Multiple ecological processes jointly drive the soil microbial community assembly in subalpine coniferous forests

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The mechanisms underlying community dynamics, which govern the complicated biogeographical patterns of microbes, have long been a research hotspot in community ecology. However, the mixing of multiple ecological processes and the one-sidedness of analytical methods make it difficult to draw inferences about the community assembly mechanisms. In this study, we investigated the driving forces of the soil microbial community in subalpine coniferous forests of the Loess Plateau in Shanxi, China, by integrating multiple analytical methods. The results of the null model demonstrated that deterministic processes (especially interspecific relationships) were the main driving force of the soil microbial community assembly in this study area, relative to stochastic processes. Based on the results of the net relatedness index (NRI) and nearest taxon index (NTI), we inferred that historical and evolutionary factors, such as climate change and local diversification, may have similar effects on microbial community structure based on the climatic niche conservatism. Based on the results of a functional traits analysis, we found that the effects of ongoing ecological processes on the microbial community assembly varied among sites. Therefore, the functional structures seemed to be more related to ongoing ecological processes, whereas the phylogenetic structures seemed to be more related to historical and evolutionary factors, as well as the tradeoff between deterministic and stochastic processes. The functional and phylogenetic structures were mainly shaped by different ecological processes. By integrating multiple ecological processes, our results provide more details of the mechanisms driving the community assembly

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2   **coniferous forests**

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22   **Abstract** The mechanisms underlying community dynamics, which govern the complicated biogeographical

23   patterns of microbes, have long been a research hotspot in community ecology. However, the mixing of multiple

24   ecological processes and the one-sidedness of analytical methods make it difficult to draw inferences about

25   the community assembly mechanisms. In this study, we investigated the driving forces of the soil microbial

26   community in subalpine coniferous forests of the Loess Plateau in Shanxi, China, by integrating multiple

27   analytical methods. The results of the null model demonstrated that deterministic processes (especially

28   interspecific relationships) were the main driving force of the soil microbial community assembly in this study

29   area, relative to stochastic processes. Based on the results of the net relatedness index (NRI) and nearest taxon

30   index (NTI), we inferred that historical and evolutionary factors, such as climate change and local diversification,

31   may have similar effects on microbial community structure based on the climatic niche conservatism. Based on

32   the results of a functional traits analysis, we found that the effects of ongoing ecological processes on the

33   microbial community assembly varied among sites. Therefore, the functional structures seemed to be more

34   related to ongoing ecological processes, whereas the phylogenetic structures seemed to be more related to

35   historical and evolutionary factors, as well as the tradeoff between deterministic and stochastic processes. The

36   functional and phylogenetic structures were mainly shaped by different ecological processes. By integrating

37   multiple ecological processes, our results provide more details of the mechanisms driving the community

38   assembly.

39   **Key words**: ecological process; community assembly; phylogenetic structure; functional traits; soil microbial

40   community

41   **Introduction**

42   Understanding the fundamental ecological mechanisms that drive the assembly process of microbial

43 communities is a major challenge in community ecology [1]. The assembly process of the microbial community

44 in a local community is generally influenced by two types of ecological processes, namely deterministic and

45 stochastic processes. The deterministic process hypothesizes that deterministic factors such as organism traits,

46 interspecies relationships (e.g., competition, predation, mutualisms), and environmental stresses (e.g., pH,

47 temperature, salt, and moisture) govern community succession [2-4]. For example, ecologists have traditionally

48 tended to consider that the environmental context determines the assembly process of microbial communities:

49 "Everything is everywhere, but the environment selects" [5]. For example, environmental factors such as pH [6],

50 temperature [7], and nitrogen levels [8] may be major determinants of microbial community structure. However,

51 there is no doubt that interspecies relationships may also be an important force that influences community

52 structure and dynamics [9]. Although ecologists accept that competition and environmental processes act

53 simultaneously [10], biogeographic patterns have usually been ascribed to environmental filtering alone [11].

54 Little attention has been paid to the relative contributions of competition and environmental stress.

55   For the other types of community assembly processes (i.e., stochastic processes), it is assumed that

56 community structures are independent of organism traits and are governed by birth, death, colonization,

57 extinction, drift, and speciation [12], and it is hypothesized that species are all ecologically equivalent [13]. It

58 has recently been accepted that the two ecological processes are not mutually exclusive, but rather form a

59 continuum [14]. However, Clark [11] argued that stochasticity could occur only in mathematical models and not

60 in nature, and therefore questioned the universality of the continuum hypothesis [12]. Therefore, to interpret a

61 global map of bacterial diversity patterns, more studies are necessary to characterize the biogeographic patterns

62 and assembly processes in different environmental contexts or conditions.

63   Communities at different stages of succession [15] or in different sub-communities[16] are driven by

64   different assembly processes. Most studies of ecological mechanisms have been limited to specific spatial or

65   temporal scales [6, 17-19]. For example, stochastic processes may dominate microbial community assembly

66   within successional stages, while deterministic processes may prevail during the transition periods between

67   successional stages. Moreover, some of the conclusions reached in typical examples do not apply to all

68   environmental contexts [20]. This may be related to the mixing of multiple ecological processes and the one-

69   sidedness of analytical methods.

70   The biogeographic patterns of the community are the aggregate of multiple ecological processes (e.g., the

71   evolutionary process [21, 22] and ongoing ecological processes [23]) operating on multiple axes. This

72   aggregation severely complicates the identification of causal relationships in a local community [20]. Therefore,

73   the aggregation of multiple ecological processes makes it difficult to draw inferences about the community

74   assembly [20].

75   On the other hand, the one-sidedness of analytical methods also makes it difficult to draw inferences about

76   the ecological processes. Ecologists investigating the community assembly process mainly rely on community

77   β-diversity [2, 24-26], phylogenetic structure [6, 27], and functional traits [28]. First, the β-null deviation

78   measure can be used to create stochastically assembled communities from the regional species pool and

79   investigate the degree to which the observed β-diversity patterns deviate from the stochastic assembly [2, 25,

80   26]. However, it is difficult to precisely and robustly disentangle the different multiple ecological processes

81   structuring communities [26]. Second, the net relatedness index (NRI) [29] and nearest taxon index (NTI) are

82   two important indexes that can be used to characterize phylogenetic structure. Both the NRI and NTI increase

83   with increasing clustering and become negative with over-dispersion [29]. However, little attention has been

84   given to the differences or relationships between NRI and NTI. In most cases, this approach completely relies

85   on phylogenetic relatedness being a strong proxy of ecological similarity. The result of this is the aggregation of

86   multiple processes. Thus, it is difficult to draw inferences of the complete community assembly process. Finally,

87   the functional traits should be closely linked to ongoing ecological processes [30]. Functional trait analyses have

88   followed an almost identical trajectory to the phylogenetically-based analyses [28, 30]. However, the measured

89   traits cannot represent the whole functioning of an organism. It would be incorrect to describe the process of

90   community assembly only according to functional traits. In essence, the different analytic methods infer the

91   process of community assembly based on different perspectives. Therefore, by integrating these three analytical

92   methods, more information is provided regarding the biogeographic distribution patterns of the community.

93   In this study, soil was sampled from 23 soil plots in subalpine coniferous forests located on the Loess

94   Plateau in Shanxi Province, China. Microbial communities have a high taxonomic and metabolic diversity [31,

95   32], and perform important ecological functions [33]. Thus, microorganisms are ideal research objects for the

96   study of community assembly mechanisms. The 16S ribosomal RNA genes of bacteria were analyzed using

97   high-throughput sequencing. Linking data on soil microbial communities to data on the community turnover

98   rate, historical or evolutionary factors, and ongoing ecological processes to investigate the community assembly

99   process may provide more evidence of the biogeographic distribution patterns of a community. Specifically, we

100   aimed to (i) quantify the relative roles of deterministic and stochastic processes in bacterial community

101   dynamics; (ii) disentangle the relative importance of environmental filtering and interspecific relationships on

102   the community assembly process; and (iii) determine the effects of historical or evolutionary factors and the

103   ongoing ecological processes on the assembly of microbial communities.

104   **1.   Materials and methods**

105   **2.1 Study site and sampling**

106 A total of 23 soil plots were sampled (Figure 1) in August 2016 and 2017. The plots were located in

107 subalpine coniferous forests at an altitude between 1900 and 3055 m above mean sea level. In addition, the

108 distance between the samples of each plot was more than 50 m. All samples were collected from the 0–10 cm

109 soil horizon. Soil samples were sealed in plastic bags and refrigerated, immediately transported to the laboratory,

110 and sieved through a 2-mm mesh. The soil samples were stored at -80°C prior to analysis.

111 The soil samples were subsampled for a molecular analysis, with the extraction of 1 g of soil using an

112 E.Z.N.A. ® Soil DNA Kit (Omega Bio-tek, Inc., Norcross, GA USA). The quality and quantity of DNA extracts

113 were then measured by an Infinite 200 PRO plate reader (Tecan, Männedorf, Switzerland). The DNA purity was

114 assessed by a determination of the A260/A280 absorbance ratios, and only DNA extracts with absorbance ratios

115 of 1.8–2.0 were used in further analyses. Three DNA samples were extracted from each soil sample and were

116 then mixed and sequenced on the Illumina MiSeq sequencing platform (Majorbio Biotechnology Co.,

117 Ltd.,Shanghai, China) in the bacterial v3-v4 hypervariable region using the bacterial 16S universal primers (341F

118 5′-ACTCCTACGAGGAGCA-3′ and 805R 5′- TTACCGCGGCTGCTGGCAC -3′) [34].

119 **2.2 Bioinformatics analysis**

120 The sequencing data were analyzed by the QIIME (v1.8.0, http://qiime.org/) pipeline [35]. The filtered

121 sequence alignments were denoised by DeNoiser [36] and then screened for chimeras by UCHIME [37]. The

122 Eukaryota, Archaea, and unknown sequences were removed. The sequences were clustered into operational

123 taxonomic units (OTUs) at a 97% similarity level by the average neighbor method and taxonomy was blasted to

124 the SILVA database by the k-mer searching method using MOTHUR [38]. The OTU table was rarefied to 4020

125 sequences per sample. Ten independent maximum-likelihood phylogenetic trees, with the Jukes-Cantor distance,

126 were then constructed using FastTree2 [39] after removing gaps and hypervariable regions using a Lane mask

127 wrapped within QIIME to support the phylogenetic diversity (pd) calculations.

2.3 Environmental variables

129 In the laboratory, soil total carbon (TC), total nitrogen (TN) and total sulfur (TS) were measured using an

130 elemental analyzer (Vario EL/ MACRO cube, Elementar, Hanau, Germany); nitrate nitrogen (NO_3^-_N),

131 ammonium nitrogen (NH_4^+_N), and nitrite nitrogen (NO_2^-_N) were measured using an automated discrete

132 analysis instrument (CleverChem 380, DeChem-Tech. GmbH, Hamburg, Germany). After shaking the soil :

133 water (1:2.5 mass/volume) suspension for 30 min, the soil pH was measured by a pH meter (Hl 3221, HANNA

134 Instruments Inc., Woonsocket, RI, USA). The soil organic carbon in each soil sample was measured by the

135 potassium dichromate volumetric method [40].

2.4 Null model analysis

137 The null model accounted for such changes in β-diversity, while controlling for stochastic variation and

138 associated changes in α-diversity (i.e., local species richness) [25]. We considered the null deviation to be the

139 relative difference between the observed β-diversity and the null-model β-diversity, $\beta_{obs} - E(\beta_{null})$, and the β-

140 diversity was measured as the Sorenson-Czekanowski dissimilarity [26]. As such, null deviation values may

141 represent communities that are more similar than expected by chance (a negative null deviation value), less

142 similar than expected by chance (a positive null deviation value), or close to the chance expectation (values near

143 zero). The detailed calculation process is provided in previous studies [15, 26, 41, 42].

2.5 Phylogenetic analysis

145 The pd was determined with the method of Stegen et al. using the picante library for R [43]. The NRI and

146 the NTI were used to quantify the phylogenetic structure. The NRI measures the mean pairwise phylogenetic

147 distance between all species or individuals in a sample (MPD), while the NTI measures the mean phylogenetic

148   distance between a species or individual and its closest relative mean nearest taxon distance (MNTD), in both

149   cases adjusting for the null-model expectation by random sampling from a species pool. They are calculated as

150   follows:

151
$$-1 \times \frac{r_{obs} - mean(r_{rand})}{sd(r_{rand})}$$

152   where r_{obs} is the observed NRI/NTI and r_{rand} is the MPD/MNTD from a null model, which is built by permuting

153   the species labels across a phylogeny covering all species in a given species pool and using the "taxa labels in

154   phylum level" null model in picante, to preserve the community structure and achieve a reliable randomization

155   [44]. Positive values represent phylogenetic clustering, whereas species in the community are more closed

156   related than expected. The negative values indicate phylogenetic over-dispersion, where species in the

157   community are more distantly related than expected.

158   **2.6 Functional attributes**

159   Functional community structure was calculated based on a single functional trait of key importance in

160   microbial communities, e.g., niche breadth [45]. This was because the niche breadth was the only trait broadly

161   available for the species studied. The formula below was used:

162
$$B_j = \frac{1}{\sum_{i=1}^{N} P_{ij}^2}$$

163   where B_j indicates niche breadth and P_{ij} is the relative abundance of species j present in habitat i [46, 47].

164   Niche breadth is the sum of all the resources that can be used by organisms. The abundance of a given bacterial

165   species is the result of the balance between its growth rate and loss factors [48]. Thus, the niche breadth can

166   reflect important functional information of the community.

167   A functional traits analysis was conducted in the same way that the NRI was calculated [49], but using the

168 functional niche breadth-based dendrogram. A similarity distance matrix for the niche breadth of all species was

169 then constructed, comparing the niche breadth values of all pairs of species using the Euclidian distance.

170 Subsequently, we conducted a cluster analysis (i.e., a complete linkage method that identified similar clusters)

171 of this distance matrix and constructed a dendrogram based on the results of the cluster analysis. Finally,

172 functional trees were calculated.

173 **2.7 Statistical analysis**

174 All statistical analyses were performed in the R environment using the vegan, gplots, ggpubr. and corrplot

175 packages. A Venn diagram was used to show the shared OTUs among the sites. A correlation matrix graph was

176 used to demonstrate the correlation between soil physicochemical factors. A multivariate regression tree (MRT)

177 analysis was used to explain the relationship between bacterial α-diversity estimates and environmental variables

178 in a visualized tree, and the diversity indices were normalized to the same mean before performing the MRT

179 analysis [50]. To test the effects of soil physical and chemical factors on NRI across all datasets, we used a

180 generalized additive mixed model (GAMM). The GAMM was fitted using the "gamm" function in the "mgcv"

181 R package. A combination of soil physicochemical data and community matrices were used in a redundancy

182 analysis (RDA) to visualize the e☐ects of soil physicochemical properties on the structure of soil microbial

183 communities (Hellinger-transformed data) using the vegan package in R. The forward selection of the principal

184 coordinates of neighbor matrices (PCNM) variables based on permutation tests was chosen to identify two of

185 the 23 extracted PCNM variables, which could significantly (P <0.05) explain the spatial structure. The PCNM

186 eigenfunctions, which represent the 'spectral decomposition of the spatial relationship across sampling

187 locations', were considered to be the spatial variables in the ordination-based analysis. The contributions of

188 environmental filtering and the space variable (PCNM) to the variation of the bacterial community composition

189   were calculated using the variance partitioning analysis (VPA) (CANOCO for Windows Version 5.0).

190   **Results**

191   **Physicochemical properties of the soils at different sites**

192   The soil physicochemical properties varied across the different sampling sites (Figure 2). Briefly, the

193   ammonium nitrogen and nitrite nitrogen concentrations were highest in LY sites and lowest in WT sites (P

194   <0.05). The nitrate nitrogen, SOC, TC, and TN concentrations were highest in WT sites and lowest in LY sites

195   (P <0.05). TN was significantly positively correlated with TC and SOC (P <0.05) and significantly negatively

196   correlated with pH (P <0.05). TC and pH were significantly negatively correlated (P <0.05). SOC was

197   significantly positively correlated with nitrate nitrogen (P <0.0.5), and was significantly negatively correlated

198   with nitrite nitrogen (P <0.05). Ammonium nitrogen was significantly negatively correlated with ammonium

199   nitrogen, nitrate nitrogen, and nitrite nitrogen (P <0.05). The difference in the environmental factors formed an

200   ecological gradient along the different sites. Based on this ecological gradient, the study aimed to investigate the

201   assembly process of the bacterial community by integrating multiple analytical methods.

202   **Dynamics of the bacterial community composition and diversity**

203   A total of 4258 OTUs were identified by 1,062,241 high-quality sequences recovered from 23 soil samples.

204   Good's coverage index ranged from 95.19 to 99.75%, indicating that the sequences identified represented the

205   majority of the bacterial sequences in the soil samples.

206   As shown in the Venn diagram, 869 bacterial shared OTUs were observed from all sampling sites. There

207   were 46 bacterial phylum identified (Figure 3). There were 15 bacterial phylum with a relative abundance of

208   more than 0.01%. The highest abundance at all sites was recorded for *Proteobacteria* (mean relative abundance

209   = 30.59%), followed by *Acidobacteria* (19.63%), *Actinobacteria* (16.51%), and *Chloroflexi* (13.22%). The mean

210 relative abundance of *Proteobacteria* was highest in PQG sites (34.39%), while the abundance of *Actinobacteria*

211 was highest in LY sites (26.29%). The mean relative abundances of *Acidobacteria* (28.68%) and *Chloroflexi*

212 (16.09%) were highest in WT sites. There were 20 bacterial classes with a relative abundance of more than

213 0.01% in the study area. The sample plots for each site could be roughly clustered together (Figure 3d).

214   The community α-diversity indices varied at the different sites (Figure 4). Briefly, the pd and the number of

215 observed species (sobs) were greater in WT sites (P <0.05). There was no significant differences in the Ace,

216 Chao, Shannon, and Simpson indexes among the different sites (P >0.05).

217   **Effects of environmental factors on microbiome dynamics**

218   Redundancy analyses were used to identify the abiotic environmental drivers that influenced bacterial

219 community composition. The results demonstrated that *Proteobacteria* and *Cyanobacteria* were mainly shaped

220 by pH, while SOC, TC, and TN were the main abiotic drivers of *Parcubacteria* and *Planctomycetes* (Figure 5).

221 In addition, SOC made the largest contribution to the microbial community structure (i.e., the arrow had the

222 longest length). From the MRT analysis (Figure 6), we found that normalized diversity estimates were mainly

223 split by SOC, which explained 36.75% (in the first spilt), followed by pH (6.68%).

224   The variation partitioning analysis showed that environmental factors (20.3%) and the spatial variables

225 (1.9%) were minor contributors to the bacterial biogeographic distribution pattern, because there was a 78.6%

226 contribution from an unexplained variable (Figure 7).

227   Because the NRI is a standardized measure of the mean pairwise phylogenetic distance of taxa in a given

228 sample [29] and its calculation relies on both phylogenetic and species abundance information. The NRI can

229 effectively reflect the process of community assembly. To investigate the effect of environmental factors on the

230 NRI across all datasets, we used a GAMM (Table 1). Our results showed that SOC had a slight (the estimate

231   was 0.004) but significant ($P < 0.05$) effect on the NRI.

Nonrandom co-occurrence patterns of the microbial community

233   Network analysis was applied to explore interspecific relationship patterns in the complex microbial

234   communities. The results demonstrated that the number of edges (595675), vertices (4014), and the average

235   degree (296.799) were greater in the WT sites (Figure 8). The diameter (5) and modularity (0.975) were greater

236   in the LY site. The significant and strongly correlated OTUs were mainly distributed in the different modules in

237   the network. The modules were more frequently observed in PQG sites (9), followed by WT sites (7) and LY

238   sites (6). A module is a group of OTUs that are highly connected within the group, but with very few connections

239   outside the group [51]. Thus, we considered that the changes of modules represented changes in interspecific

240   relationships.

The bacterial community assembly process

242   According to the null model analysis, our results demonstrated that the null deviation values varied at

243   different sites (ranging from 0.29 to 0.57) (Figure 9). Positive null deviation values can represent communities

244   that are more dissimilar than the null expectation [2, 25]. The bacterial communities in WT sites deviated

245   significantly from the null expected value (relative null deviation = 0.45) and there were more communities in

246   WT sites than in the LY and PQG sites (relative null deviation = 0.32 and 0.34) ($P < 0.05$).

247   The functional community structure ($NRI_{(FUN)}$) varied among the different sites (ranging from -1.02 to -

248   0.31,) ($P > 0.05$). The $NRI_{(FUN)}$ was negative, indicating a traits divergence in communities. The ($NRI_{(FUN)}$) was

249   lowest in WT sites (-0.75).

250   The NRI (ranging from -0.94 to -0.38) and NTI (ranging from -1.04 to -0.46) varied among the different

251   sites ($P > 0.05$). Both the NRI and NTI were negative, indicating over-disperse phylogenetic patterns. There were

252   no significant differences between the NRI and NTI ($P >0.05$).

253   **Disscussion**

254   Microorganisms typically form diverse communities of interacting species, whose activities have a

255   tremendous impact on the plants, animals, and humans they associate with [52]. The mechanism or ecological

256   processes that drives the structure of these complex communities is crucial to understanding and managing them.

257   The integration of multiple ecological processes can provide more clues for drawing the biogeographic patterns

258   of communities. The results of this study indicate that the ongoing ecological processes and historical or

259   evolutionary factors, as well as the trade-off between deterministic and stochastic processes, jointly drive the

260   assembly processes of the soil microbial community in subalpine coniferous forests on the Loess Plateau, China.

261   By integrating multiple analytical methods, the one-sidedness of a single method can be avoided and a more

262   scientific and accurate conclusion can be drawn. There are some inconsistencies in the results obtained for the

263   same microbial community data when using different analytical methods, and this may be the main reason why

264   the universality of ecological mechanisms is often challenged. What these conclusions have in common is that

265   interspecific relationships are driving factors in the process of community assembly.

266   **Trade-off between deterministic and stochastic process in driving the community assembly process**

267   In the null model analysis, the degree of deviation from the random expectation is understood to reflect

268   community assemble processes through environmental filtering (negative values; communities less dissimilar

269   than expected by chance) or competitive interactions (positive values; communities more dissimilar than

270   expected by chance) [2, 26]. The large deviations from the random expectation could be interpreted as reflecting

271   communities structured by deterministic assembly mechanisms [26]. The results demonstrated that null deviation

272   values varied among sites ($P <0.05$); thus, we inferred that the trade-offs between deterministic and stochastic

273   processes drove the composition of microbial communities in the study area [25, 26]. Previous studies have

274   confirmed that the trade-off could be dependent on varying environmental conditions or the characteristics

275   of organisms [53]. The null deviation in WT sites significantly deviated from the stochastic assembly model to

276   a greater extent than for the other two sites, indicating a stronger deterministic process. From the VPA, the spatial

277   variables (1.9%) were found to be the minimal contributor to the bacterial biogeographic distribution pattern,

278   indicating the minimal role of stochastic processes. Therefore, we inferred that a deterministic process was

279   predominant for governing the biogeographic distribution patterns of the microbial community in current study.

280   **Relative to environmental filtering, interspecific relationships dominate the biogeographic patterns of**

281   **microbial communities**

282   Environmental factors, such as salinity [54], pH [55, 56], C/N ratio [57], soil C [58], soil N [59], and the

283   structure of the plant community [60] may be major determinants of microbial community structure. Our results

284   demonstrated that pH, SOC, TC, and TN were the main abiotic drivers of microbial community composition.

285   More importantly, it was SOC that had the most significant effects on community diversity (MRT analysis),

286   structures (RDA), and NRI (GAMM). We observed that SOC was significantly different at different sites, and

287   was significantly correlated with nitrate nitrogen, nitrite nitrogen, and TN ($P <0.05$). Thus, SOC was found to

288   be closely related to many soil environmental factors and had the highest weighting. Along the northern slope

289   of the Changbai Mountains, the SOM decomposition rate had a significant positive relationship with the total

290   microbial, bacterial, and *Actinomycetes* PLFAs and soil enzyme activity [61]. Thus, SOC was closely related to

291   microbial community structure, composition, and diversity [62], and was therefore related to the community

292   assembly process.

293   The VPA showed that environmental factors and spatial variables were minor contributors to the bacterial

294   biogeographic distribution pattern, explaining only 21.4% of the total variation. The sample plots used in this

295   study were established under subalpine coniferous forest. The environmental context of subalpine regions

296   includes pronounced climatic gradients and climosequences within short distances, with a high level of

297   environmental heterogeneity [17]. However, crowded coniferous forests can block most of the sunlight and

298   reduce wind, reducing environmental heterogeneity. In addition, the current study was initiated in a subalpine

299   coniferous forest soil, where the composition of litter was relatively simple. Although the sites were different,

300   there was little variation in the aboveground vegetation (dominant species: C. *breviculmis*, *Stipa capillata* Linn.).

301   This could be the reason why environmental filtering made only a minor contribution to the bacterial

302   biogeographic distribution pattern.

303   The unexplained variation in VPA (78.6%) could also be due to unmeasured environmental variables and

304   unincorporated neutral factors. We inferred that this was more related to the interspecific relationship.

305   We observed that the null deviations were positive in the null model, which were interpreted as showing

306   competitive interactions within the community, because communities were more dissimilar than expected by

307   chance [2, 25]. In addition, we also observed an over-dispersion of phylogenetic patterns (NRI and NTI) and

308   trait divergence in communities (NRI $_{(fun)}$) [10]. By integrating the results of the three analytical methods, we

309   inferred a consistent conclusion that interspecific relationships were the driving factor of community assembly

310   processes rather than environmental filtering. The driving effect of interspecific relationships in the process of

311   community assembly can be represented by the changes of modules in the network analysis (Figure 8).

312   Many analytical methods can be used to separate the relative roles of competition and abiotic filtering, but

313   their distinction is frequently fuzzy. This may be because competitive interaction and environmental stress act

314   synchronously, as suggested by the existence of a balance between stress tolerance and nutrient access [11].

315   Many ecologists tend to appreciate that environmental filtering is the dominant process in community assembly,

316   because such conditions have traditionally been ascribed to environmental filtering alone in most cases [9].

317   However, it is not correct to ignore the roles of interspecific relationships to infer the process of community

318   assembly. Many studies have confirmed the importance of interspecific relationships. For microorganisms,

319   competition is most important under conditions of high resource availability while abiotic filtering prevails

320   during periods of high environmental stress [63]. For macroorganisms, the interactions due to competition were

321   more important than the regional climate in governing long-term changes in tree mortality [64]. In the current

322   study, the dense coverage of coniferous forest litter on the surface of the soil formed an unventilated

323   environment, which was conducive to the accumulation of soil nutrients. This nutrient accumulation promoted

324   substrate availability. The high resource availability then accelerated interspecies competition [63].

325   **Effects of historical factors and ongoing ecological processes on community assembly**

326   The phylogenetic patterns could reflect the imprints of evolutionary and biogeographic history on

327   community structure [65]. The NRI primarily reflects the structure in deeper parts of the phylogeny, while NTI

328   mainly reflects the shallow parts of the phylogeny [29, 49]. For example, previous studies found that broad scale

329   deep-time intercontinental migration (inferred by the NRI index), together with climatic niche conservatism,

330   appear to influence the tree community phylogenetic structure in East Asian forests, with a shallow phylogenetic

331   imprint of local diversification (inferred by the NTI index) [27]. This may be because patterns of relatedness,

332   where related taxa have disjunctive occurrences, often occur at the genus or higher taxonomic levels and could

333   reflect historical factors (migrations that occurred millions of years ago) [66]. Climatic conservatism is a

334   necessary component in maintaining such disjunctions [27]. The results of the current study demonstrated that

335   there were no significant differences between the NRI and NTI, indicating little difference between the deeper

336   and the shallow parts of the phylogeny. We therefore inferred that historical and evolutionary factors, such as

337   the paleoclimate, current climate, and local diversification, have similar effects on microbial community

338   structure based on climatic niche conservatism [67, 68]. This may be because there is no significant difference

339   between the paleoclimate and current climate, or that the difference between the two is not the driving factor in

340   the process of microbial community assembly.

341   The functional traits should be directly linked to ongoing ecological processes [30]. Because niche breadth

342   is the sum of all the resources that can be used by organisms, it is an ideal proxy of the functional traits. The

343   functional community structure (NRI_{fun}) also showed functional dispersal patterns (traits divergence) [69]. The

344   effects of ongoing ecological processes on microbial community assembly were greater in WT sites. Both the

345   null deviation and the NRI_{fun} were greater in WT sites, which is probably related to the larger elevation gradient

346   in WT sites (Table S1). The ongoing ecological processes may be related to the community turnover. The

347   functional and phylogenetic structure were shaped by divergent processes, which is consist with the results of

348   previous studies [27, 28].

349   **Conclusion**

350   The most important finding in this study was that deterministic processes (especially interspecific

351   relationships) drove the bacterial community assembly in subalpine coniferous forests on the Loess Plateau,

352   China. Historical and evolutionary factors, such as the paleoclimate and current climate, had similar effects on

353   microbial community structure based on climatic niche conservatism. The effects of ongoing ecological

354   processes on microbial community assembly were largest in WT sites. The functional and phylogenetic

355   structures were shaped by divergent processes. The results of this study will improve our understanding of the

356   trade-off between deterministic versus stochastic process in bacterial community assemblages and the shaping

357 of bacterial biogeography from multiple dimensions.

365

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521

Figure 1(on next page)

Figure 1 Geographic distribution of the 23 sampling plots located on Loess Plateau, China

WT: Wutai mountain; LY: Luya mountain; PQG: Yunding mountain located on Pang Quangou National Nature Reserve
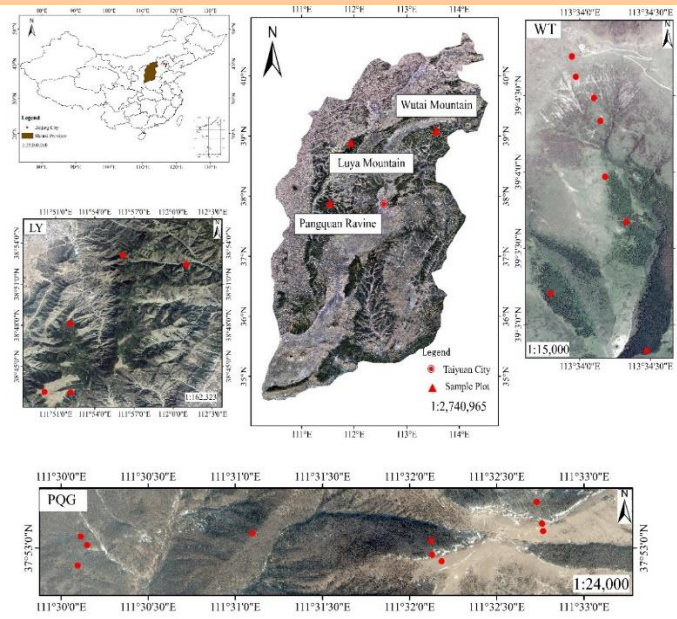
Figure 2(on next page)

Figure 2 Barplots (a) show the soil physicochemical factors of each sites. Correlation matrix graph (b) shows the correlation between soil physicochemical factors
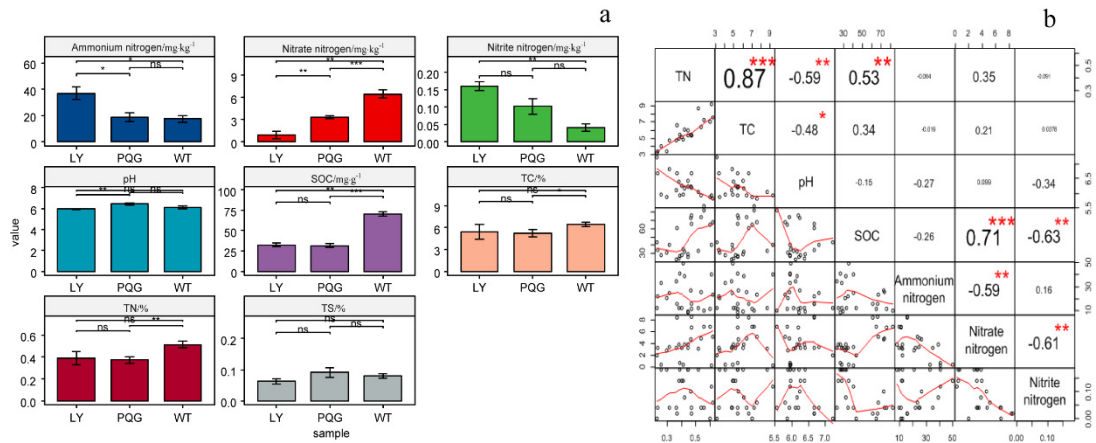
Figure 3(on next page)

Figure 3 Relative abundance of the dominant bacterial phylum (a) and class (c) across the sites. Venn Diagram (b) showed the shared OTUs in all plots. The heat map (d) shows clustering patterns in different plots
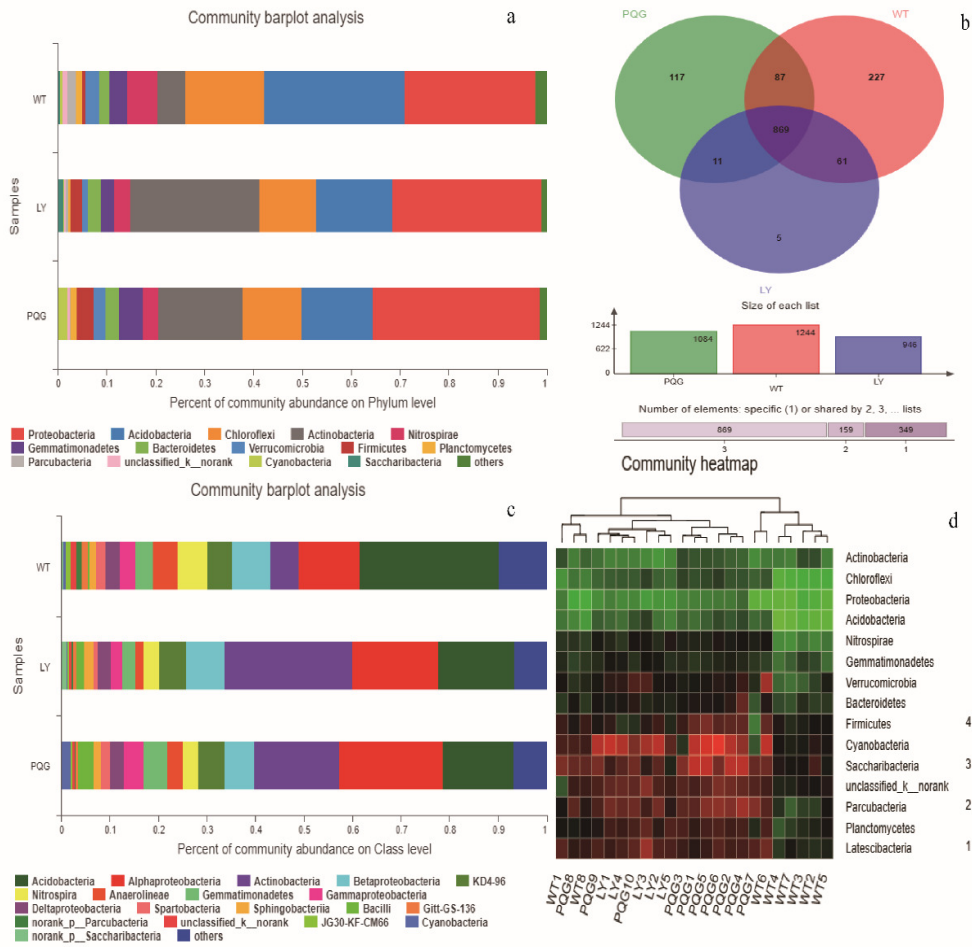
Figure 4(on next page)

Figure 4 Bacterial community diversity on different sites

ace: ACE index; chao: Chao index; shannon: Shannon index; simpson: Simpson index
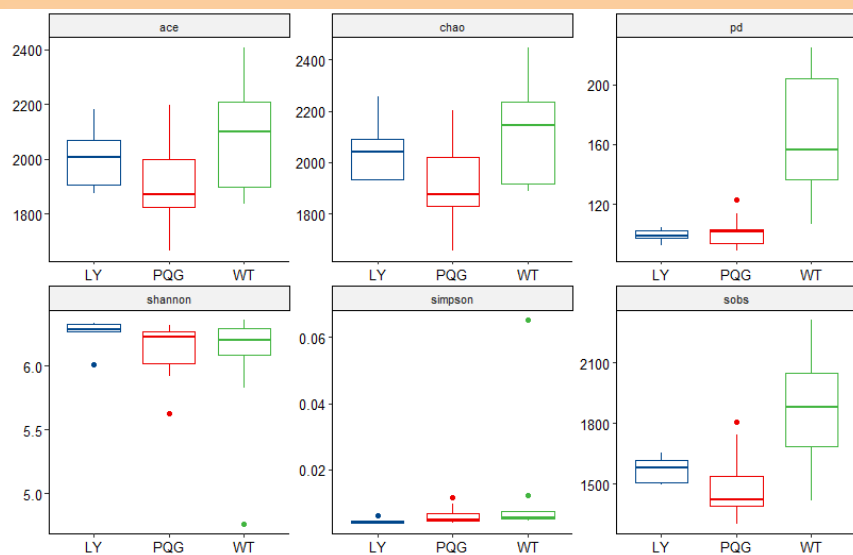
Figure 5(on next page)

Figure 5 Redundancy analysis (RDA) plots of bacterial communities and the response of these communities to significant soil physicochemical properties
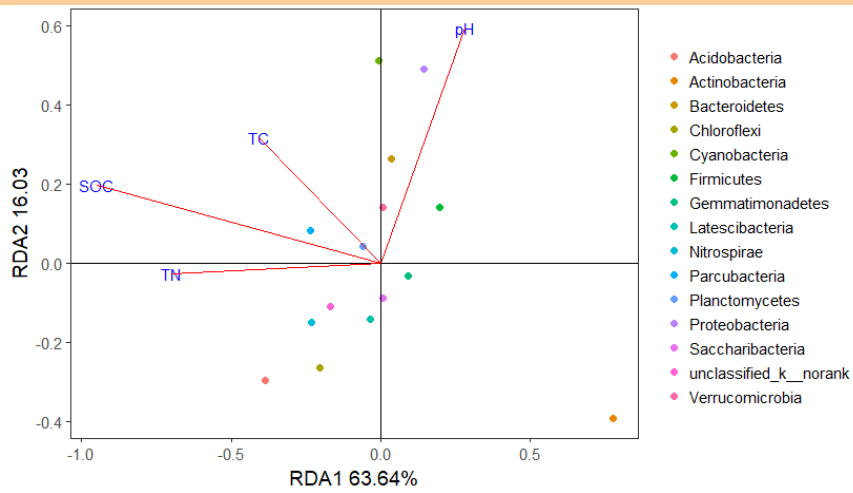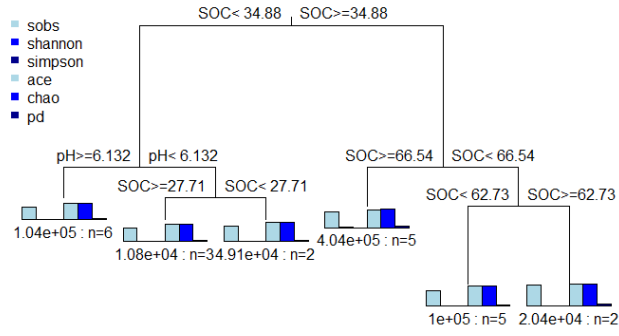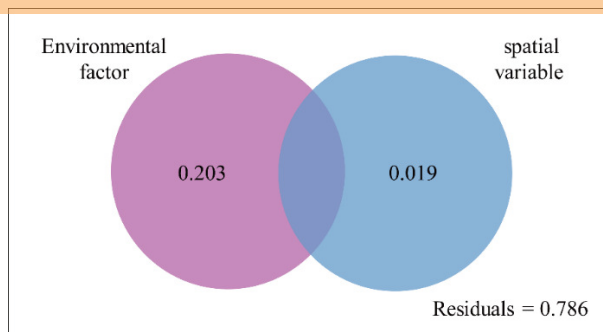
Figure 6(on next page)

Figure 6 Multivariate regression tree (MRT) of bacterial α-diversity data associated with key environmental factors

Figure 7(on next page)

Figure 7 Variation partitioning analysis showing the percentages of variance in bacterial communities explained by environment factor, spatial variable (PCNM)

Values <0 not shown

Figure 8(on next page)

Figure 8 Network of co-occurring OTUs colored by modularity class

A connection stands for a strong (Spearman's ρ > 0.6) and significant (p-value < 0.01)
correlation network. For each panel, the size of each node is proportional to the betweenness
centrality; the thickness of each connection between two nodes (edge) is proportional to the
value of Spearman's correlation coefficients (> 0.6)
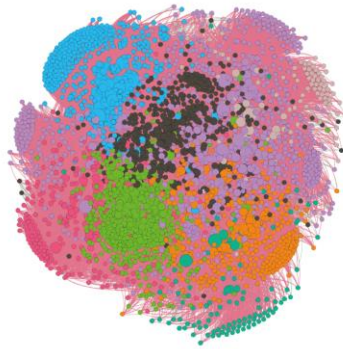
LY   PQG   WT

Table 1(on next page)

Table 1 Generalized Additive Mixed Model fitted to the NRI data across all sites

Table 1 Generalized Additive Mixed Model (GAMM) fitted to the

NRI data across all sites

☐	Estimate	SE	T	Pr(>|t|)
(Intercept)	-1.044	0.676	-1.546	0.141
TN	-0.129	0.578	-0.223	0.826
TC	0.014	0.034	0.411	0.686
pH	0.023	0.096	0.24	0.813
SOC	**0.004**	**0.002**	**2.472**	**0.024 ***
PCNM	0.0001	0.0001	1.158	0.263

SE: standard error

1

Figure 9(on next page)

Figure 9 The community assembly preocesses in different sites

a: null deviation values; b:NRI(fun); c: NRI(phy); d: NTI