

338

339 3.3 Soil microbial β-diversity on different parental materials

The first two axes of a PCoA based on Bray-Curtis dissimilarity explained 340 60.6% of the variation in the bacterial community structure (Fig. 7a). PERMANOVA 341 based on Bray-Curtis dissimilarity showed that the bacterial communities differed 342 significantly between these two parental materials ($R^2=0.39$, F=33.90, p=0.001). It is 343 particularly noteworthy that bacterial communities at 1200m and 2100m on limestone 344 345 were highly similar, even though they were at very different altitudes (Fig. 7A). Whilst just 14.8 % of the variation of the fungal community structure could be 346 explained by the first two axes of the PCoA ordination (Fig. 7c), PERMANOVA 347 showed that the fungal communities differed significantly between these two parental 348 materials ($R^2=0.07$, F=3.85, p=0.001). The RDA results indicated that soil pH is the 349 most important environmental factor for controlling bacterial community composition 350

351 (β-diversity) (Fig. 7a & b), although SOC, TN, MAT, and others were also significant.

352 SOC exerted the most influence on fungal communities, although other factors were

also significant e.g., TN, MAT, AGB etc. (Fig. 7c & d).

354

355 4. Discussion

Climate is thought to structure soil microbial communities along elevational 356 gradients, and the potential role of parental material variation is not well understood. 357 358 We have revealed how parental material (specifically limestone and clasolite) and climate jointly structure soil microbial communities. Whilst climate and vegetation 359 did not differ significantly between limestone and clasolite sites, microbial biomass 360 and diversity differed significantly on different soils (pH, clay, and SOC etc.). Soil 361 362 microbial communities showed marginal elevational trends on both parental materials, further undermining the effects of climate on soil microbial communities. Instead, our 363 results highlight the importance of bottom-up control of parental material on 364 microbial biomass and diversity. 365

366

367 4.1 Effects of parental material on soil microbial communities

Our results indicate that parental material influenced soil microbial communities 368 along a subtropical elevational gradient, which aligns with previous studies revealing 369 the significant effects of parental material on soil microbial biomass and diversity 370 (Deng et al., 2015; Sun et al., 2016; Xiao et al., 2022). In a similar subtropical 371 monsoon climate, Deng et al. (2015) found that microbial biomass and α -diversity in 372 soil derived from granite were significantly higher than from quaternary red earth and 373 374 tertiary red sandstone, and that parental material explained more variation in 375 microbial biomass and α -diversity than land use types. Sun et al. (2016) showed that agricultural soils derived from granite supported more microbial biomass than 376 quaternary red clay soil and purple sandy shale, even after 40 years of agricultural use 377 (Sun et al., 2016). Karst mature forests in southwest China have been shown to be 378 significantly higher in diazotroph richness and Shannon index than non-karst soils 379

parental materials drive the spatial variation of soil microbial biomass and α -diversity. 381 382 Parental material affected the composition (β -diversity) of soil microbial communities in our study, which is consistent with previous studies (Ulrich and 383 Becker, 2006; Lamarche et al., 2007; Eskelinen et al., 2009; Kooijman et al., 2020). 384 We found that soil microbial communities on the same parental material were very 385 similar, even when the climatic conditions differed between distant sites. For example, 386 387 soil microbial communities (including bacteria and fungi) on limestone grouped in a significant cluster (Fig. 7), even though the sites were at 1200 m and 2100 m. This 388 result can be explained by the differing soil conditions between limestone sites and 389 clasolite sites, including soil pH, SOC concentration, and soil clay content, which 390 corresponds with the fact that differences in mineral and element composition or soil 391 texture are important in shaping microbial community composition (Barton et al., 392 2007; Tytgat et al., 2016). 393

(Xiao et al., 2022). Together these results provide compelling evidence that soil

380

Our results suggest that it was soil pH resulting from different parental materials 394 395 that affected biomass, α - and β - diversity of soil microbial communities. The importance of soil pH for structuring soil microbial communities has been elucidated 396 by various studies (Fierer and Jackson, 2006; Lamarche et al., 2007; Chu et al., 2010; 397 Wang et al., 2017; Tripathi et al., 2018; Shen et al., 2020; Ni et al., 2021). Soil pH is 398 particularly important for microorganisms, largely because the intracellular pH of 399 most microorganisms is usually within 1 pH unit of neutral, and any significant 400 deviation in environmental (extracellular) pH stresses the microorganisms, especially 401 single-celled prokaryotes (Fierer and Jackson, 2006). Moreover, the strong correlation 402 403 between soil pH and microbial communities could result from the integration of soil pH with other soil variables. For example, soil pH is an efficient integrator of soil 404 nutrient availability since differences in hydrogen ion concentrations affect the 405 capacity to hold charged ions in soils (Glassman et al., 2017). Typically, a broad range 406 of soil pH values, especially in the range of acidic to neutral, is a necessary condition 407 for the strong correlation between soil pH and microbial communities (Fierer and 408

Jackson, 2006; Fierer, 2017). Soils derived from limestone are neutral or weakly 409 alkaline, producing a broad range of soil pH in acidic subtropical soils (Lazzaro et al., 410 2009). This explains why parental material influences soil pH and thus soil microbial 411 community biomass and diversity. We found that the effect of soil pH for structuring 412 fungal community diversity (both α - and β -diversity) is weaker compared to bacterial 413 communities. This may also be a reason for the smaller variation in fungal community 414 diversity along the elevational gradient in this study, as well as the lower explanatory 415 power of the models for its variation. The strong influence of pH on bacterial 416 communities is thought to be due to the narrow pH ranges for optimal growth of 417 bacteria, as opposed to the weaker influence on fungi, which generally exhibit a wider 418 pH tolerance (Rousk et al., 2010). This has been supported by numerous studies, for 419 instance, Acidobacteria, as a dominant phylum in bacterial communities, are 420 commonly found in soils with low pH (Jones et al., 2009). 421

422

423 4.2 Effects of climate on soil microbial communities

424 Contrary to our hypothesis, we found that soil bacterial biomass showed no significant trend with soil MAT on either parental material type. This finding is 425 inconsistent with previous studies reporting that soil bacterial biomass significantly 426 increased with decreasing MAT in subtropical elevational gradients (He et al., 2020). 427 This inconsistency is probably due to contrasting trends of soil pH and SOC along this 428 elevational gradient, both of which usually have positive effects on soil bacterial 429 430 biomass. The fact that soil MAT had no effect on fungal α -diversity on either parental material is consistent with previous studies (Ji et al., 2022; Ma et al., 2022). Although 431 432 it should be noted that temperature has been found to play an important role in controlling soil fungal α -diversity (Looby and Martin, 2020; Shen et al., 2020). 433 Evidently, further study is needed to understand how soil fungal communities respond 434 435 to temperature gradients.

We did observe some effects of MAT gradients on soil microbial community
parameters, but these differed on the different parental materials. Soil fungal biomass

correlated positively with MAT on clasolite but showed no significant correlation on 438 limestone. Given the importance of SOC in controlling soil fungal biomass, this could 439 be caused by the significant increase of SOC with MAT on the clasolite. The fact that 440 SOC accumulated on the clasolite but not on the limestone suggests that SOC on 441 clasolite is more sensitive to climate change. We also found soil bacterial α -diversity 442 was significantly negatively correlated with MAT on clasolite, but showed no 443 significant correlation on limestone. This is because soil bacterial α -diversity is 444 445 correlated with soil pH in acid soils (Fierer and Jackson, 2006). These differing effects of temperature on soil fungal biomass and bacterial α-diversity on contrasting 446 parental materials suggest that models of soil microbial responses to climate change 447 should include parental material as an important mediator. 448

449

We acknowledge that the ideal sampling design to study the influence of 450 parental material differences on the elevational pattern of soil microbial communities 451 would be to have two independent elevational transects located on each of the 452 453 different parental materials, with matching elevations for paired comparisons. In this study, our confidence in our ability to separate the impact of parental material on soil 454 microbial communities from the influence of climate is mainly based on two results: 455 Firstly, we found that there were no significant differences in the average values of 456 climate factors between the two parental materials. We nevertheless found significant 457 differences in multiple characteristics of soil microbial communities and soil 458 459 physicochemical properties between the two parental materials. Secondly, it is possible for soil microbial community compositions from the same parental material 460 under differing climates to be very similar. These results confirm the significant 461 influence of parental material on soil microbial communities. However, our site 462 design does have certain limitations, such as the difficulty in verifying whether there 463 is a significant interaction between parental material and climate on soil microbial 464 communities. Therefore, future research should focus on more comprehensive site 465 designs to systematically study the interaction of multiple soil-forming factors, such 466

467 as parental material and climate, on soil microbial communities.

468

469 **5. Conclusion**

470	Our goal was to clarify that climate and parental material jointly control the
471	biomass and composition of soil microbial communities using microbial lipid
472	biomarkers and high-throughput amplicon sequencing. Parental material exerted
473	significant effects on soil microbial biomass, α -diversity, and β -diversity. Although
474	climate exerted weaker effects, it was jointly responsible for structuring the soil
475	microbial communities along a subtropical karst elevational gradient. Soil pH was the
476	most important factor affecting soil microbial biomass, α -diversity, and even β -
477	diversity of bacterial communities directly, and soil pH varied with parental material
478	and climate, which underlines the need to consider both parental material and climatic
479	variation in space-for-time studies of soil microbial communities along elevational
480	gradients.
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- 713

715 **Table 1 Comparisons of climate, soil, and vegetation on contrasting parental materials**

716 (mean±se). Different characters after the values indicate significant differences between two

parental materials in Wilcoxon test (p < 0.05), while the same character indicates no

significant difference (p > 0.05). Measures with significant differences are in bold font.

	Clasolite	Limestone
MAT (°C)	11.30 ± 0.33 a	11.91 ± 0.43 a
MAM (%)	21.15 ± 0.49 a	20.79 ± 0.95 a
Above ground biomass (Mg ha ⁻¹)	142.55 ± 6.70 a	152.76 ± 9.05 a
Plant Shannon diversity	$3.11\pm0.04~a$	$2.95\pm0.08\ a$
Soil pH	$3.94\pm0.13~a$	$6.08\pm0.21~b$
SOC (g kg ⁻¹)	$45.84\pm4.59\ a$	$65.99 \pm 3.12 \text{ b}$
TN (g kg ⁻¹)	$1.11 \pm 0.08 \ a$	$1.69\pm0.08~b$
TP (g kg ⁻¹)	$0.65\pm0.03~a$	$0.84\pm0.06\ b$
Clay (%)	34.34 ± 1.19 a	$39.24 \pm 1.11 \text{ b}$
Silt (%)	30.26±0.87 a	30.41±0.37 a
Sand (%)	35.39±1.78 a	30.34±1.24 a
Bacterial biomass (ug g ⁻¹)	$14.46 \pm 0.68 \text{ a}$	$30.19\pm1.45\ b$
Fungal biomass (ug g ⁻¹)	$0.38\pm0.02~a$	$0.60\pm0.03~b$
Bacteria to Fungi biomass ratio	$4.11 \pm 0.05 \ a$	$4.33\pm0.08\ b$
Bacterial Shannon diversity	$5.95\pm0.05~a$	$6.19\pm0.04\ b$
Fungal Shannon diversity	$3.56\pm0.10\ a$	$3.97\pm0.13\ b$

719 MAT: mean annual temperature; MAM: mean annual soil moisture; SOC: soil organic carbon

720 concentration; TN: soil total nitrogen concentration; TP: soil total phosphorus concentration.

721

723 Figure 1 Distribution of sampling sites along the subtropical elevational transect.

Red points indicate limestone sites and blue points indicate clasolite sites. Numbers
 near the dots indicate the elevation values. The base map is a coloured DEM map

derived from SRTM 90 m data. [This figure is 2/3rd column].



Figure 2 Effects of temperature on soil microbial parameters on clasolite and

limestone, respectively. (a) soil bacterial biomass; (b) soil fungal biomass; (c) soil bacterial Shannon index; (d) soil fungal Shannon index. Solid and dashed lines indicate significant (p < 0.05) and insignificant (p > 0.05) linear regression relationships, respectively. [This figure is double column.]





740 Figure 3 Relative importance of predictors of soil microbial community

parameters quantified using random forest models. [This figure is double column.]



744 MAT: mean annual soil temperature; MAM: mean annual soil moisture; SOC: soil organic carbon

- concentration; TN: soil total nitrogen concentration; TP: soil total phosphorus concentration.

Figure 4 Relationship between soil pH and soil microbial community 748

parameters. (a) soil bacterial biomass; (b) soil fungal biomass; (c) soil bacterial 749

Shannon index; (d) soil fungal Shannon index. Solid and dashed lines indicate 750

significant (p < 0.05) and insignificant (p > 0.05) linear regression relationships, 751

respectively. Red lines indicate relationship on limestone; blue lines indicate 752

- 753 relationship on clasolite; and black lines indicate relationship on all sites. [This figure
- is double column.] 754
- 755





Figure 5 Structural equation modelling evidencing direct and indirect effects of 758 climate and parental material on soil bacterial community biomass and α -759 diversity, respectively. In (a) and (b), the thickness of the arrows indicates the 760 strength of the causal relationship, supplemented by a path coefficient. R² values 761 denote the amount of variance explained by the model for the response variables. ***, 762 **** indicates significance at the 99.9%, and 99.99% levels, respectively. (c) and (d): 763 standardized total effects of climate, parental material and soil properties on soil 764 microbial biomass and α -diversity, respectively. [This is figure is double column.] 765 766



Figure 6 Structural equation modelling evidencing direct and indirect effects of climate and parental material on soil fungal community biomass and α -diversity, respectively. In (a) and (b), the thickness of the arrows indicates the strength of the causal relationship, supplemented by a path coefficient. R² values denote the amount of variance explained by the model for the response variables. ***, **** indicates significance at the 99.9%, and 99.99% level, respectively. (c) and (d): standardized total effects of climate, parental material, and soil properties on soil microbial biomass and α -diversity, respectively. PD: plant diversity. [This figure is double column.]



Figure 7 Redundancy analysis (RDA) of the relationship between predictor 782 variables and the Bray-Curtis dissimilarity distance between microbial 783 communities. In (a) and (c), dots indicate individual samples; blue dots indicate 784 clasolite sites, red dots indicate limestone sites; the arrow lengths and directions 785 correspond to the variance explained by the individual variables; only the variables 786 with significant effects are shown in the ordinary plots. R^2 in (b) and (d) indicate the 787 proportion of variation of soil bacterial and fungal communities explained by the 788 predictor variables, respectively; star above a bar indicates it is statistically significant 789 (p < 0.05). [This figure is double column.] 790

