New insight into the divergent responses of plants to warming in the context of root endophytic bacterial and fungal communities

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Plant adaptation under climate changes is critical to the maintenance of terrestrial ecosystem structure and function. Studying the response of the endophytic community to climate warming is a novel way to reveal the mechanism of host environmental adaptability because of the prominent role endophytes play in host nutrient acquisition and stress tolerance. However, host performance was generally neglected in previous relevant research, which limits our understanding of the relationships between the endophytic community and host responses to climate warming. The present study selected two plants with different responses to climate warming. *Elymus nutans* is more suitable for growing in warm environments at low altitude compared to *Kobresia pygmaea*. *K. pygmaea* and *E. nutans* were sampled along an altitude gradient in the natural grassland of Qinghai-Tibet Plateau, China. Root endophytic bacterial and fungal communities were analyzed using high throughput sequencing. The results revealed that hosts growing in more suitable habitats held higher endophytic fungal diversity. Elevation and host identity significantly affected the composition of the root endophytic bacterial and fungal community. 16S rRNA functional prediction demonstrated that hosts that adapted to lower temperatures recruited endophytic communities with higher abundance of genes related to cold resistance. Hosts that were more suitable for warmer and drier environments recruited endophytes with higher abundance of genes associated with nutrient absorption and oxidation resistance. We associated changes in the endophytic community with hosts adaptability to climate warming and suggested a synchronism of endophytic communities and hosts in environmental adaptation.

1 **New insight into the divergent responses of plants to warming in the context of root**

2 **endophytic bacterial and fungal communities**

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22 **Abstract**

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24 structure and function. Studying the response of the endophytic community to climate warming

25 is a novel way to reveal the mechanism of host environmental adaptability because of the

26 prominent role endophytes play in host nutrient acquisition and stress tolerance. However, host

27 performance was generally neglected in previous relevant research, which limits our

28 understanding of the relationships between the endophytic community and host responses to

29 climate warming. The present study selected two plants with different responses to climate

30 warming. *Elymus nutans* is more suitable for growing in warm environments at low altitude

31 compared to *Kobresia pygmaea*. *K. pygmaea* and *E. nutans* were sampled along an altitude

32 gradient in the natural grassland of Qinghai-Tibet Plateau, China. Root endophytic bacterial and

33 fungal communities were analyzed using high throughput sequencing. The results revealed that

34 hosts growing in more suitable habitats held higher endophytic fungal diversity. Elevation and

35 host identity significantly affected the composition of the root endophytic bacterial and fungal

36 community. 16S rRNA functional prediction demonstrated that hosts that adapted to lower

37 temperatures recruited endophytic communities with higher abundance of genes related to cold

38 resistance. Hosts that were more suitable for warmer and drier environments recruited

39 endophytes with higher abundance of genes associated with nutrient absorption and oxidation

40 resistance. We associated changes in the endophytic community with hosts adaptability to

41 climate warming and suggested a synchronism of endophytic communities and hosts in

42 environmental adaptation.

44 **Introduction**

45   Plant endophytes are microorganisms that colonize the intercellular space of plants and

46 establish a harmonious association with their host plants (*Odelade and Babalola, 2019*). The

47 genome of the host and its microbiome are considered a unit according to the hologenome theory

48 (*Zilber-Rosenberg and Rosenberg, 2008*). Studies on plant-associated microbiota recently

49 exhibited phenomenal expansion in the areas of natural and agricultural ecosystems due to their

50 great potential in improving host fitness, including nutrient acquisition (*van der Heijden, 2016*),

51 stress tolerance (*Liu et al., 2020*) and disease defense (*Dini-Andreote, 2020; Carrión, 2019*).

52 Endophytic community diversity was closely associated with multiple ecosystem functions

53 (*Laforest-Lapointe et al.,* 2017).

54   Recently, great attention has been paid to the environmental problems caused by climate

55 warming in terrestrial ecosystems, such as the loss of biodiversity, changes in vegetation

56 composition and community succession. Climate changes also affect plant ranges and allow

57 some species to expand or narrow their ranges (*Morin, Viner & Chuine, 2008*). The formation of

58 new interspecific relationships between plant species under climate change may drive these

59 ecological processes (*Little et al., 2016*), and endophytes may modulate the interspecific

60 interactions and vegetation composition (*Porras-Alfaro, 2011; Jackrel, 2020*).

61   Plant endophytic communities are dynamic and variable among seasons (*Li, Yang & Zhao,*

62 *2005*) and environments (*Laforest-Lapointe, 2016*) and reflect the adaptability of plants to

63 changing environments. The recent ''cry for help'' hypothesis suggests that plants selectively

64   recruit beneficial microbes from their surroundings under biotic or abiotic stress (*Neal et al.*

65   *2012; Liu et al. 2019*). *Pseudomonas* colonization in the roots of the desert plant *Alhagi*

66   *sparsifolia* increased under drought conditions and promoted drought resistance (*Zhang et al.,*

67   *2020*). Different plant species show unique abilities in the process of recruitment. For example,

68   most plants form symbioses with arbuscular mycorrhizal fungi (AMF) to increase phosphorus

69   availability. *Arabidopsis thalian*, which lose the ability of symbiosing with AMF, recruit

70   Helotiales and *Colletotrichum tofieldiae* under phosphorus-limited conditions (*Almario et al.,*

71   *2017*; *Hiruma et al., 2016*).

72   However, studies on the impacts of climate change on plant endophyte communities are

73   insufficient and many findings are conflicting. Endophytes and their hosts are "communities of

74   interest". Previous studies focused only on endophytic communities and host responses to

75   climate change were generally neglected (*Qian et al., 2018*; *Cordier et al., 2012*), which limits

76   our understanding of the ecological significance of endophytic community changes. Host

77   selection is critical because plant species (*Yao et al., 2019*; *Laforest-Lapointe, Messier & Kembel,*

78   *2016*) and compartments (leaf, stem, root) of the same plant greatly affect endophytic

79   community composition (*Qian et al., 2019*). Fujimura also concluded that the conflicting results

80   between studies on the effects of warming on endophytic communities may due to differences in

81   host responses (*Fujimura, Egger & Henry, 2008*). Therefore, plant species that are sensitive to

82   climate changes should be selected to examine the responses of endophyte communities and

83   improve our understanding of plant-microbe interactions and elucidate the mechanisms of plant

84   adaptation to climate change from the perspective of endophytes.

85   The Qinghai-Tibet Plateau is the highest plateau in the word, and it is experiencing rapid

86   climate warming at rate more than twice the global level (*Ma et al., 2017*), which threatens

87   biodiversity and ecosystem function in this area. *Kobresia pygmaea* and *Elymus nutans* are two

88   common plants in the Qinghai-Tibet Plateau that respond differently to climate warming.

89   *Kobresia pygmaea* is a carpet-like sedge species that is highly adaptable to high-altitude

90   environments, and it primarily grows on the alpine meadow of the Qinghai-Tibet Plateau

91   between altitudes of 3 000 m and 6 000 m (*Wu et al., 2017*; *Miehe et al., 2008*). The *K. pygmaea*

92   alpine meadow, which is dominated by *K. pygmaea*, covers 17% of the total area of the Qinghai-

93   Tibet Plateau (*Miehe et al., 2008*), which makes it an important species in maintaining the

94   stability of the local ecosystem. *Elymus nutans* is a dominant and constructive grass of meadow

95   steppe, and it is characterized by strong drought, cold and pest resistance. It is widely distributed

96   and survives at elevations ranging from 450 m to 4 500 m. *E. nutans* is better suited to low

97   altitudes and warmer habitats than *K. pygmaea,* and an increase in temperature from high to low

98   elevations promotes the growth of *E. nutans* (*Qi et al., 2020*). *K. pygmaea* primarily grows at

99   high altitudes in humid and cold habitats (*Wu et al., 2017*). Warming increased the proportion of

100   *Elymus nutans* and decreased the proportion of *K. pygmaea* in alpine grassland ecosystems (*Liu*

101   *et al., 2018; Niu et al., 2019*).

102   The present study examined the adaptability of plants to climate warming by analyzing

103   changes in endophytic community diversity and composition. We used elevation gradients where

104   climatic conditions, vegetation and soil characteristics show regular changes over short

105   geographic distances to investigate the responses of terrestrial ecosystems to climate warming

106   (*Korner, 2007*). *K. pygmaea* and *E. nutans* were sampled along an elevation gradient in the

107   Qinghai-Tibet Plateau, and the root endophytic bacterial and fungal communities were analyzed

108   based on the high-throughput sequencing. The innovation of this study lies in the selection of the

109   two hosts with opposing responses to climate warming to elucidate how endophytic communities

110   change during the promotion or inhibition of host growth by climate warming. We addressed the

111   following questions: 1) how endophytic community diversity in the roots of *K. pygmaea* and *E.*

112   *nutans* changed with climate warming (decreasing elevation); 2) whether climate warming

113   affected endophytic community composition in *K. pygmaea* and *E. nutans*; and 3) how changes

114   in the endophytic community explain host responses to climate warming.

115   **Materials and Methods**

116   **Study site and sampling**

117   Fieldwork was performed in August 2019 in the middle of the Qilian Mountains (37.68° N,

118   100.75° E) near Qilian County, which is located in the northeastern part of Qinghai Province,

119   China. With an average elevation over 3 000 m, this area experiences a typical plateau

120   continental climate. The mean annual and growing season temperatures (from June to August)

121   are 1°C and 13°C, respectively. The annual mean precipitation is 480 mm, with 80% occurring

122   during the growing season. The climate and vegetation composition change significantly with

123   elevation in the Qilian Mountains. We sampled at elevations of 3 350 m, 3 460 m, 3 570 m, 3

124   680 m, and 3 800 m above sea level (a.s.l.) on the south slope of the mountain. The temperature

125   decreases and the precipitation increases with increasing altitude in this area (*Jin et al., 2017*; *Li*

126   *et al., 2018*). According to the vegetation investigation, the grassland was classified as alpine

127   meadows at elevations of 3 350 m, 3 460 m, and 3 570 m and alpine shrub meadows at

128   elevations of 3 680 m and 3 800 m.

129   We defined three plots at each elevation, with each plot 20 m away from the other plots. The

130   roots of *K. pygmaea* and *E. nutans* were sampled around each plot. *K. pygmaea* always

131   aggregates in grasslands and forms slightly yellow green patches because of its dense network of

132   roots, which are produced clonally. Therefore, patches of *K. pygmaea* and clumps of *E. nutans*

133   were dug using a sterilized shovel from a depth of 10 cm. We reserved the belowground parts,

134   including the soil and roots, when sampling, and separated the roots after transported to the

135   laboratory. We reserved the belowground parts, including the soil and roots, when sampling, and

136   separated the roots after transport to the laboratory. Five individuals of each plant species per

137   plot composed one sample, which resulted in 30 root samples (2 species × 5 elevations × 3

138   replicates). All samples were placed in labeled sterile plastic bags and immediately stored in an

139   incubator with dry ice. Samples were transported to the laboratory and stored at -80°C until

140   further processing. The roots of *K. pygmaea* and *E. nutans* were separated in the laboratory using

141   sterilized scissors and brushes then transferred to 50-mL sterile centrifuge tubes containing 30

142   mL of 0.01 M sterilized PBS (136 mM NaCl, 8 mM Na_2HPO_4, 2 mM KH_2PO_4, 2.6 mM KCl, pH

143   7.2). The tubes were placed on a shaking platform (180 rpm) for 20 min to remove the soil

144   attached to the root. The roots were rinsed in deionized water and surface sterilized according to

145   the following steps: immersion in sterile water twice for 30 s, 75% ethanol for 1 min, 3.25%

146   sodium hypochlorite for 3 min, 75% ethanol for 30 s, sterile water for 1 min, and a final rinse

147   with sterile water. The surface-sterilized roots were stored in a freezer at -80°C.

Genomic DNA extraction, amplification and purification

148

149   Endophytic genomic DNA was extracted from 0.5 g of frozen surface-sterilized roots using

150   the MOBIO PowerSoil® DNA Isolation Kit (MOBIO Laboratories, Carlsbad, CA, USA). The

151   integrity and purity of the extracted DNA were tested on 1% agarose gels, and a NanoDrop One

152   was used to measure the concentration and purity of the extracted DNA. The 16S rRNA gene

153   V5-V7 region was amplified using the primers 799F (5'-AACMGGATTAGATACCCKG-3') and

154   1193R (5'-ACGTCATCCCCACCTTCC-3') with a 12-bp barcode (*Horton et al., 2014*). The

155   primer set 799F-1193R reduces co-amplification levels of mitochondrial gene during endophytic

156   16S rRNA gene PCR amplification (*Wang et al., 2018*). The internal transcribed spacer 1(ITS1)

157   region of fungal rRNA was amplified using the forward primer 5'-

158   CTTGGTCATTTAGAGGAAGTAA-3' and the reverse primer 5'-

159   GCTGCGTTCTTCATCGATGC-3' (*Horton et al., 2014*). Primers were constructed by

160   Invitrogen (Carlsbad, CA, USA). A 50-μL reaction containing 25 μL of 2x PremixTaq (Takara

161   Biotechnology, Dalian Co. Ltd., China), 1 μL of each primer (10 mM) and 3 μL of DNA

162   template (20 ng/μL) was used for PCR amplification in a BioRad S1000 (Bio-Rad Laboratory,

163   CA). The following thermal cycling process was used: 5 min at 94°C for initialization; 30 cycles

164   of denaturation at 94°C for 30 s; annealing at 52°C for 30 s; extension at 72°C for 30 s; and final

165   elongation at 72°C for 10 min. The PCR products of 3 replicates of each sample were mixed, and

166   1% agarose gel electrophoresis was used to detect the quality of the PCR products. The products

167   were mixed in equidensity ratios according to GeneTools analysis software (version 4.03.05.0,

168   SynGene). An EZNA Gel Extraction Kit (Omega, USA) was used for purification of the mixed

169   PCR products.

170   **Library preparation and sequencing**

171   Libraries were processed with the NEBNext® Ultra™ DNA Library Prep Kit for Illumina®

172   (New England Biolabs, USA). The libraries were assessed using a Qubit@ 2.0 fluorometer

173   (Thermo Scientific) and an Agilent Bioanalyzer 2100 system, and sequenced on the Illumina

174   HiSeq 2500 platform to generate 250-bp paired-end reads. High-quality clean reads were

175   obtained following the Trimmomatic quality control process (V0.33,

176   http://www.usadellab.org/cms/?page=trimmomatic). The clean paired-end reads from which the

177   barcodes and primers were removed, were merged using FLASH (V1.2.11,

178   https://ccb.jhu.edu/software/FLASH/). Effective clean tags were retained after filtering the

179   spliced sequences using Trimmomatic software.

180   **OTU cluster and species annotation**

181   Usearch software (V8.0.1517, http://www.drive5.com/usearch/) was applied for sequence

182   analysis. Sequences with ≥97% similarity were assigned to the same operational taxonomic unit

183   (OTU). Singletons were removed using usearch

184   (http://www.drive5.com/usearch/manual/chimera_formation.html) after OTU clustering, and the

185   chimera sequences were detected and removed using the UCHIME *de novo* algorithm

186   (http://www.drive5.com/usearch/manual/uchime_algo.html). The Greengenes

187   (http://greengenes.secondgenome.com/) and UNITE V8.0 databases were consulted based on the

188   RDP Classifier algorithm and the assign_taxonomy.py script

189   (http://qiime.org/scripts/assign_taxonomy.html) in QIIME for taxonomic annotation (the

190   confidence threshold was set to 0.8). OTUs that were annotated as chloroplasts or mitochondria

191   (16S rRNA) and could not be annotated at the kingdom level were removed.

192   Data analysis

193   The Shannon index of the endophytic bacterial and fungal communities was calculated using

194   QIIME (V1.9.1), and the Kruskal-Wallis test at the $P < 0.05$ level was used to examine the

195   effects of elevation and species on the diversity of endophytic bacterial and fungal community.

196   Nonmetric multidimensional scaling (NMDS) analysis based on Bray-Curtis distance was

197   performed to visualize the dissimilarities of endophytic bacterial and fungal community

198   composition in different species and at different elevations. Permutational multivariate analysis

199   of variance (PerMANOVA) using the "Anosim" function in R (3.6.1) with 999 random

200   permutations was performed to compare differences in endophytic community structure between

201   plant species and elevations. The correlations of elevation and the relative abundances of the top

202   20 genera were analyzed using the Spearman method. Samples from different elevations were

203   combined for further analysis of the differences in endophytic bacterial and fungal communities

204   between the two plant species. The linear discriminant analysis (LDA) effect size (LEfSe)

205   method with a threshold of 4 was used to investigate indicator species that exhibited significant

206   differences in relative abundance between plant species. KEGG functional analysis of

207   endophytic bacteria was performed using Phylogenetic Investigation of Communities by

208   Reconstruction of Unobserved States (PICRUSt) (*Langille et al., 2013*). We also analyzed genes

209   associated with cold resistance, antioxidant enzymes (catalase, peroxidase and superoxide

210   dismutase), nitrogen metabolism (nitrogen fixation, nodulation protein, nitrogen regulatory

211   protein, and nitrate reductase) and phosphorus metabolism (phosphatase). Ca^{2+} signaling

212   (*Monroy, Sarhan & Dhindsa, 1993*), cold shock proteins, HSP20 family proteins (*Elkelish et al.,*

213   *2020*), glutathione (*Liu et al., 2020*), glycerol-3-phosphate acyltransferases (*Gomes et al., 2000*),

214   glycine betaine (*Annunziata et al., 2019*; *Chen and Murata, 2011*), glycosyltransferases (*Shi et*

215   *al., 2020*), protein kinases (histidine kinase, serine protein kinase, and tyrosine kinase) (*Martín*

216   *and Busconi, 2001*), trehalose (*Liu et al., 2020*), nitric oxide (*Farnese et al., 2016*), and

217   antioxidant enzymes (*Baier et al., 2019*) have been reported to improve plant cold resistance.

218   **Results**

219   **Diversity of endophytic bacterial and fungal communities**

220   The rarefaction curves of the Shannon index reached a saturation plateau in all samples,

221   which suggested that the sampling was sufficient to obtain most of the OTUs (Fig. S1). We

222   found a higher Shannon index of the root endophytic bacterial and fungal communities in *K.*

223   *pygmaea* than *E. nutans*. Elevation significantly affected the Shannon index of root endophytic

224   bacteria in *K. pygmaea*, but not *E. nutans*by (Fig. 1A). The Kruskal-Wallis test revealed that

225   elevation had a significant effect on the Shannon index of root endophytic fungi in *E. nutans* (*P*

226   = 0.021) but not *K. pygmaea* (*P* > 0.05). The Shannon index of endophytic fungi in *E. nutans*

227   roots decreased significantly with increasing altitude, but an inconspicuous increasing trend was

228   observed for *K. pygmaea* roots (Fig. 1B). There was no significant difference in the Shannon

229   index of root endophytic fungi between *K. pygmaea* and *E. nutans* at lower elevations (3 350-3

230   570 m). The Shannon index of root endophytic fungi was much higher in *K. pygmaea* than *E.*

231   *nutans* at higher elevations (3 680 and 3 800 m).

Community compositions of endophytic bacteria and fungi

232

233   The NMDS ordination (Fig. 2) and analysis of similarities (ANOSIM) (Table 1) showed

234 that endophytic bacterial and fungal community composition in *K. pygmaea* was significantly

235 different than *E. nutans* (*R*=0.78, *P*=0.001; *R*=0.58, *P*=0.001) (Fig. 2A, 2B). Elevation also

236 obviously influenced root endophytic bacterial and fungal communities in *E. nutans* (*R*=0.42,

237 *P*=0.001; *R*=0.84, *P*=0.001) (Fig. 2C, 2D) and *K. pygmaea* (*R*=0.76, *P*=0.001; *R*=0.98, *P*=0.001)

238 (Fig. 2E, 2F). The root endophytic bacterial community composition in *K. pygmaea* was more

239 sensitive to elevation than *E. nutans*, which was validated by the R value of ANOSIM (Table 1).

240 Greater the R values indicate a more significant influence of altitude on community composition.

241 The endophytic fungal community was more sensitive to elevation than the bacterial community

242 for each species. Plant identity had a greater influence on the endophytic bacterial community

243 than the fungal community.

244   Proteobacteria was the predominant phylum in *E. nutans* and *K. pygmaea* roots (Fig. S2A).

245 The average relative abundance of Proteobacteria was 58% in *K. pygmaea* roots, which was

246 much lower than *E. nutans* (80%). Actinobacteria was the subdominant phylum in *K. pygmaea*

247 roots and accounted for 30% of the bacterial reads, which was much higher than its abundance in

248 *E. nutans* roots (1.4%). There were significant differences in root endophytic bacterial

249 community composition at the order level between *K. pygmaea* and *E. nutans* (Fig. 3A). For *E.*

250 *nutans* roots, Pseudomonadales was the most abundant order, with the relative abundance

251 ranging from 36% to 69% along the elevation gradient. The endophytic bacterial community

252 composition was more uniform in *K. pygmaea* roots than *E. nutans* roots. Rhizobiales accounted

253   for 20% of the total community abundance, and there was no obvious difference between

254   elevations. Pseudomonadales was highly abundant (50%) at the elevation of 3 800 m, and

255   Pseudonocardiales showed low abundance at this elevation.

256   Most endophytic fungi in all samples belonged to ascomycetes, with average 76% for *E.*

257   *nutans* roots and 61% for *K. pygmaea* (Fig. S2B). Helotiales were the dominant order in *E.*

258   *nutans* roots and were more abundant at high altitudes, which was different from that in *K.*

259   *pygmaea* (Fig. 3B). Pleosporales were more abundant in *K. pygmaea* roots than *E. nutans* roots,

260   especially at lower altitudes (3 350-3 570 m). Agaricales were enriched in *K. pygmaea* roots at

261   higher altitudes (3 680 m and 3 800 m) and more abundant in *E. nutans* roots (Fig. 3B). The

262   relative abundance of Hypocreales in *K. pygmaea* roots were 18.55% and 8.1% at 3 350 m and 3

263   460 m, respectively, which was higher than *E. nutans* roots at the same elevations (Fig. 3B).

264   We compared the abundances of the top 20 OTUs which covered more than 50% of the

265   total reads in all samples at different altitudes and found that plants tended to be enriched with

266   specific endophytes at different elevations (Fig. S3A-D). Species with significant differences in

267   relative abundance between *K. pygmaea* and *E. nutans* were analyzed according to LEfSe (Fig.

268   4). The results indicated that bacterial species belonging to Actinobacteria (Frankiales,

269   Pseudonocardiales and Micromonosporales) and Rhizobiales (Rhizobiaceae) were highly

270   enriched in *K. pygmaea* roots, and Gammaproteobacteria (Burkholderiaceae), Pseudomonadales

271   (Pseudomonadaceae) and Flavobacteriales (Flavobacteriaceae) were highly enriched in *E. nutans*

272   roots (Fig. 4A). Fungal species belonging to Dothideomycetes (Pleosporales), Eurotiomycetes

273   (Chaetothyriales), and Sordariomycetes (Coniochaetales, Hypocreales) were highly enriched in

274   *K. pygmaea* roots, and Helotiales (Hyaloscyphaceae, Helotiaceae) and Tremellales

275   (Bulleribasidiaceae) were highly enriched in *E. nutans* roots (Fig. 4B).

276   The correlations of the relative abundances of the top 20 genera and elevation were

277   analyzed (Table 2). For bacterial communities, the relative abundances of *Pantoea*,

278   *Sphingomonas*, and *Paenibacillus* in *E. nutans* roots showed positive correlations with elevation.

279   The relative abundances of *Pseudomonas*, *Pantoea*, *Serratia*, and *Paenibacillus* in *K. pygmaea*

280   roots positively correlated with elevation, and *Rhizobium*, *Acidibacter*, *Actinophytocola*, and

281   *Cryptosporangium* negatively correlated with elevation. For fungal communities, the relative

282   abundances of *Mortierella* and *Cistella* showed negative correlations with elevation in *E. nutans*

283   roots. The relative abundances of *Mycena*, *Mortierella*, *Ilyonectria*, *Sebacina*, and *Leptosphaeria*

284   showed positive correlations with elevation in *K. pygmaea* roots.

285   The results of 16S rRNA functional prediction indicated that the endophytic bacterial

286   community in *E. nutans* roots had a higher abundance of genes associated with nitrogen fixation,

287   phosphatase activity and antioxidase activity and a lower abundance of genes associated with

288   cold resistance than *K. pygmaea* roots (Fig. 5). Genes associated with nutrient absorption,

289   including nitrogen fixation, nodulation, and phosphatase activity, were enriched in *K. pygmaea*

290   roots at higher elevations. These genes were enriched at lower elevations in *E. nutans* roots, (Fig.

291   S4).

292   **Discussion**

293   **Diversity of the endophytic community along an elevation gradient**

294   Most studies on the influence of climate change on plant associated microbial communities

295 overlooked host responses and did not link the responses of hosts and endophytes (*Qian et al.,*

296 *2018*; *Cordier et al., 2012*). Therefore, the significance of changes in the endophytic community

297 of the host in response to climate change were not well explained. Two plant species showing

298 contrasting responses to climate warming were selected in the present study to fill in this

299 knowledge gap. The increase in endophytic fungal diversity in *E. nutans* and decrease in *K.*

300 *pygmaea* with warming (i.e., decreasing elevation) indicated that the endophytic fungal diversity

301 of plants growing in suitable environments was higher than plants growing in unsuitable habitats.

302 Our findings demonstrated that changes in endophytic fungal diversity under climate warming

303 were closely related to host adaption, and endophytic diversity decreased when the climate

304 became unfavorable for plant growth. A previous study indicated that endophytic diversity in

305 sugar maple seedlings was higher within its natural range than at the edge of species' elevational

306 range (*Wallace, Laforest-Lapointe & Kembel, 2018*), which was consistent with the results of our

307 study. We hypothesized that the loss of endophytic diversity in adverse environments was related

308 to host selection. The symbiotic relationships between the host and some endophytes break down,

309 and some endophytes that are beneficial to host survival are selectively enriched (*Werner et al.,*

310 *2018*).

311   Plant adaptability to environmental changes is very complex and affected by many factors.

312 We just found a phenomenon that fungal diversity was higher in the habitat that suitable for host

313 growth. However, no direct cause-and-effect relationship between fungal diversity and host

314 adaptation was demonstrated in the present study. Whether higher endophytic diversity

315 contributes to host growth is worthy to study in the future.

316 **Species and elevation significantly affected the root endophytic bacterial and fungal**

317 **communities**

318 Previous studies indicated that plant endophytes showed significant host preferences (*Toju,*

319 *Kurokawa & Kenta, 2019*; *Yao et al., 2019*). Different plant species sharing similar environments

320 recruit different microbial communities in roots (*Aleklett et al., 2015*), which may be related to

321 the metabolic characteristics of the host. Because root exudates contain important factors that

322 shape endophytic microbiome assembly, such as salicylic acid (*Lebeis et al., 2015*) and jasmonic

323 acid (*Carvalhais et al., 2015*). Environmental parameters are also key factors thatcause changes

324 in the endophytic community (*Carper et al., 2018*; *Bei et al., 2019*; *Chen et al., 2019*). Climate

325 characteristics, soil nutrients and vegetation composition change along altitude gradients, which

326 may cause variations in the endophytic community (*Cai et al., 2020*; *Zarraonaindia et al., 2015*).

327 Plant species and altitude significantly affected the root endophytic bacterial and fungal

328 community composition in the present study. The bacterial community composition was affected

329 more strongly by plant species, and elevation more strongly affected the fungal community.

330 Previous studies also concluded that fungal communities were more susceptible to geographic

331 distance than bacterial communities (*Coleman-Derr et al., 2016*; *Meiser, Bálint & Schmitt, 2014*).

332 Among the endophytic bacteria, Proteobacteria was the predominant phylum in *E. nutans*

333 and *K. pygmaea* roots, which was consistent with many other studies involving different plant

334 species (*Bulgarelli et al., 2012*; *Beckers et al., 2017*; *Carrell and Frank, 2015*). However, *K.*

335 *pygmaea* roots had a higher abundance of Actinobacteria than *E. nutans* roots. At the order level,

336 the relative abundances of Pseudonocardiales, Frankiales and Rhizobiales in *K. pygmaea* roots

337   were much higher than *E. nutans* roots. However, *E. nutans* roots had a higher abundance of

338   Pseudomonadales. These root endophytes promote host plant growth and help plants resist biotic

339   and abiotic stress (*Hasegawa, et al., 2006*; *Santoyo et al., 2016*). *Pseudomonas* participates in

340   nitrogen fixation (*Yan et al., 2008*) and iodoacetamide (IAA) synthesis (*Taghavi et al., 2009*),

341   which promote plant growth. In addition to symbiosis with leguminous plants, *Rhizobium* acts as

342   an endophyte of nonleguminous plants to promote nitrogen and phosphorus uptake and improve

343   the photosynthetic rate (*Yanni et al., 2001*; *Chi, 2006*). Helotiales, which promote phosphorus

344   absorption in nonmycorrhizal plants (*Almario et al., 2017*), were enriched in *E. nutans* roots.

345   Some genera, such as *Mortierella,* showed positive correlations with elevation in *K. pygmaea* but

346   negative correlations with elevation in *E. nutans*, which also reflected the plant selectivity.

347   **Functional differences of root endophytic bacteria in *K. pygmaea* and *E. nutans***

348   Chilling stress is an important environmental factor that affects plant growth and

349   geographical distribution (*Lv et al., 2018*), which is especially true on the Qinghai-Tibet Plateau.

350   A previous study also demonstrated that temperature and altitude factors primarily affected the

351   distribution of alpine meadow grass (Lu et al., 2020). Symbiosis with bacteria that could improve

352   the cold tolerance of the host represents a survival strategy in alpine regions with high elevations

353   (*Acuna-Rodriguez et al., 2020*; *Tiryaki, Aydin & Atici, 2019*). Beirinckx *et al*. also indicated that

354   the root microbiome promoted maize growth under chilling conditions (*Beirinckx et al., 2020*).

355   The present study provided new insight into the role of endophytic bacteria in host adaptation to

356   climate warming in terms of bacterial functional genes associated with cold resistance and

357   nutrient absorption.

358   The study area in the present study exhibited a cold and wet climate at high altitudes and a

359   warm and dry climate at low altitudes. Temperature is the main factor affecting plant growth at

360   high elevations. Increased recruitment of bacteria with cold resistance ability in *K. pygmaea*

361   roots made this plant more adaptable to high altitude than *E. nutans* although the abundance of

362   genes associated with nutrient uptake was higher in *E. nutans* than *K. pygmaea*. Because the

363   expression of these genes and the enzymes activity involved in nutrient absorption are restricted

364   by low temperatures. Therefore, symbiosis with high abundance of these endophytes in *E. nutans*

365   cost plant carbohydrates without getting the expected return.

366   The outcomes of symbiosis with microbiomes are environmentally dependent (*Rubin et al.,*

367   *2020*). For example, arbuscular mycorrhizal fungi significantly enhance phosphorus absorption

368   and promote plant growth in phosphorus deficient soils, but the contribution of mycorrhizal

369   pathways to phosphorus uptake in plants is weakened under high P soil condition (*Chu et al.,*

370   *2020*). Climate warming relieves cold stress at high elevations and exacerbates heat and drought

371   stress at lower elevations (Reinhardt et al., 2011; Moyes et al., 2013). With climate warming or

372   decreasing altitude, plant growth is freed from the cold limitation. Selectively symbiosis with

373   bacteria that have the ability to facilitate host nutrient absorption, such as nitrogen fixation and

374   phosphorus solubilization, in *E. nutans* roots made this plant more adaptable to warming

375   environments than *K. pygmaea*.

376   Endophytes do not always establish harmonious symbioses with plant hosts. They also cause

377   host disease and other adverse effects. There in a 'balance of antagonisms' relationship between

378   fungal and plant partners (*Schulz and Boyle 2005*). We only focused on the "good" side of

379   endophytes for their importance in maintaining a stable symbiotic relationship. Exploring

380   beneficial roles of endophytes to their hosts in special environments such as the Qinghai-Tibet

381   Plateau contributes a lot to the development and application of microbial resources. However,

382   functional prediction alone is not sufficient to directly demonstrate the role of endophytic

383   bacteria in host responses to climate changes. Further studies should concentrate more on the

384   interactions between endophytic community and host to rich our understanding of responses of

385   terrestrial ecosystem to global warming.

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389   **Conclusions**

390   The present study demonstrated that the diversity of the endophytic community was higher

391   in hosts growing in habitats that were conducive to its growth. *K. pygmaea*, with higher

392   endophytic diversity and greater abundance of genes associated with cold resistance, was suited

393   for growth in cold areas at high altitudes, and its growth was suppressed by warming. Higher

394   endophytic diversity and greater abundance of genes associated with nutrient absorption and

395   oxidation resistance in warmer environments (lower altitude) may contribute to the growth of *E.*

396   *nutans* under global warming (Fig. 6). We also found that the relative abundances of the same

397   taxa in different hosts showed different correlations with elevation, which demonstrated that the

398   ability to recruit endophytes differed between the hosts when the habitat changed. Our study

399   highlights the importance of plant endophytes in the responses of terrestrial ecosystems to

400 climate change.

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404 **Conflict of interest:**

405 The authors declare there are no competing interests

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Table 1. Significance test of community composition differences among elevations and hosts determined by permutational multivariate analysis of variance using distance matrices (PERMANOVA) according to Bray-Curtis distances.

1 **Table 1.** Significance test of community composition differences among elevations and hosts

2 determined by permutational multivariate analysis of variance using distance matrices

3 (PERMANOVA) according to Bray-Curtis distances.

			Anosim	
			R	*P*
Bacteria	Elevation	*E. nutans*	0.416	0.001
		K. pygmaea	0.756	0.001
	Host species	*E. nutans* vs *K. pygmaea*	0.78	0.001
Fungi	Elevation	*E. nutans*	0.84	0.001
		K. pygmaea	0.984	0.001
	Host species	*E. nutans* vs *K. pygmaea*	0.579	0.001

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Spearman correlation analysis between elevation and the relative abundance of bacterial and fungal at genus level.

1 **Table 2.** Spearman correlation analysis between elevation and the relative abundance of bacterial

2 and fungal at genus level.

	Genus	K. pygmaea		E. nutans	
		R	P	R	P
Bacteria	*Pseudomonas*	**0.731**	**0.002**	0.153	0.587
	Pantoea	**0.633**	**0.011**	**0.556**	**0.031**
	Rhizobium	**-0.546**	**0.035**	0.011	0.969
	Acidibacter	**-0.676**	**0.006**	-0.207	0.458
	Actinophytocola	**-0.524**	**0.045**	0.022	0.938
	Cryptosporangium	**-0.676**	**0.006**	-0.016	0.954
	Serratia	**0.535**	**0.040**	0.491	0.063
	Sphingomonas	-0.262	0.346	**0.644**	**0.010**
	Paenibacillus	**0.742**	**0.002**	**0.764**	**0.001**
Fungi	*Mycena*	**1.000**	**0.000**	-0.700	0.188
	Mortierella	**0.900**	**0.037**	**-0.900**	**0.037**
	Cistella	0.700	0.188	**-0.900**	**0.037**
	Ilyonectria	**0.900**	**0.037**	-0.800	0.104
	Sebacina	**0.900**	**0.037**	-0.700	0.188
	Leptosphaeria	**0.975**	**0.005**	-0.616	0.269

Figure 1

Shannon index of root endophytic bacteria (A) and fungi (B) in *K. pygmaea* and *E. nutans* at different elevations.

Averages ± SE are displayed (n=3). Different letters indicate significant differences between elevations

A

E. nutans

K. pygmaea

Bacteria shannon index

Elevations: 3350, 3460, 3570, 3680, 3800

B

E. nutans

K. pygmaea

Fungi shannon index

Elevations (m)
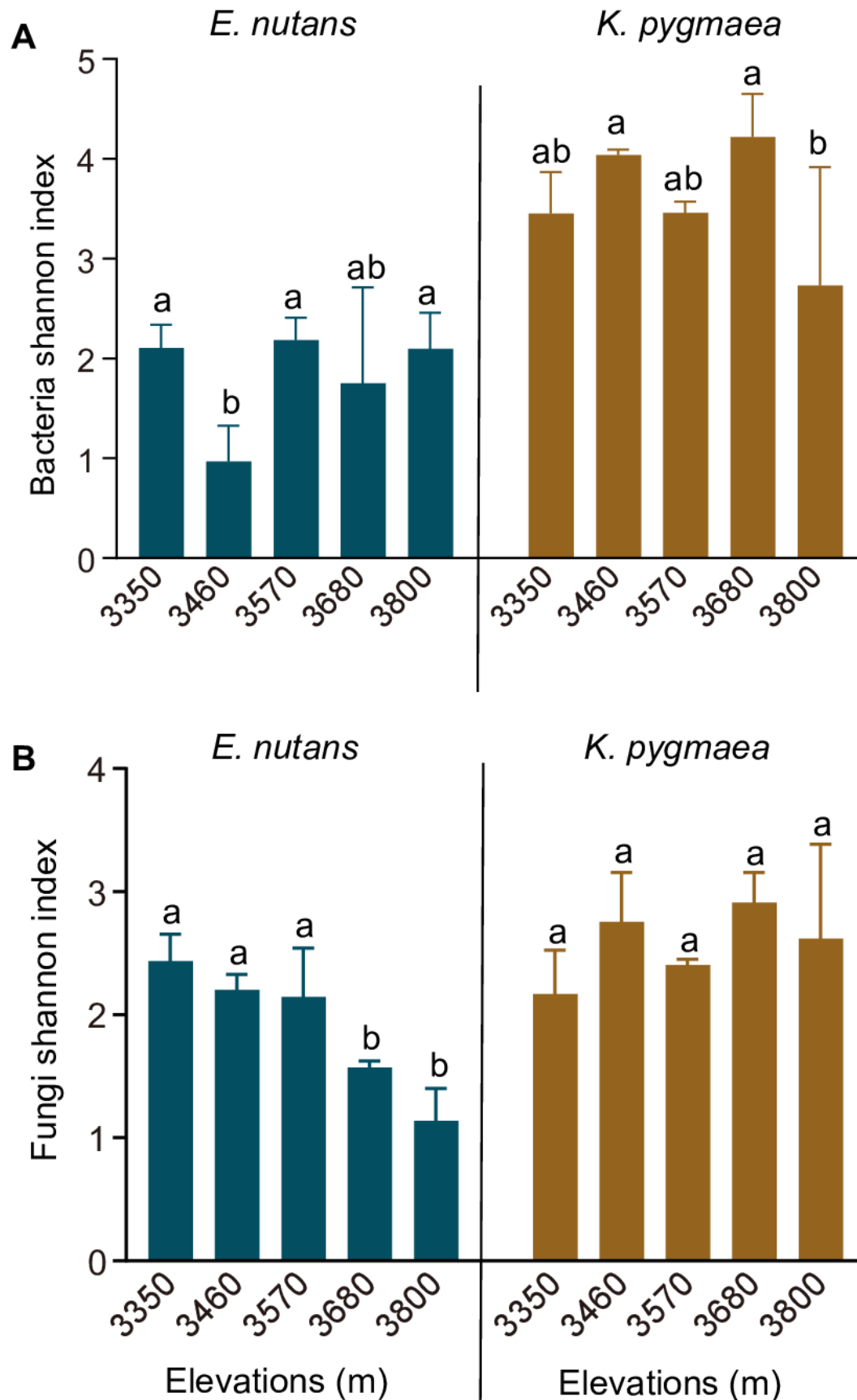
Figure 2

Non-metric multidimensional scaling (NMDS) ordination of the community composition of endophytic bacteria and fungi based on Bray-Curtis distances.

A and B indicated fungal and bacterial community composition in *E. nutans* and *K. pygmaea* root regardless of elevations; C and D indicated fungal and bacterial community composition at different elevations in *E. nutans* root; E and F indicated fungal and bacterial community composition at different elevations in *K. pygmaea* root. Plant species were distinguished by colors, and elevations were distinguished by shapes.
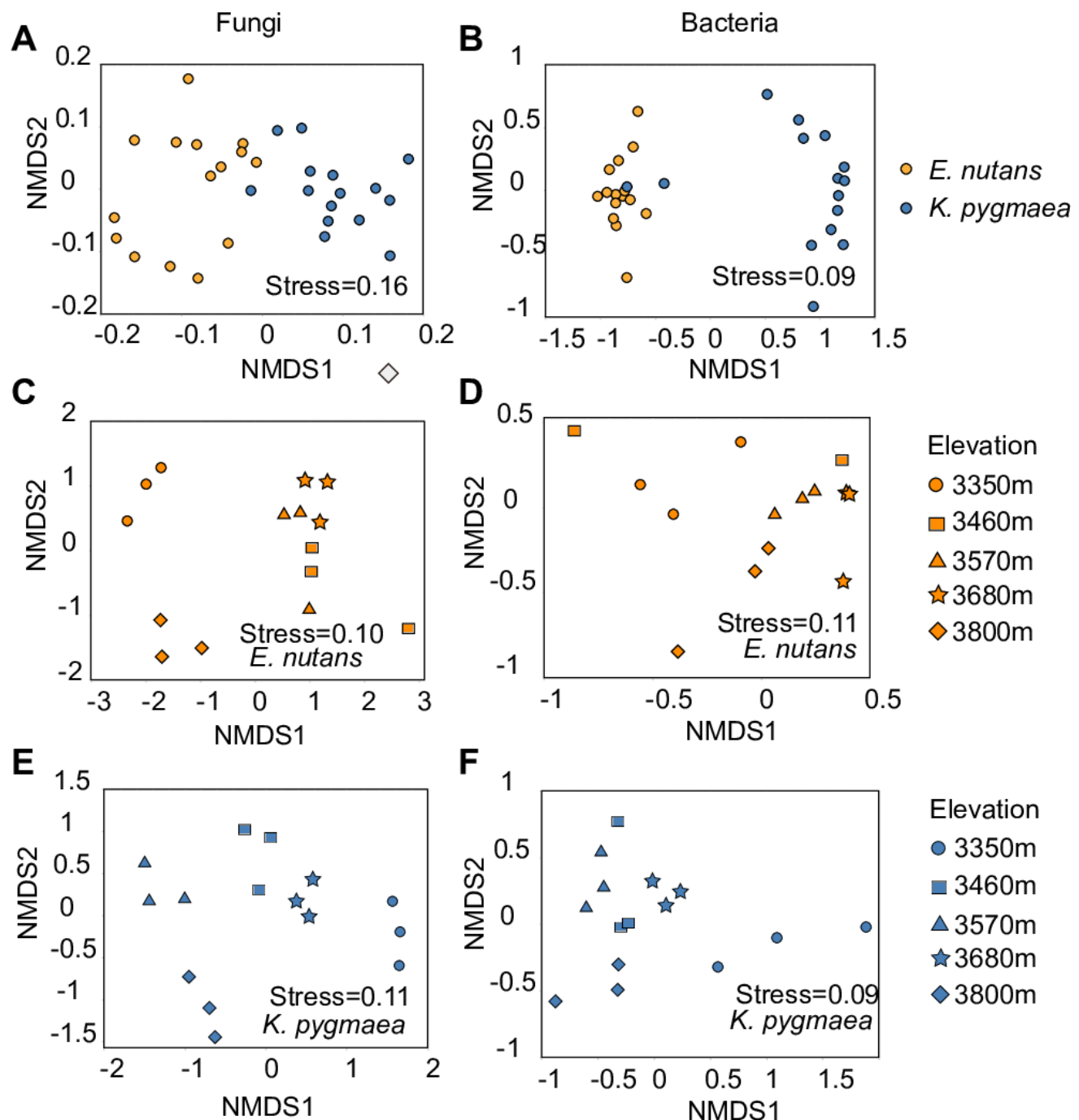
Figure 3

Relative abundance of bacterial (a) and fungal taxa (b) at the order level.

ER *E. nutans root*; KR *K. pygmaea root*; the numbers represent elevations. For example, ER3350 indicate *E. nutans* root at the elevation of 3 350 m; KR3350 indicate *K. pygmaea* root at the elevation of 3 350 m. The same as below.
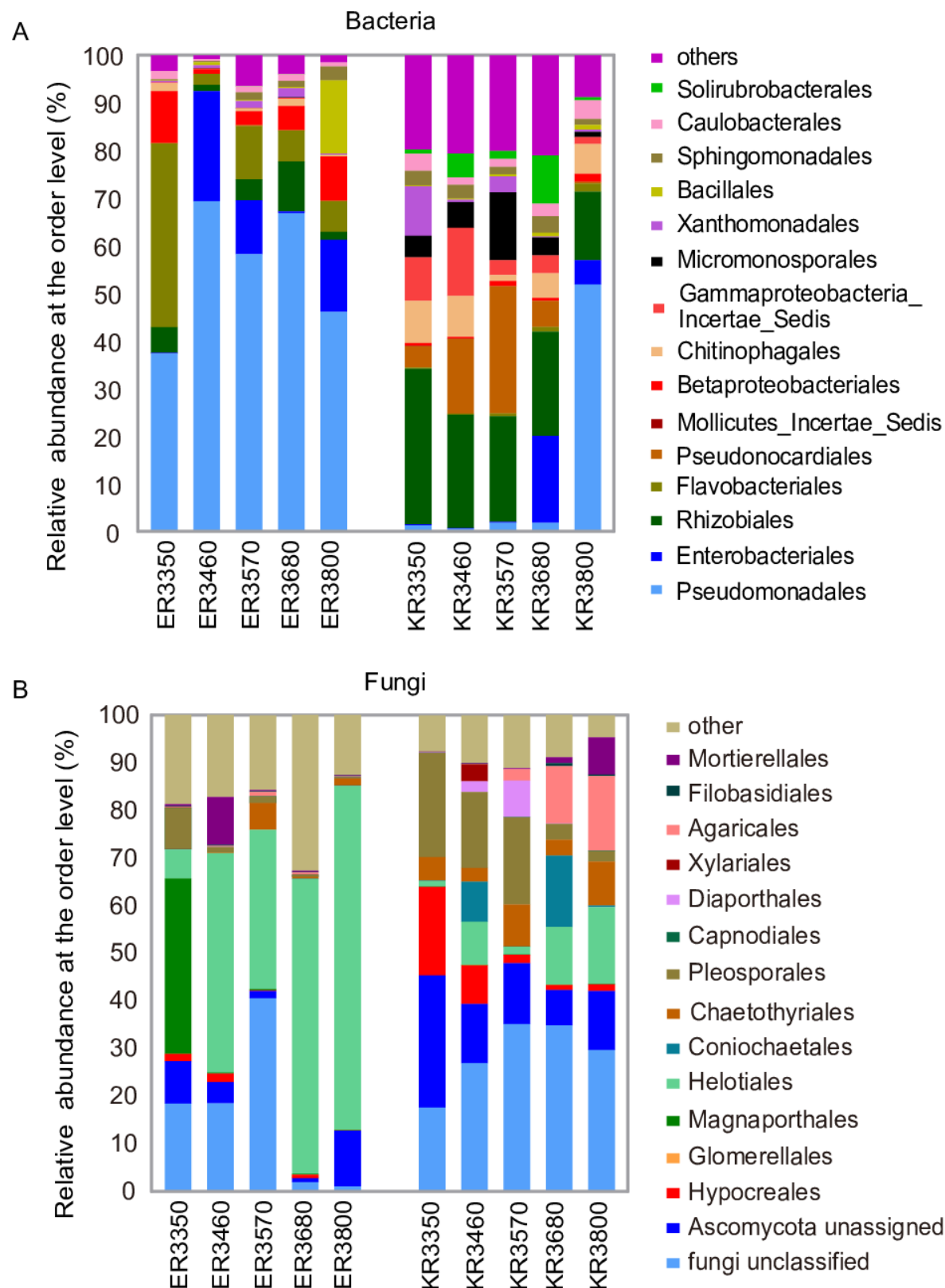
Figure 4

Cladograms of LEfSe showing bacterial and fungal taxa with significant differences in relative abundance between *K. pygmaea* and *E. nutans*.

The filled circles from inside to outside indicate the taxonomic levels with phylum, class, order, family, genus, and species. Circles or nodes shown in color corresponding to different plant species represented a significantly more abundant group. Yellow circles indicate species with no significant differences in relative abundance.

Bacteria

A

■ *E. nutans*
■ *K. pygmaea*

Thermoleophilia

Actinobacteria
Actinobacteria

Gammaproteobacteria

Alphaproteobacteria

Proteobacteria

- a: Cryptosporangium
- b: Cryptosporangiaceae
- c: Frankiales
- d: Micromonosporaceae
- e: Micromonosporales
- f: Actinophytocola
- g: Pseudonocardia
- h: Pseudonocardiaceae
- i: Pseudonocardiales
- j: 67_14
- k: Solirubrobacterales
- l: Chitinophaga
- m: Chitinophagaceae
- n: Chitinophagales
- o: Flavobacterium
- p: Flavobacteriaceae
- q: Flavobacteriales
- r: Allorhizobium_Neorhizobiu
- s: Rhizobiaceae
- t: Xanthobacteraceae
- u: Rhizobiales
- v: Burkholderiaceae
- w: Betaproteobacteriales
- x: Acidibacter
- y: Unknown_Family
- z: Gammaproteobacteria_Inc
- a0: Pseudomonas
- a1: Pseudomonadaceae
- a2: Pseudomonadales
- a3: Steroidobacter
- a4: Steroidobacteraceae
- a5: Steroidobacterales

Fungi

B

Ascomycota
Leotiomycetes
Eurotiomycetes
Dothideomycetes

- a: Massarinaceae
- b: unidentified
- c: unidentified
- d: Pleosporales
- e: Cladophialophora
- f: Herpotrichiellaceae
- g: Chaetothyriales
- h: unidentified
- i: Helotiaceae
- j: Hyaloscyphaceae
- k: unidentified
- l: unidentified
- m: Helotiales
- n: Coniochaeta
- o: Coniochaetaceae
- p: Coniochaetales
- q: Diaporthales
- r: Hypocreales_fam_Incertae_
- s: Hypocreales

Figure 5

Differences in relative abundance of functional genes associated with cold resistance, nitrogen absorption, phosphatase and antioxidase between *K. pygmaea* and *E. nutans*.
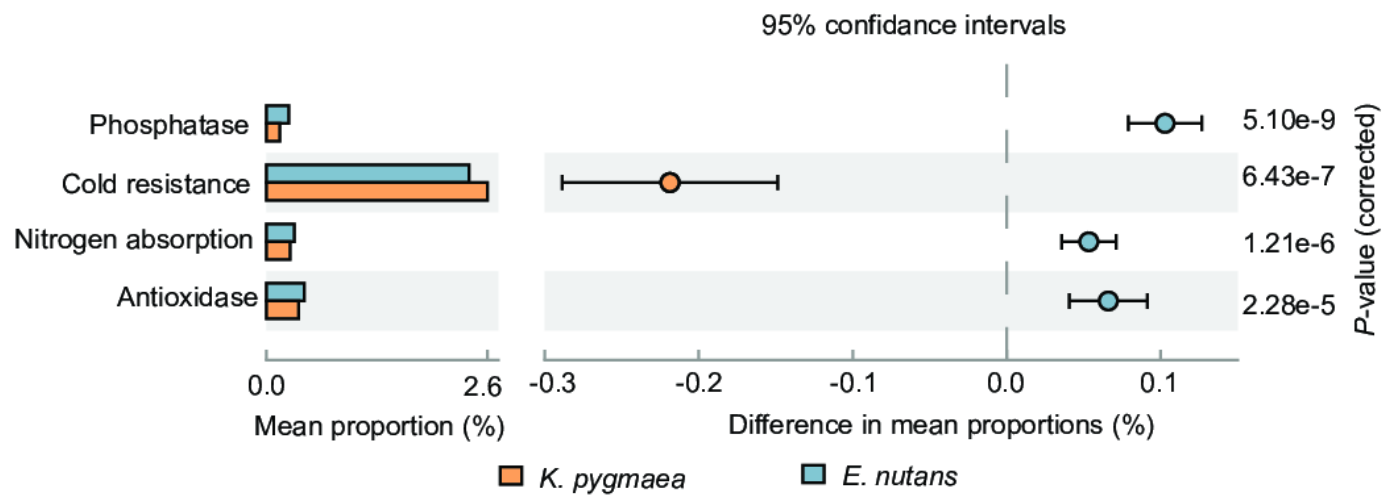
Figure 6

Frame diagram of response of endophytic bacterial and fungal community to altitude

The proportion of *Elymus nutans* in vegetation community as well as its endophytic fungal diversity decreased with elevation increasing. While, the proportion of *Kobresia pygmaea* as well as its endophytic fungal diversity showed a slight increase trend with elevation increasing. The Shannon index of root bacterial and fungal community in *Kobresia pygmaea* were higher than that in *Elymus nutans*. Besides, bacteria in *Kobresia pygmaea* root had more genes associated with cold resistance, and in *Elymus nutans* root had more genes associated with nutrient absorption.