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Key microbial phylum influencing the priming effects of variously degraded alpine meadow soils enriched with animal excrements

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ABSTRACT：

Tibetan sheep (*Ovis aries*) and plateau pika (*Ochotona curzoniae*) excrements are important organic materials that influence soil carbon cycling in the Qinghai-Tibetan Plateau. However, their exact priming effects (PE) on soil and mechanisms of influence in alpine meadows are subject to their health status. To fill this knowledge gap, we carried out a 45-day incubation experiment using alpine meadow soils with differing degrees of degradation that had been enriched with these two types of excrement. Soil PE was assessed via the natural abundance method, while soil microbial communities and their compositions were examined through high-throughput sequencing. The findings indicated that severely degraded alpine meadow soils exhibit significantly stronger positive priming effects (PE) from Tibetan sheep and plateau pika excrements than non-degraded soils ($P <$ 0.05). These excrements restructure soil microbial communities, reducing bacterial α-diversity while increasing β-diversity and microbial biomass C:N ratios. Key microbial drivers of PE include Actinobacteriota (positive correlation, r = 0.70) and Proteobacteria (negative correlation, r = −0.52), whose abundances are modulated by soil properties: the C:N ratio promotes Proteobacteria (r = 0.39), while a lower NO_3^--N:NH_4^+-N ratio suppresses Actinobacteriota (r = −0.18). Meadow degradation thus indirectly intensifies PE by altering these microbial phylum and stoichiometric balances. These findings support Qinghai-Tibetan Plateau grassland management through: (1) restricting grazing/pika activity in degraded zones to reduce carbon loss, (2) optimizing soil C:N

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ratios to suppress priming effect (PE)-enhancing microbes, and (3) conserving intact meadows for

carbon sequestration—collectively enabling sustainable grassland management.

Keywords: Soil microbia phylum, degree of meadow degradation, soil priming effects, excrement supplements

1. Introduction

Renowned as the "Roof of the World" and the "Third Pole of the Earth", the Tibetan Plateau, with an average elevation of 4,000 m above sea level, is the highest plateau in the world (Qiu, 2008), where the land cover is dominated by alpine meadows. They offer a multitude of invaluable ecological services, including the preservation of biodiversity and water resources (Liu et al., 2018), the mitigation of greenhouse gas emissions, and the safeguarding of habitats for endangered species (Chen et al., 2018; Wang et al., 2020). However, about 80% of the alpine meadows have been degraded to varying degrees (Li et al., 2013; Zhu et al., 2022). Degraded alpine meadows exhibit a decreased plant cover and biodiversity (Urak et al., 2017; Yang et al., 2013), accompanied by a decline in soil nutrients and microbial biomass (Korkanc and Korkanc, 2016; Nunes et al., 2012; Shang et al., 2016). These changes have subsequently altered soil microbial composition and structure (Li et al., 2021), ultimately affecting the capacity of the alpine meadows to sequester soil carbon (Kou et al., 2019; Wang et al., 2002).

In an effort to restore the compromised meadow ecosystem functions, various measures have been attempted, such as supplementation of exogenous organic matter. After its application to the soil, it may promote or inhibit soil organic carbon mineralization temporally, a phenomenon termed the priming effect (PE) (Chen et al., 2014; Fontaine et al., 2004; Fontaine et al., 2003; Kuzyakov et al., 2000; Smith et al., 2014). Previous studies have indicated that the nature of exogenous organic matter, the existing soil nutrient status, and microbial composition are all factors influential to the magnitude and duration of PE (Blagodatskaya and Kuzyakov, 2008). Moreover, alterations in soil microbial composition exert a significant impact on ecosystem carbon cycling (Chen et al., 2021). Researchers have discovered that specific microbial

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58　phylum, such as Proteobacteria and Acidobacteria, can facilitate the decomposition of

59　soil organic matter (SOM) and may play a crucial role in modulating soil PE

60　(Razanamalala et al., 2018; Tao et al., 2020).

61　　　As a widespread and readily available soil amendment, animal excrement not only

62　influences soil physicochemical properties and enzyme activities but also profoundly

63　alters the composition of soil microbial communities (Ai et al., 2018; Ramirez et al.,

64　2012; Wang et al., 2017) and dominant microbial communities in soil, and subsequently

65　influences the efficiency of organic carbon cycling (Zhang et al., 2015). For instance,

66　r-strategist microbes have a high demand for nitrogen and prefer easily accessible

67　substrates, while oligotrophic K-strategist microbes favor recalcitrant carbon

68　substrates, and have a lower nitrogen requirement (Fierer et al., 2007; Fontaine et al.,

69　2003). They can thrive in soils of a high stoichiometric C:N ratio, and release

70　extracellular enzymes to extract nitrogen from SOM (Heitkötter et al., 2017; Hicks et

71　al., 2019; Zechmeister-Boltenstern et al., 2015). Conversely, they tend to dominate

72　substrates with a lower stoichiometric C:N ratio that match the growth rates and activity

73　demands of fast-growing r-strategist microbes (Chen et al., 2014; Fang et al., 2018;

74　Hessen et al., 2004), thereby altering SOM decomposition. So far, it has been confirmed

75　that exogenous supplements with a low C:N ratio are more likely to influence soil

76　microbial composition as they are mineralized more rapidly (Xu et al., 2016;

77　Zimmermann et al., 2012), even though the rate and mechanisms of soil PE are also

78　subject profoundly to the structure and composition of the microbial communities

79　(Blagodatskaya and Kuzyakov, 2008; Pascault et al., 2013).

80　　　In recent decades, the alpine meadows in the Tibet Plateau have been degraded to

81　various levels owing mostly to overgrazing and rodent outbreaks (Wei et al., 2023).

82　Alpine meadows that have been degraded to varying degrees harbor different densities

83　of livestock and plateau pikas (*Ochotona curzoniae*), naturally resulting in widely

84　ranging quantities of excrement. It plays a distinct role in nutrient cycling (Wei et al.,

85　2023). As one of the primary grazing livestock on the Qinghai-Tibet Plateau, Tibetan

86　sheep (*Ovis aries*) and their excrement, if properly decomposed, are significant

87　contributors to the nutrient cycling in the meadows (Liu et al., 2023). Additionally, the

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88 excrement is also a crucial source of organic fertilizer, often utilized for the restoration

89 of degraded grasslands (Liu et al., 2023). The plateau pika is the most common small

90 burrowing mammal in the alpine meadows of the Plateau (Qin et al., 2015; Thomson

91 and Simpson, 2007; Zhang et al., 2014). Despite the wide distribution of pika excrement

92 in some severely degraded alpine meadows, the reconstructed vegetation established

93 via artificial seeding is still highly prone to degradation (Davidson and Lightfoot, 2008;

94 Lai and Smith, 2003; Smith and Foggin, 1999). This suggests that the difficulty in

95 restoring degraded grasslands could not be attributed primarily to low soil fertility (Li

96 et al., 2013; Liu et al., 2018). To some degree, the soil nutrients can be absorbed by

97 plants only after they have been properly decomposed by the activities of microbes that

98 are abundant in the soil. Nevertheless, their impact on soil carbon cycling and their

99 intrinsic links with soil fungus communities in the alpine meadows with varying

100 degrees of degradation remain unknown at present. On the other hand, such knowledge

101 can provide theoretical insights into the mechanisms of soil PE induced by the

102 excrements. The research in this area also can offer data support for the scientific

103 utilization and management of these excrements in meadows degraded to different

104 degrees.

105   This study aims to explore the influence of these two types of excrement

106 supplements on soil fungal communities in the alpine meadows with different degrees

107 of degradation through high-throughput sequencing. The specific objectives are: (1) to

108 explore how the level of soil degradation impacts the PE; (2) to identify major microbial

109 phylum that are critical to enhancing soil PE; and (3) to develop a theory to explain

110 how animal excrement supplements can be best used for the effective restoration of

111 degraded meadows based on the relationship between microbial communities and soil

112 PE.

113 **2. Materials and methods**

114 *2.1 Collection of soil samples and excrements*

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115   The research site is in Menyuan County, Qinghai Province (37°46′ N; 101°21′ E)

116 whose altitude ranges approximately from 3200 to 3700 m a.m.s.l.. Its typical plateau

117 alpine climate enables alpine meadow to thrive year-round, especially in the warm

118 season of June-September. Distributed in this meadow are such common plants as

119 tarragon grasses (*Kobresia myosuroides*), tussock grasses (*Carex* spp.), and lancewood

120 (*Elymus dahuricus*).

121   In July 2022, typical alpine meadows representative of non-degraded (N), and three

122 degrees of degradation (L - lightly degraded, M - moderately degraded, and S - severely

123 degraded) were selected. This classification was based primarily on plant coverage in

124 accordance with the GB19377-2003 standards for grading natural grassland

125 degradation (Su et al., 2003). The vegetation coverage exceeded 80% in non-degraded

126 alpine meadows, ranged between 65% and 80% in lightly degraded areas, between 40%

127 and 65% in moderately degraded areas, and dropped below 40% in severely degraded

128 alpine meadows (Fig. S1). For each level of degradation, three plots of 50 m × 50 m

129 were chosen for analysis. Within each plot, ten 1 m × 1 m subplots spaced at > 1 m

130 apart were randomly established. Five S-shaped cores of 0-20 cm soil samples were

131 randomly collected using a soil auger with a diameter of 4.5 cm. The soil samples from

132 a total of 150 cores in the three plots were thoroughly mixed. In the laboratory, the

133 samples were cleaned by removing plant remnants, stones, and other impurities.

134 Following air-drying, they were sieved through a 20-mesh sieve. The sieved soil was

135 then utilized for assessing physicochemical properties and the PE (Table 1).

136   Tibetan sheep excrement (T) and plateau pika excrement (P) were collected from

137 the alpine meadows in the study area. After natural air-drying, the excrements were

138 crushed and passed through a 20-mesh sieve for physicochemical property

139 determination and soil PE analyses (Table 1).

2.2 Pre-incubation experiments

141   Since the maximum summer temperature in the alpine meadow area is around

142 20 °C, all incubation experiments in this study were conducted at this temperature.

143 Initially, 120 grams (g) of soil samples were placed in 1-liter incubation bottles,

144 adjusted to 50% of their water-holding capacity, and pre-incubated at 20 °C for seven

145 days to stabilize soil microbial activities. Water was replenished using a gravimetric

146 method during this period. Subsequently, a preliminary test was performed by adding 3

147 g, 9 g, and 15 g of Tibetan sheep excrement (T) and plateau pika excrement (P) to

148 separate soil batches. The results of this test showed that the intensity of the PE reached

149 its peak at a dosage of 9 g of Tibetan sheep excrement, while no clear correlation was

150 observed with the dosage of plateau pika excrement. To ensure a consistent supplement

151 dosage and based on the study by Gross and Glaser (2021), the final dosage for the

152 main experiment was determined to be 9 g (Table S1).

153 *2.3 Experimental design*

154 The experimental design consisted of five soil substrates: a control (quartz sand),

155 and four types of soils (N, L, M, S), combined with three excrement supplementation

156 treatments: a control (no excrement added), Plateau pika excrement, and Tibetan sheep

157 excrement. Each of the 5×3 treatment combinations was replicated three times,

158 resulting in a total of 45 incubation bottles. Thus, a total of 45 incubation bottles were

159 set up, with a 50 mL vial suspended at the top of each, containing 20 mL of NaOH to

160 absorb CO_2 released from the soil. The incubation bottles were then placed in a constant

161 temperature incubator at 20°C for further incubation. On days 1, 3, 5, 7, 9, 11, 13, 17,

162 21, 25, 29, 37, and 45 of incubation, phenolphthalein was used as an indicator to

163 precipitate carbonates with 20 mL of 1 M $BaCl_2$. The NaOH solution was titrated using

164 a standard 0.5 M hydrochloric acid solution. The precipitate was obtained by

165 centrifugation at 1800 r·min^{-1} for 15 minutes, dried at 55°C, and analyzed for $\delta^{13}C$

166 values using an isotope mass spectrometer (Qiao et al., 2014). Additionally, another set

167 of soil samples was incubated under the same conditions and protocol. During the peak

168 periods of CO_2 release from the excrements (days 9, 21, and 45), samples were collected

169 to assess soil physicochemical properties, microbial composition, and diversity.

170 *2.4 Measured indicators*

171 *2.4.1 Soil priming effect*

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172   The ratio of CO_2-C (α) from soil respiration was calculated using the two-

173   component mixing model (Pausch et al., 2013):

174                $\alpha = (\delta_t - \delta_s)/(\delta_{ck} - \delta_s)$ (1)

175   In this context, δ_t represents the $\delta^{13}C$ value of the total CO_2-C released from the

176   soil after the addition of excrements. δ_s represents the $\delta^{13}C$ value of the excrement itself,

177   while δ_{ck} represents the $\delta^{13}C$ value of the total CO_2-C released from the control soil.

178   The PE, which stands for the proportion of SOC respiration induced by the added

179   excrements, was calculated using the following formula:

180                $PE = \alpha \times R_t - R_{ck}$ (2)

181   In this context, R_t represents the total CO_2-C released from respiration in the soil

182   after the addition of excrements, while R_{ck} represents the total CO_2-C released from the

183   control treatment (i.e., the same soil substrate with no excrement added). Both R_t and

184   R_{ck} are measured in milligrams of carbon (mg C).

185   *2.4.2 Determination of soil physicochemical properties*

186   Soil organic carbon (SOC) content was measured using the potassium dichromate-

187   sulfuric acid oxidation method. Total nitrogen (TN) was measured through digestion

188   with concentrated sulfuric acid followed by distillation. Total phosphorus (TP) was

189   measured spectrophotometrically after digestion with $HClO_4$-H_2SO_4. Total potassium

190   (TK) was measured spectrophotometrically using the flame photometric method,

191   following digestion with HF-$HClO_4$. Concentrations of NH_4^+-N and NO_3^--N were

192   determined using a continuous flow injection analyzer after extraction with 1 mol·L^{-1}

193   KCl. Available phosphorus (AP) was measured spectrophotometrically following

194   extraction with $NaHCO_3$. Available potassium was measured spectrophotometrically

195   after extraction with NH_4OAc (Sparks and Johnston, 1996). Microbial biomass carbon

196   (MBC) and microbial biomass nitrogen (MBN) were determined by chloroform

197   fumigation at a 4:1 water-soil ratio, potassium sulphate leaching, and the TOC-L

198   analyzer (Jenkinson and Powlson, 1976).

199   *2.4.3 Soil microbial determination*

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200   The diversity and composition of soil bacterial and fungal communities were

201   ascertained   with   the   help   of   Personalbio   Inc.   platform   in   Beijing

202   (https://www.genescloud.cn/). The V3-V4 region of bacterial 16S rDNA and the ITSV1

203   of   fungal   ITS   rDNA   were   amplified   by   specific   primers   for   F:

204   ACTCCTACGGGGAGGCAGCA   R:GGACTACHVGGGTWTCTAAT   and

205   F:GGAAGTAAAAGTCGTAACAAGG   R:GCTGCGTTCTTCATCGATGC   region

206   (Claesson et al., 2009). Microbiome biological information was analyzed using

207   QIIME2 version 2019.4. Soil bacteria and fungi were analyzed through the Silva

208   database (Release 132, http://www.arb-silva.de) and the UNITE database (Release 8.0,

209   https://unite.ut.ee/), respectively, by comparing the ASV characteristic sequences to the

210   reference   sequences   in   the   databases   to   obtain   the   corresponding   taxonomic

211   information corresponding to each ASV.

212   *2.5 Data analysis*

213   Analysis of variance (ANOVA) was utilized to detect significant differences

214   between various treatments. IBM SPSS Statistics 26.0 (IBM SPSS Inc.) was employed

215   for statistical analyses, with significance set at $P < 0.05$. Graphical representations were

216   crafted using Graphpad Prism 7.0. Chao1 and Shannon indices were analyzed through

217   ANOVA, whereas principal coordinates analysis (PCoA) was conducted using

218   PERMANOVA. Soil microbial community composition at the amplicon sequence

219   variant (ASV) level was explored through PCoA (employing Bray-Curtis distance) and

220   PERMANOVA tests. Pearson correlation analysis was conducted to assess the

221   relationships between soil physicochemical factors and the relative abundances of

222   bacterial and fungal phyla. SPSS 26.0 was used for data analysis, and Origin 2019b,

223   Graphpad Prism 7.0, and the Personalbio company's cloud platform were utilized for

224   graphical visualizations. Mean values were compared using the Tukey test ($P < 0.05$).

225   A correlation network was built using Spearman analysis and analyzed using Gephi.

226   Significance P-values and correlation coefficients (R-values) were computed using the

227   RMT package in R. Based on these coefficients and significance, a modular network

228   and a ZiPi plot were developed. Structural equation modeling (SEM) was applied to

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229 identify the impacts among multiple variables. Prior to modeling, all parameters were

230 logarithmically transformed to ensure normality and enhance linearity. Binary

231 relationships between variables were evaluated to incorporate linear relationships into

232 the model and avoid strong multicollinearity ($r < 0.8$). Gradually, insignificant

233 pathways were eliminated, retaining only those that significantly contributed to the final

234 model. Since some model input variables were not normally distributed, Bootstrap

235 resampling was performed to determine the probability that a path coefficient differed

236 from zero. Model fit was assessed using the comparative fit index (CFI), goodness of

237 fit index (GFI), standardized fit index (NFI), and approximate root-mean-square error

238 (RMSEA). The final model was deemed good based on the following criteria: (i) CFI

239 between 0.97 and 1.00; (ii) GFI between 0.95 and 1.00; (iii) NFI between 0.95 and 1.00;

240 (iv) CMIN/df between 1 and 3, with P-value between 0.05 and 1.00; (v) RMSEA $\leq$

241 0.05, with *P*-value between 0.10 and 1.00. All SEM models were created using IBM

242 SPSS Amos Version 24.

3. Results

3.1 Soil priming effects and physicochemical properties

245   After introducing the two types of excrement, a generally positive PE was

246 observed over the 45-day incubation period, with only occasional negative PE at

247 specific times (Fig. 1a). The addition of plateau pika excrement resulted in a

248 significantly higher cumulative PE in soils at various levels of degradation than that

249 Tibetan sheep excrement (Fig. 1b). Initially, following the application of Tibetan sheep

250 excrement, a negative PE was noted. Furthermore, non-degraded alpine meadow soil

251 exhibited a significantly higher cumulative PE than severely degraded soil ($P < 0.05$).

252 Conversely, upon adding plateau pika excrement, a predominantly positive PE was

253 observed throughout the incubation period. Notably, severely degraded alpine meadow

254 soil showed a significantly higher cumulative PE than non-degraded soil ($P < 0.05$).

255   During the early incubation period (9 d), there were significant differences in C:N

256 ratio, MBC:MBN ratio, and NO_3^--N:NH_4^+-N ratio between non-degraded and severely

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257 degraded soils (Fig. 2a-c). As the incubation period lengthened, all these ratios

258 significantly decreased. The addition of excrement significantly increased the soil C:N

259 ratio, but significantly reduced the NO_3^--N:NH_4^+-N ratio in both non-degraded and

260 severely degraded soils on 9 d and 45 d ($P < 0.05$).

261 *3.2 Changes in microbial community composition*

262 On 9 d, 21 d, and 45 d of incubation, compared to the control soils, non-degraded,

263 mildly degraded, and moderately degraded alpine meadow soils were not significantly

264 impacted by the addition of the two types of excrement in their Chao1 and Shannon

265 indiced (Fig. 3a and 3b). In severely degraded alpine meadow soil, the addition of

266 Tibetan sheep excrement significantly reduced the Chao1 and Shannon indices of

267 bacteria ($P < 0.05$). On the 9 d, 21 d, and 45 d of incubation, the added Tibetan sheep

268 excrement significantly increased the Chao1 and Shannon indices of fungi in non-

269 degraded, mildly degraded, and moderately degraded alpine meadow soils ($P < 0.05$)

270 in comparison with the control soil, but not the severely degraded alpine meadow soil

271 (Fig. 3c and 3d).

272 After the addition of the two types of excrement, differences showed up in the

273 composition of soil bacterial and fungal communities, with PCo1 and PCo2 explaining

274 28.4% and 36.0% of the total variance in bacterial and fungal communities, respectively

275 (Fig. 4a and 4b). PERMANOVA analysis revealed significant differences in bacterial

276 communities between soils with and without the amendment of Tibetan sheep

277 excrement in non-degraded meadow soil ($P < 0.05$). In moderately degraded alpine

278 meadows, significant differences were observed in bacterial communities between soils

279 with and without the addition of plateau pika excrement ($P < 0.05$). In severely

280 degraded alpine meadows, the β diversity of bacterial communities showed significant

281 differences after the addition of both types of excrement compared to the control soils

282 ($P < 0.05$). Across all four types of alpine meadow soils, the β diversity of the fungal

283 communities significantly differed between excrement-amended soils and unamended

284 soils ($P < 0.05$).

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285   The addition of the two types of excrement increased the relative abundance of

286   Proteobacteria and Actinobacteria, while decreasing the relative abundance of

287   Acidobacteria and Gemmatimonadetes ($P < 0.05$) (Fig. 5a). Following the addition of

288   excrement, the relative abundance of Ascomycota increased to over 90% to become the

289   dominant fungal phylum in all treatments. The relative abundance of

290   Mortierellomycota decreased to below 5%, but still remained as the second most

291   abundant fungal phylum. The relative abundance of Basidiomycota dropped to below

292   2% to rank the third in terms of fungal phylum abundance in all treatments (Fig. 5b).

293   *3.3 Soil microbial co-occurrence networks and key phylum*

294   Separate networks were constructed for soil bacteria and fungi, a control network

295   without excrement (CK), a network with Tibetan sheep excrement (T), and a network

296   with plateau pika excrement (P) (Fig. 6). The addition of excrement increased total

297   nodes, and the proportion of positive edges (Table 2). The ZiPi plot indicated that the

298   key bacterial phylum without the excrement supplement and with the Tibetan sheep

299   excrement supplement were Proteobacteria, Acidobacteriota, Actinobacteriota,

300   Gemmatimonadota, and Chloroflexi. The key bacterial phylum with the addition of

301   plateau pika excrement were Proteobacteria, Acidobacteriota, Actinobacteriota,

302   Gemmatimonadota, and Bacteroidota. The key fungal phylum of all treatments (without

303   excrement, with Tibetan sheep excrement, or plateau pika excrement) were identified

304   as Ascomycota, Mortierellomycota, and Basidiomycota.

305   We also constructed soil bacterial and fungal networks for alpine meadows in four

306   states: non-degraded, lightly degraded, moderately degraded, and severely degraded

307   (Fig. 7), and analyzed these networks. Compared with severely degraded soil, non-

308   degraded soil had an increased number of network edges and average degree (Table 2).

309   The ZiPi plot revealed that the dominant bacterial phyla in non-degraded, lightly

310   degraded, and moderately degraded soils were Proteobacteria, Acidobacteriota,

311   Actinobacteriota, Gemmatimonadota, and Chloroflexi, respectively. In contrast, the

312   dominant bacterial phyla in severely degraded soil were Actinobacteriota,

313   Proteobacteria, Acidobacteriota, Gemmatimonadota, and Chloroflexi. The main fungal

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314 phyla in non-degraded, lightly degraded, and moderately degraded soils were

315 Ascomycota, Basidiomycota, and Mortierellomycota. However, in severely degraded

316 soil, the dominant fungal phyla were Ascomycota and Mortierellomycota.

317 *3.4 Effects of stoichiometric ratios and soil microbial composition*

318 The addition of the two types of excrement had a direct negative impact on PEs (r

319 = -0.94). Key microbial communities in the soil, namely Actinobacteriota,

320 Proteobacteria, and Chloroflexi, showed a direct positive influence on soil PEs (r =

321 0.21, r = 0.29, r = 0.22). The soil C:N ratio positively affected the key microbial

322 community Proteobacteria (r = 0.39), thereby influencing PEs, while the soil NO_3^-

323 N:NH_4^+-N ratio negatively impacted the key microbial community Actinobacteriota (r

324 = -0.18), subsequently affecting PEs. The degree of meadow degradation influenced the

325 key microbial communities, Actinobacteriota and Proteobacteria, as well as the soil C:N

326 ratio, indirectly affecting the soil PEs (r = 0.70, r = -0.52, r = -0.26) (Fig. 8).

4. Discussion

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328 *4.1 Impact of degradation degree*

329 In alpine meadow soils with varying degrees of degradation, severely degraded

330 soils were more susceptible to soil PEs than non-degraded soils (Fig. 1a and 1b). When

331 the soil exhibited a positive PE, it was minimal in non-degraded soil and maximal in

332 severely degraded soil. However, when the soil exhibited a negative PE, it was maximal

333 in non-degraded soil and minimal in severely degraded soil, an outcome that was

334 aligned with previous findings (Bernal et al., 2016; Chen et al., 2019; Chen et al., 2022;

335 Zhang et al., 2017). That is, PE is related to plant coverage (degree of degradation),

336 with PE in bare soil being typically stronger than that in vegetated soil. Additionally, it

337 may also be associated with the NO_3^--N:NH_4^+-N ratio. After the addition of excrement,

338 compared to non-degraded soil, severely degraded soil had a higher NO_3^--N:NH_4^+-N

339 ratio. Previous studies have shown that soil C and N availability markedly influences

340 soil microbial community composition and microbial functions related to soil C and N

341 cycling (Bowles et al., 2014; Koranda et al., 2011; Zhou et al., 2017).

342   Compared to non-degraded soil, severely degraded meadow soil had a higher

343 MBC:MBN ratio (Fig. 2), reduced soil α-diversity (Fig. 3), no significant change in

344 microbial community structure (Fig. 3), but a significant change in soil bacterial

345 community composition (Fig. 4). This aligns with the findings of others in the literature

346 (Sullivan and Hart, 2013), and indicates that the addition of exogenous carbon alters

347 the size and composition of microbial communities in different soils. After excrement

348 supplementation, the relative abundance of Proteobacteria and Actinobacteria

349 significantly increased. They are typically considered r-strategists (Fierer et al., 2007;

350 Peiffer et al., 2013). Compared to severely degraded soil, non-degraded soil had a

351 higher relative abundance of Proteobacteria. As a key bacterial community in soil

352 ecosystems, Proteobacteria promotes the dissolution of organophosphates and soil

353 nitrogen fixation (Pascault et al., 2013). Both Proteobacteria and Actinobacteria prefer

354 nutrients-rich environments and play crucial roles in cycling soil nutrients (Ren et al.,

355 2018), particularly in decomposing excrements in which they are responsible for

356 degrading organic matter (Pascault et al., 2013). The excrement supplements used in

357 this study provided a rich nutrient source for Proteobacteria, potentially leading to an

358 increase in its relative abundance. Actinobacteria have strong decomposition abilities

359 for recalcitrant organic components in soil, such as chitin and cellulose, facilitating soil

360 carbon cycling (Eilers et al., 2010). Additionally, their spore production endows them

361 with robust metabolic capabilities in low-temperature environments (Yergeau et al.,

362 2010), making Actinobacteria a dominant microbial community in the alpine meadow

363 soil. After excrement supplementation, the relative abundance of K-strategists such as

364 Acidobacteria and Gemmatimonadota decreased. Therefore, the increase in easily

365 utilizable carbon sources led to an increase in r-strategist abundance and a decrease in

366 K-strategist abundance. In degraded grasslands, however, nutrient depletion in plant

367 roots reduced the availability of easily utilizable carbon sources, resulting in a decrease

368 in r-strategist abundance and an increase in K-strategist abundance.

369   In this study, after excrement supplementation, the relative abundance of

370 Ascomycota increased, while the relative abundance of Basidiomycota decreased in the

371 alpine meadow soil suffering varying degrees of degradation. As the degree of

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372 degradation intensified, the relative abundance of Ascomycota in the soil also

373 increased, whereas the relative abundance of Basidiomycota decreased (Fig. 4). In the

374 literature (Kabuyah et al., 2012), both Ascomycota and Basidiomycota typically

375 dominate soils rich in lignin and cellulose due to their effective decomposition of these

376 recalcitrant components in vegetation residues. In particular, Ascomycota is able to

377 grow in arid and nutrient-poor environments, and is often a key fungal decomposer in

378 livestock excrement and agricultural waste compost (Chen et al., 2017; Duan et al.,

379 2019; Yu et al., 2015), which may explain its increased abundance after excrement

380 addition. Basidiomycota is considered a K-strategist (Bastian et al., 2009; Yelle et al.,

381 2008), and often constitutes a major component of the fungal community in alpine

382 meadow soil due to its high lignin content (van der Wal et al., 2006). Basidiomycota

383 tends to dominate nutrient-rich ecosystems (Sterkenburg et al., 2015). Therefore, as the

384 meadow degrades and soil and plant organic carbon dwindle, the abundance of

385 Basidiomycota declines, in contrast to the more resilient Ascomycota that rises.

386 *4.2 Mechanisms of microbial communities influencing soil priming effects*

387   The excrement supplement increased the complexity and stability of the microbial

388 symbiotic network in the degraded alpine meadow soil. Non-degraded alpine meadow

389 soil exhibited a more complex and stable microbial symbiotic network than severely

390 degraded alpine meadow soil (Figs. 5 and 6), characterized by an increase in the number

391 of edges and average degree (Banerjee et al., 2019). They represent microbial

392 interactions, which are crucial for the stability and resilience of microbial communities

393 (Olesen et al., 2007; Shi et al., 2016). This may be attributed to the increased organic

394 matter content in the soil following excrement addition, which promotes microbial

395 metabolism (Hu et al., 2017). However, in severely degraded soil, the soil microbial

396 symbiotic network was very simple, which may explain why vegetation restoration is

397 difficult to suceed in severely degraded "Black Soil Beach" despite the presence of large

398 amounts of pika excrement.

399   SEM revealed complex interactions among stoichiometric ratios, key microbial

400 phylum, and SOC PEs (Fig. 7). Previous studies have shown that the microbial

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mechanisms of PEs include "cometabolism," "N-mining," and "stoichiometric decomposition" (Blagodatskaya and Kuzyakov, 2008; Chen et al., 2014; Craine et al., 2007; Fang et al., 2018; Hessen et al., 2004). In this study, the excrement supplements were the main influencer of soil PEs in the alpine meadows degraded to various degrees (r = -0.94) (Fig. 7). The microbial community activities associated with different stoichiometries in the excrements were consistent across the alpine meadows regardless of their degree of degradation. The degree of degradation affected only key microbial phylum such as Actinobacteriota (r = 0.70), Proteobacteria (r = -0.52), and C:N ratio(r = -0.26), indirectly influencing PEs.

Based on the analysis of soil symbiotic networks, the key phylum were identified at different phylum levels. These key microorganisms, including Actinobacteriota (r = 0.21), Proteobacteria (r = 0.29), and Chloroflexi (r = 0.22), directly and positively influenced PEs. Actinobacteria participate in nitrogen and organic matter decomposition and cycling processes (Sunagawa et al., 2015), which aligns with our findings. The soil $NO_3^--N:NH_4^+-N$ ratio negatively affected the key microbial phylum Actinobacteriota (r = -0.18), thereby influencing soil PE. Proteobacteria promote the dissolution of organophosphates and soil nitrogen fixation (Pascault et al., 2013), which is consistent with our results. Soil C:N ratio positively influenced the key microbial phylum Proteobacteria (r = 0.39), thereby affecting PEs. Chloroflexi can survive in extreme environments, performs anaerobic photosynthesis (Zeng et al., 2021; Zeng et al., 2015), possesses carbon fixation capabilities (Vavourakis et al., 2019; Zorz et al., 2019), and can reduce N_2O in the nitrogen cycle (Park et al., 2017).

Previously, this paper has demonstrated that the supplementation of both Tibetan sheep excrement and plateau pika excrement elevated the relative abundance of crucial soil microorganisms and bolstered microbial community stability. Consequently, the added excrements modulated PEs through alterations in the composition of key microbial communities. This research further reveals that, in contrast to plateau pika excrement, Tibetan sheep excrement boasted carbon stability more and a lower carbon-to-nitrogen ratio (C:N ratio). Based on the N mining hypothesis, the lower C:N ratio of Tibetan sheep excrement may induce a comparatively lower positive PE for soil organic

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431 carbon accumulation (Jones et al., 2011; Kuzyakov and Bol, 2006). This attribute favors

432 soil carbon sequestration, implying that Tibetan sheep excrement addition may offer

433 superior benefits than plateau pika excrement in restoring degraded meadows in the

434 context of soil remediation and enhancement.

5. Conclusions

436 Compared to non-degraded alpine meadow soils, the MBC:MBN ratio in severely

437 degraded meadow soils exhibited a significant increase, accompanied by a notable

438 decrease in bacterial α-diversity and pronounced changes in bacterial composition and

439 β-diversity, the microbial symbiotic network in these degraded soils was simpler and

440 less stable. Although the addition of excrements enhanced the soil organic carbon

441 accumulation and improved the complexity and stability of the soil microbial symbiotic

442 network, the microbial community in severely degraded soils remained unstable.

443 Simultaneously, degradation of alpine meadows impacts key microbial phylum such as

444 Actinobacteriota and Proteobacteria in the soil, which directly affects soil PEs. As a

445 result, severely degraded soils were more susceptible to PEs than non-degraded soils.

446 Of all the factors influencing soil PEs, key microbial phylum like Actinobacteriota,

447 Proteobacteria, and Chloroflexi have a direct positive impact. Soil stoichiometric ratios

448 (C:N ratio, NO_3^--N:NH_4^+-N ratio) indirectly influence soil PEs by affecting these key

449 microbial phylum. Therefore, in lightly and moderately degraded alpine meadow soils,

450 the addition of both types of excrement is beneficial for soil restoration, with Tibetan

451 sheep excrement showing superior effects to plateau pika excrement. These findings

452 inform targeted grassland management strategies for the Qinghai-Tibetan Plateau:

453 limiting livestock grazing and plateau pika activity in severely degraded zones reduces

454 carbon loss by minimizing priming effects; optimizing soil C:N ratios through organic

455 amendments suppresses PE-enhancing microbes like Actinobacteriota; and prioritizing

456 conservation of intact meadows enhances regional carbon sequestration potential.

457 Collectively, these practices support sustainable alpine ecosystem restoration and

458 carbon stewardship.

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Data availability

The data will be made available upon reasonable request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Qinyao Li: Data curation, Investigation, Software, Writing—original draft; Wenquan Yang: Conceptualization, Supervision, Writing—review & editing; Jiancun Kou: Conceptualization, Project administration, Funding acquisition, Supervision, Writing—review & editing; Qian Liu: Investigation; Yangcan Zhang: Investigation; Weiliang Kou: Investigation; Jiaqing Liu: Investigation; Xilai Li: Conceptualization, Formal analysis, Methodology; Jing Zhang: Conceptualization, Formal analysis, Methodology.

Acknowledgments

References

Ai, C., Zhang, S., Zhang, X., Guo, D., Zhou, W., Huang, S., 2018. Distinct responses of soil bacterial and fungal communities to changes in fertilization regime and crop rotation. Geoderma 319:156-66. http://doi.org/10.1016/j.geoderma.2018.01.010.

Banerjee, S., Walder, F., Buchi, L., Meyer, M., Held, A.Y., Gattinger, A., Keller, T., Charles, R., van der Heijden, M.G.A., 2019. Agricultural intensification reduces microbial network complexity and the abundance of keystone taxa in roots. Isme J. 13:1722-36. http://doi.org/10.1038/s41396-019-0383-2.

Bastian, F., Bouziri, L., Nicolardot, B., Ranjard, L., 2009. Impact of wheat straw decomposition on successional patterns of soil microbial community structure. Soil Biol. Biochem. 41:262-75. http://doi.org/10.1016/j.soilbio.2008.10.024.

18

489 Bernal, B., McKinley, D.C., Hungate, B.A., White, P.M., Mozdzer, T.J., Megonigal, J.P., 2016. Limits
490   to soil carbon stability; Deep, ancient soil carbon decomposition stimulated by new labile organic
491   inputs. Soil Biol. Biochem. 98:85-94. http://doi.org/10.1016/j.soilbio.2016.04.007.
492 Blagodatskaya, E., Kuzyakov, Y., 2008. Mechanisms of real and apparent priming effects and their
493   dependence on soil microbial biomass and community structure: critical review. Biol. Fertil. Soils
494   45:115-31. http://doi.org/10.1007/s00374-008-0334-y.
495 Bowles, T.M., Acosta-Martinez, V., Calderon, F., Jackson, L.E., 2014. Soil enzyme activities, microbial
496   communities, and carbon and nitrogen availability in organic agroecosystems across an
497   intensively-managed agricultural landscape. Soil Biol. Biochem. 68:252-62.
498   http://doi.org/10.1016/j.soilbio.2013.10.004.
499 Chen, B.B., Jiao, S., Luo, S.W., Ma, B.B., Qi, W., Cao, C.D., Zhao, Z.G., Du, G.Z., Ma, X.J., 2021.
500   High soil pH enhances the network interactions among bacterial and archaeal microbiota in alpine
501   grasslands of the Tibetan Plateau. Environ. Microbiol. 23:14. http://doi.org/10.1111/1462-
502   2920.15333.
503 Chen, L., Liu, L., Qin, S., Yang, G., Fang, K., Zhu, B., Kuzyakov, Y., Chen, P., Xu, Y., et al., 2019.
504   Regulation of priming effect by soil organic matter stability over a broad geographic scale. Nat.
505   Commun. 10:5112. http://doi.org/10.1038/s41467-019-13119-z.
506 Chen, L.Y., Liu, L., Mao, C., Qin, S.Q., Wang, J., Liu, F.T., Blagodatsky, S., Yang, G.B., Zhang, Q.W.,
507   et al., 2018. Nitrogen availability regulates topsoil carbon dynamics after permafrost thaw by
508   altering microbial metabolic efficiency. Nat. Commun. 9:3951. http://doi.org/10.1038/s41467-
509   018-06232-y.
510 Chen, R., Senbayram, M., Blagodatsky, S., Myachina, O., Dittert, K., Lin, X., Blagodatskaya, E.,
511   Kuzyakov, Y., 2014. Soil C and N availability determine the priming effect: microbial N mining
512   and stoichiometric decomposition theories. Global Change Biol. 20:2356-67.
513   http://doi.org/10.1111/gcb.12475.
514 Chen, X., Lin, J.J., Wang, P., Zhang, S., Liu, D., Zhu, B., 2022. Resistant soil carbon is more vulnerable
515   to priming effect than active soil carbon. Soil Biol. Biochem. 168:108619.
516   http://doi.org/10.1016/j.soilbio.2022.108619.
517 Chen, Y.L., Xu, T.L., Veresoglou, S.D., Hu, H.W., Hao, Z.P., Hu, Y.J., Liu, L., Deng, Y., Rillig, M.C., et
518   al., 2017. Plant diversity represents the prevalent determinant of soil fungal community structure
519   across temperate grasslands in northern China. Soil Biol. Biochem. 110:12-21.
520   http://doi.org/10.1016/j.soilbio.2017.02.015.
521 Claesson, M.J., O'Sullivan, O., Wang, Q., Nikkilae, J., Marchesi, J.R., Smidt, H., de Vos, W.M., Ross,
522   R.P., O'Toole, P.W., 2009. Comparative analysis of pyrosequencing and a phylogenetic microarray
523   for exploring microbial community structures in the human distal intestine. PLoS One 4:e6669.
524   http://doi.org/10.1371/journal.pone.0006669.
525 Craine, J.M., Morrow, C., Fierer, N., 2007. Microbial nitrogen limitation increases decomposition.
526   Ecology 88:2105-13. http://doi.org/10.1890/06-1847.1.
527 Davidson, A.D., Lightfoot, D.C., 2008. Burrowing rodents increase landscape heterogeneity in a desert
528   grassland. J. Arid. Environ. 72:1133-45. http://doi.org/10.1016/j.jaridenv.2007.12.015.
529 Duan, Y.M., Awasthi, S.K., Liu, T., Chen, H.Y., Zhang, Z.Q., Wang, Q., Ren, X., Tu, Z., Awasthi, M.K.,
530   et al., 2019. Dynamics of fungal diversity and interactions with environmental elements in
531   response to wheat straw biochar amended poultry manure composting. Bioresour. Technol.
532   274:410-17. http://doi.org/10.1016/j.biortech.2018.12.020.
533 Eilers, K.G., Lauber, C.L., Knight, R., Fierer, N., 2010. Shifts in bacterial community structure
534   associated with inputs of low molecular weight carbon compounds to soil. Soil Biol. Biochem.
535   42:896-903. http://doi.org/10.1016/j.soilbio.2010.02.003.
536 Fang, Y., Nazaries, L., Singh, B.K., Singh, B.P., 2018. Microbial mechanisms of carbon priming effects
537   revealed during the interaction of crop residue and nutrient inputs in contrasting soils. Global
538   Change Biol. 24:2775-90. http://doi.org/10.1111/gcb.14154.
539 Fierer, N., Bradford, M.A., Jackson, R.B., 2007. Toward an ecological classification of soil bacteria.
540   Ecology 88:1354-64. http://doi.org/10.1890/05-1839.
541 Fontaine, S., Bardoux, G., Abbadie, L., Mariotti, A., 2004. Carbon input to soil may decrease soil
542   carbon content. Ecol. Lett. 7:314-20. http://doi.org/10.1111/j.1461-0248.2004.00579.x.
543 Fontaine, S., Mariotti, A., Abbadie, L., 2003. The priming effect of organic matter: a question of
544   microbial competition? Soil Biol. Biochem. 35:837-43. http://doi.org/10.1016/s0038-
545   0717(03)00123-8.

19

546 Gross, A., Glaser, B., 2021. Meta-analysis on how manure application changes soil organic carbon
547   storage. Sci. Rep. 11. http://doi.org/10.1038/s41598-021-82739-7.
548 Heitkötter, J., Heinze, S., Marschner, B., 2017. Relevance of substrate quality and nutrients for
549   microbial C-turnover in top-and subsoil of a Dystric Cambisol. Geoderma 302:89-99.
550   http://doi.org/10.1016/j.geoderma.2017.04.029.
551 Hessen, D.O., Agren, G.I., Anderson, T.R., Elser, J.J., De Ruiter, P.C., 2004. Carbon, sequestration in
552   ecosystems: The role of stoichiometry. Ecology 85:1179-92. http://doi.org/10.1890/02-0251.
553 Hicks, L.C., Meir, P., Nottingham, A.T., Reay, D.S., Stott, A.W., Salinas, N., Whitaker, J., 2019. Carbon
554   and nitrogen inputs differentially affect priming of soil organic matter in tropical lowland and
555   montane soils. Soil Biol. Biochem. 129:212-22. http://doi.org/10.1016/j.soilbio.2018.10.015.
556 Hu, X.J., Liu, J.J., Wei, D., Zhu, P., Cui, X., Zhou, B.K., Chen, X.L., Jin, J., Liu, X.B., et al., 2017.
557   Effects of over 30-year of different fertilization regimes on fungal community compositions in the
558   black soils of northeast China. Agric. Ecosyst. Environ. 248:113-22.
559   http://doi.org/10.1016/j.agee.2017.07.031.
560 Jenkinson, D.S., Powlson, D.S., 1976. The effects of biocidal treatments on metabolism in soil—V: A
561   method for measuring soil biomass,. Soil Biol. Biochem. 8:209-13.
562   http://doi.org/https://doi.org/10.1016/0038-0717(76)90005-5.
563 Jones, D.L., Murphy, D.V., Khalid, M., Ahmad, W., Edwards-Jones, G., DeLuca, T.H., 2011. Short-
564   term biochar-induced increase in soil CO_2 release is both biotically and abiotically mediated. Soil
565   Biol. Biochem. 43:1723-31. http://doi.org/10.1016/j.soilbio.2011.04.018.
566 Kabuyah, R.N.T.M., van Dongen, B.E., Bewsher, A.D., Robinson, C.H., 2012. Decomposition of lignin
567   in wheat straw in a sand-dune grassland. Soil Biol. Biochem. 45:128-31.
568   http://doi.org/10.1016/j.soilbio.2011.10.014.
569 Koranda, M., Schnecker, J., Kaiser, C., Fuchslueger, L., Kitzler, B., Stange, C.F., Sessitsch, A.,
570   Zechmeister-Boltenstern, S., Richter, A., 2011. Microbial processes and community composition
571   in the rhizosphere of European beech - The influence of plant C exudates. Soil Biol. Biochem.
572   43:551-58. http://doi.org/10.1016/j.soilbio.2010.11.022.
573 Korkanc, S.Y., Korkanc, M., 2016. Physical and chemical degradation of grassland soils in semi-arid
574   regions: A case from Central Anatolia, Turkey. J. Afr. Earth. Sci. 124:1-11.
575   http://doi.org/10.1016/j.jafrearsci.2016.08.021.
576 Kou, D., Ding, J.Z., Li, F., Wei, N., Fang, K., Yang, G.B., Zhang, B.B., Liu, L., Qin, S.Q., et al., 2019.
577   Spatially-explicit estimate of soil nitrogen stock and its implication for land model across Tibetan
578   alpine permafrost region. Sci. Total Environ. 650:1795-804.
579   http://doi.org/10.1016/j.scitotenv.2018.09.252.
580 Kuzyakov, Y., Bol, R., 2006. Sources and mechanisms of priming effect induced in two grassland soils
581   amended with slurry and sugar. Soil Biol. Biochem. 38:747-58.
582   http://doi.org/10.1016/j.soilbio.2005.06.025.
583 Kuzyakov, Y., Friedel, J.K., Stahr, K., 2000. Review of mechanisms and quantification of priming
584   effects. Soil Biol. Biochem. 32:1485-98. http://doi.org/10.1016/s0038-0717(00)00084-5.
585 Lai, C.H., Smith, A.T., 2003. Keystone status of plateau pikas (*Ochotona curzoniae*): effect of control
586   on biodiversity of native birds. Biodivers. Conserv. 12:1901-12.
587   http://doi.org/10.1023/a:1024161409110.
588 Li, H.Y., Qiu, Y.Z., Yao, T., Han, D.R., Gao, Y.M., Zhang, J.G., Ma, Y.C., Zhang, H.R., Yang, X.L.,
589   2021. Nutrients available in the soil regulate the changes of soil microbial community alongside
590   degradation of alpine meadows in the northeast of the Qinghai-Tibet Plateau. Sci. Total Environ.
591   792:148363. http://doi.org/10.1016/j.scitotenv.2021.148363.
592 Li, X.L., Gao, J., Brierley, G., Qiao, Y.M., Zhang, J., Yang, Y.W., 2013. Rangeland degradation on the
593   Qinghai-Tibet Plateau: implications for rehabilitation. Land Degrad. Dev. 24:72-80.
594   http://doi.org/10.1002/ldr.1108.
595 Liu, J., Schneider, R.L., Morreale, S.J., Wang, H.M., Wang, R.X., Wang, F., Li, Z.G., 2023. The roles
596   for branch shelters and sheep manure to accelerate the restoration of degraded grasslands in
597   northern China. Front. Environ. Sci. 10:1089645. http://doi.org/10.3389/fenvs.2022.1089645.
598 Liu, S.B., Zamanian, K., Schleuss, P.M., Zarebanadkouki, M., Kuzyakov, Y., 2018. Degradation of
599   Tibetan grasslands: Consequences for carbon and nutrient cycles. Agric. Ecosyst. Environ.
600   252:93-104. http://doi.org/10.1016/j.agee.2017.10.011.
601 Nunes, J.S., Araujo, A.S.F., Nunes, L., Lima, L.M., Carneiro, R.F.V., Salviano, A.A.C., Tsai, S.M.,
602   2012. Impact of land degradation on soil microbial biomass and activity in northeast brazil.
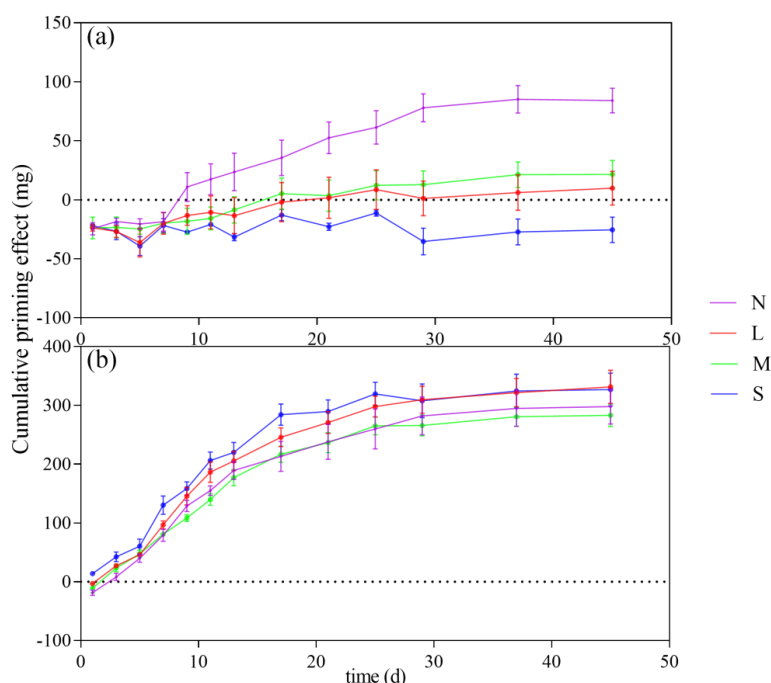603   Pedosphere 22:88-95. http://doi.org/10.1016/s1002-0160(11)60194-x.

20

604 Olesen, J.M., Bascompte, J., Dupont, Y.L., Jordano, P., 2007. The modularity of pollination networks.
605   Proceedings of the National Academy of Sciences of the United States of America 104:19891-96.
606   http://doi.org/10.1073/pnas.0706375104.
607 Park, D., Kim, H., Yoon, S., 2017. Nitrous oxide reduction by an obligate aerobic bacterium,
608   *Gemmatimonas aurantiaca* Strain T-27. Appl. Environ. Microbiol. 83:e00502-17.
609   http://doi.org/10.1128/aem.00502-17.
610 Pascault, N., Ranjard, L., Kaisermann, A., Bachar, D., Christen, R., Terrat, S., Mathieu, O., Leveque, J.,
611   Mougel, C., et al., 2013. Stimulation of different functional groups of bacteria by various plant
612   residues as a driver of soil priming effect. Ecosystems 16:810-22. http://doi.org/10.1007/s10021-
613   013-9650-7.
614 Pausch, J., Zhu, B., Kuzyakov, Y., Cheng, W., 2013. Plant inter-species effects on rhizosphere priming
615   of soil organic matter decomposition. Soil Biol. Biochem. 57:91-99.
616   http://doi.org/10.1016/j.soilbio.2012.08.029.
617 Peiffer, J.A., Spor, A., Koren, O., Jin, Z., Tringe, S.G., Dangl, J.L., Buckler, E.S., Ley, R.E., 2013.
618   Diversity and heritability of the maize rhizosphere microbiome under field conditions.
619   Proceedings of the National Academy of Sciences of the United States of America 110:6548-53.
620   http://doi.org/10.1073/pnas.1302837110.
621 Qiao, N., Schaefer, D., Blagodatskaya, E., Zou, X.M., Xu, X.L., Kuzyakov, Y., 2014. Labile carbon
622   retention compensates for CO_2 released by priming in forest soils. Global Change Biol. 20:1943-
623   54. http://doi.org/10.1111/gcb.12458.
624 Qin, Y., Chen, J.J., Yi, S.H., 2015. Plateau pikas burrowing activity accelerates ecosystem carbon
625   emission from alpine grassland on the Qinghai-Tibetan Plateau. Ecol. Eng. 84:287-91.
626   http://doi.org/10.1016/j.ecoleng.2015.09.012.
627 Qiu, J., 2008. The third pole. Nature 454:393-96. http://doi.org/10.1038/454393a.
628 Ramirez, K.S., Craine, J.M., Fierer, N., 2012. Consistent effects of nitrogen amendments on soil
629   microbial communities and processes across biomes. Global Change Biol. 18:1918-27.
630   http://doi.org/10.1111/j.1365-2486.2012.02639.x.
631 Razanamalala, K., Razafimbelo, T., Maron, P.-A., Ranjard, L., Chemidlin, N., Lelievre, M., Dequiedt,
632   S., Ramaroson, V.H., Marsden, C., et al., 2018. Soil microbial diversity drives the priming effect
633   along climate gradients: a case study in Madagascar. ISME J. 12:451-62.
634   http://doi.org/10.1038/ismej.2017.178.
635 Ren, C.J., Wang, T., Xu, Y.D., Deng, J., Zhao, F.Z., Yang, G.H., Han, X.H., Feng, Y.Z., Ren, G.X.,
636   2018. Differential soil microbial community responses to the linkage of soil organic carbon
637   fractions with respiration across land-use changes. For. Ecol. Manage. 409:170-78.
638   http://doi.org/10.1016/j.foreco.2017.11.011.
639 Shang, Z.H., Yang, S.H., Wang, Y.L., Shi, J.J., Ding, L.M., Long, R.J., 2016. Soil seed bank and its
640   relation with above-ground vegetation along the degraded gradients of alpine meadow. Ecol. Eng.
641   90:268-77. http://doi.org/10.1016/j.ecoleng.2016.01.067.
642 Shi, S.J., Nuccio, E.E., Shi, Z.J., He, Z.L., Zhou, J.Z., Firestone, M.K., 2016. The interconnected
643   rhizosphere: High network complexity dominates rhizosphere assemblages. Ecol. Lett. 19:926-36.
644   http://doi.org/10.1111/ele.12630.
645 Smith, A.P., Marin-Spiotta, E., de Graaff, M.A., Balser, T.C., 2014. Microbial community structure
646   varies across soil organic matter aggregate pools during tropical land cover change. Soil Biol.
647   Biochem. 77:292-303. http://doi.org/10.1016/j.soilbio.2014.05.030.
648 Smith, A.T., Foggin, J.M., 1999. The plateau pika (*Ochotona curzoniae*) is a keystone species for
649   biodiversity on the Tibetan plateau. Anim. Conserv. 2:235-40. http://doi.org/10.1111/j.1469-
650   1795.1999.tb00069.x.
651 Sparks, D.L., Page, A.L., Helmke, P.A., Loeppert, R.H., Soltanpour, P.N., Tabatabai, M.A.,, Johnston,
652   C.T., Sumner, M.E., , 1996. Methods of Soil Analysis. Soil Science Society of America and
653   Ameri-can Society of Agronomy, Madison.
654 Sterkenburg, E., Bahr, A., Durling, M.B., Clemmensen, K.E., Lindahl, B.D., 2015. Changes in fungal
655   communities along a boreal forest soil fertility gradient. New Phytol. 207:1145-58.
656   http://doi.org/10.1111/nph.13426.
657 Su, D.X., Zhang, Z.H., Chen, Z.Z., Hu, X.Z., 2003. Parameters for degradation, sandification and
658   salification of rangelands. The Ministry of Agriculture of the People's Republic of China GB
659   19377-2003:12.

660 Sullivan, B.W., Hart, S.C., 2013. Evaluation of mechanisms controlling the priming of soil carbon
661   along a substrate age gradient. Soil Biol. Biochem. 58:293-301.
662   http://doi.org/10.1016/j.soilbio.2012.12.007.
663 Sunagawa, S., Coelho, L.P., Chaffron, S., Kultima, J.R., Labadie, K., Salazar, G., Djahanschiri, B.,
664   Zeller, G., Mende, D.R., et al., 2015. Structure and function of the global ocean microbiome.
665   Science 348:1261359. http://doi.org/10.1126/science.1261359.
666 Tao, X., Feng, J., Yang, Y., Wang, G., Tian, R., Fan, F., Ning, D., Bates, C.T., Hale, L., et al., 2020.
667   Winter warming in Alaska accelerates lignin decomposition contributed by Proteobacteria.
668   Microbiome 8:84. http://doi.org/10.1186/s40168-020-00838-5.
669 Thomson, A.M., Simpson, I.A., 2007. Modeling historic rangeland management and grazing pressures
670   in landscapes of settlement. Hum. Ecol. 35:151-68. http://doi.org/10.1007/s10745-006-9055-8.
671 Urak, I., Hartel, T., Galle, R., Balog, A., 2017. Worldwide peatland degradations and the related carbon
672   dioxide emissions: the importance of policy regulations. Environ. Sci. Policy 69:57-64.
673   http://doi.org/10.1016/j.envsci.2016.12.012.
674 van der Wal, A., van Veen, J.A., Smant, W., Boschker, H.T.S., Bloem, J., Kardol, P., van der Putten,
675   W.H., de Boer, W., 2006. Fungal biomass development in a chronosequence of land abandonment.
676   Soil Biol. Biochem. 38:51-60. http://doi.org/10.1016/j.soilbio.2005.04.017.
677 Vavourakis, C.D., Mehrshad, M., Balkema, C., van Hall, R., Andrei, A.-S., Ghai, R., Sorokin, D.Y.,
678   Muyzer, G., 2019. Metagenomes and metatranscriptomes shed new light on the microbial-
679   mediated sulfur cycle in a Siberian soda lake. BMC Biol. 17:69. http://doi.org/10.1186/s12915-
680   019-0688-7.
681 Wang, G.X., Qian, J., Cheng, G.D., Lai, Y.M., 2002. Soil organic carbon pool of grassland soils on the
682   Qinghai-Tibetan Plateau and its global implication. Sci. Total Environ. 291:207-17.
683   http://doi.org/10.1016/s0048-9697(01)01100-7.
684 Wang, J., Song, Y., Ma, T., Raza, W., Li, J., Howland, J.G., Huang, Q., Shen, Q., 2017. Impacts of
685   inorganic and organic fertilization treatments on bacterial and fungal communities in a paddy soil.
686   Appl. Soil Ecol. 112:42-50. http://doi.org/10.1016/j.apsoil.2017.01.005.
687 Wang, J., Wang, X., Liu, G., Wang, G., Wu, Y., Zhang, C., 2020. Fencing as an effective approach for
688   restoration of alpine meadows: Evidence from nutrient limitation of soil microbes. Geoderma
689   363:114148. http://doi.org/10.1016/j.geoderma.2019.114148.
690 Wei, W.R., Yao, X., Zhang, Y., Zhen, Q.Y., Qin, M.S., Tang, Z.M., Oosthuizen, M.K., Zhang, W.G.,
691   2023. Vegetation restoration measures: Increasing plant height suppresses population densities of
692   plateau pikas. Land Degrad. Dev. 34:2201-13. http://doi.org/10.1002/ldr.4600.
693 Xu, N., Tan, G.C., Wang, H.Y., Gai, X.P., 2016. Effect of biochar additions to soil on nitrogen leaching,
694   microbial biomass and bacterial community structure. Eur. J. Soil Biol. 74:1-8.
695   http://doi.org/10.1016/j.ejsobi.2016.02.004.
696 Yang, Z.P., Gao, J.X., Zhao, L., Xu, X.L., Ouyang, H., 2013. Linking thaw depth with soil moisture and
697   plant community composition: effects of permafrost degradation on alpine ecosystems on the
698   Qinghai-Tibet Plateau. Plant Soil 367:687-700. http://doi.org/10.1007/s11104-012-1511-1.
699 Yelle, D.J., Ralph, J., Lu, F., Hammel, K.E., 2008. Evidence for cleavage of lignin by a brown rot
700   basidiomycete. Environ. Microbiol. 10:1844-49. http://doi.org/10.1111/j.1462-2920.2008.01605.x.
701 Yergeau, E., Hogues, H., Whyte, L.G., Greer, C.W., 2010. The functional potential of high Arctic
702   permafrost revealed by metagenomic sequencing, qPCR and microarray analyses. ISME J.
703   4:1206-14. http://doi.org/10.1038/ismej.2010.41.
704 Yu, M., Zhang, J., Xu, Y., Xiao, H., An, W., Xi, H., Xue, Z., Huang, H., Chen, X., et al., 2015. Fungal
705   community dynamics and driving factors during agricultural waste composting. Environ. Sci.
706   Pollut. Res. 22:19879-86. http://doi.org/10.1007/s11356-015-5172-5.
707 Zechmeister-Boltenstern, S., Keiblinger, K.M., Mooshammer, M., Penuelas, J., Richter, A., Sardans, J.,
708   Wanek, W., 2015. The application of ecological stoichiometry to plant-microbial-soil organic
709   matter transformations. Ecol. Monogr. 85:133-55. http://doi.org/10.1890/14-0777.1.
710 Zeng, Y., Nupur, Wu, N., Madsen, A.M., Chen, X., Gardiner, A.T., Koblizek, M., 2021. *Gemmatimonas
711   groenlandica* sp. Nov. Is an aerobic anoxygenic phototroph in the phylum gemmatimonadetes.
712   Front. Microbiol. 11:606612. http://doi.org/10.3389/fmicb.2020.606612.
713 Zeng, Y., Selyanin, V., Lukes, M., Dean, J., Kaftan, D., Feng, F., Koblizek, M., 2015. Characterization
714   of the microaerophilic, bacteriochlorophyll *a*-containing bacterium *Gemmatimonas phototrophica*
715   sp nov., and emended descriptions of the genus *Gemmatimonas* and *Gemmatimonas aurantiaca*.
716   Int. J. Syst. Evol. Microbiol. 65:2410-19. http://doi.org/10.1099/ijs.0.000272.

22

717 Zhang, J.P., Zhang, L.B., Liu, W.L., Qi, Y., Wo, X., 2014. Livestock-carrying capacity and overgrazing
718   status of alpine grassland in the Three-River Headwaters region, China. J. Geogr. Sci 24:303-12.
719   http://doi.org/10.1007/s11442-014-1089-z.
720 Zhang, Q., Zhou, W., Liang, G., Sun, J., Wang, X., He, P., 2015. Distribution of soil nutrients,
721   extracellular enzyme activities and microbial communities across particle-size fractions in a long-
722   term fertilizer experiment. Appl. Soil Ecol. 94:59-71. http://doi.org/10.1016/j.apsoil.2015.05.005.
723 Zhang, X., Han, X., Yu, W., Wang, P., Cheng, W., 2017. Priming effects on labile and stable soil
724   organic carbon decomposition: Pulse dynamics over two years. PLoS One 12:e0184978.
725   http://doi.org/10.1371/journal.pone.0184978.
726 Zhou, Z., Wang, C., Zheng, M., Jiang, L., Luo, Y., 2017. Patterns and mechanisms of responses by soil
727   microbial communities to nitrogen addition. Soil Biol. Biochem. 115:433-41.
728   http://doi.org/10.1016/j.soilbio.2017.09.015.
729 Zhu, B., Zhang, Z.X., Tian, J.X., Kong, R., Chen, X., 2022. Increasing negative impacts of climatic
730   change and anthropogenic activities on vegetation variation on the qinghai-tibet plateau during
731   1982-2019. Remote Sens. 14:4735. http://doi.org/10.3390/rs14194735.
732 Zimmermann, M., Bird, M.I., Wurster, C., Saiz, G., Goodrick, I., Barta, J., Capek, P., Santruckova, H.,
733   Smernik, R., 2012. Rapid degradation of pyrogenic carbon. Global Change Biol. 18:3306-16.
734   http://doi.org/10.1111/j.1365-2486.2012.02796.x.
735 Zorz, J.K., Sharp, C., Kleiner, M., Gordon, P.M.K., Pon, R.T., Dong, X., Strous, M., 2019. A shared
736   core microbiome in soda lakes separated by large distances. Nat. Commun. 10:4230.
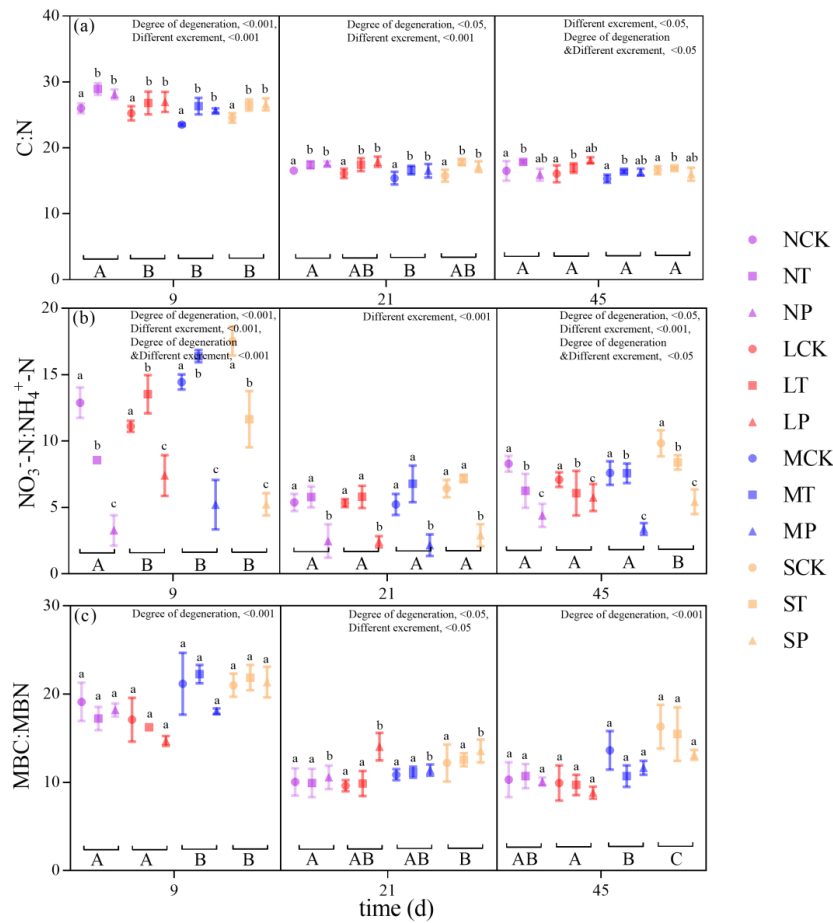737   http://doi.org/10.1038/s41467-019-12195-5.

738

739 Figure 1 Cumulative priming effects of soils from degraded alpine meadows at four levels

740 during a 45-day incubation after the addition of Tibetan sheep excrement (a) and plateau pika

741 excrement (b). The standard errors of the means are shown (n = 3). N, non-degraded; L, lightly

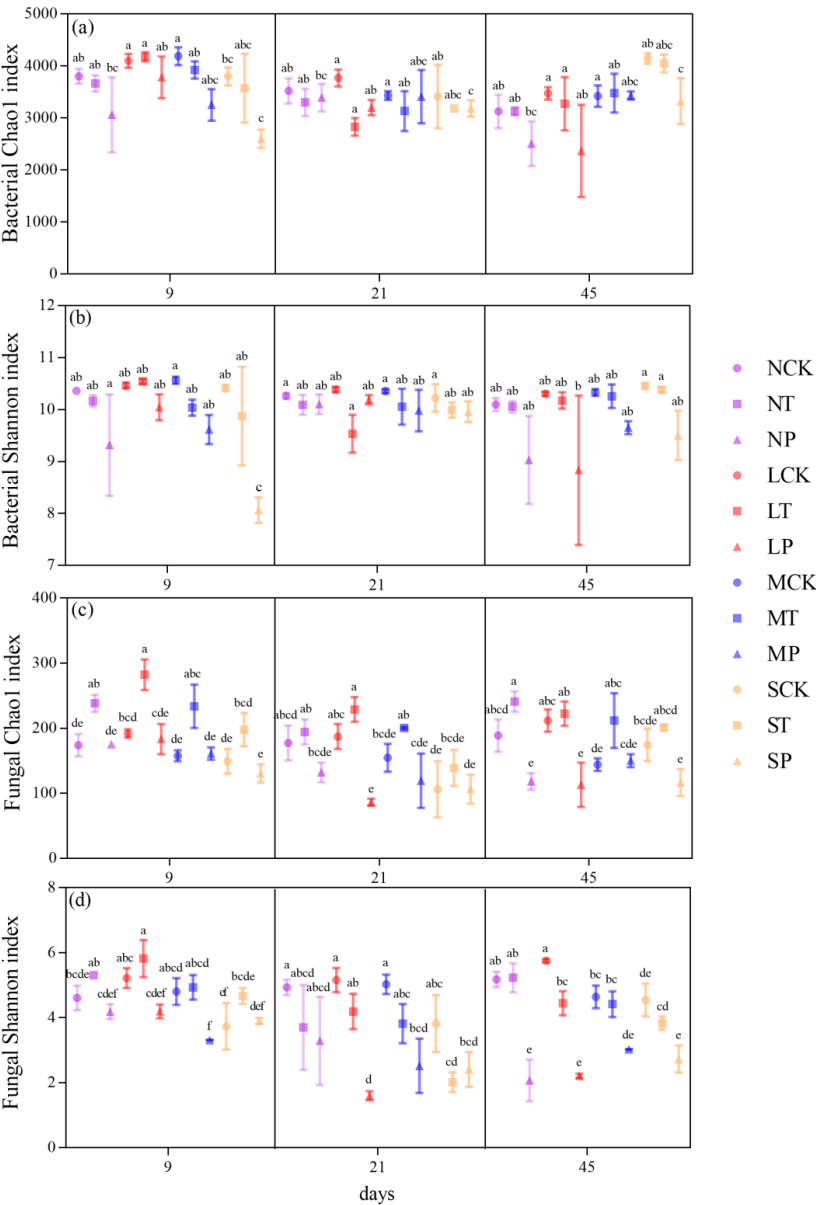742 degraded; M, moderately degraded; S, severely degraded, the same below.

23

743

744 Figure 2 C:N ratio (a), NO_3^--N:NH_4^+-N ratio (b), and MBC:MBN ratio (c) in soils from alpine

745 meadows with varying degrees of degradation without addition (CK) and with the addition of

746 two types of excrement. T, Tibetan sheep excrement; P, plateau pika excrement. Lowercase

747 letters indicate significant differences between different excrements ($P < 0.05$), while uppercase

748 letters indicate significant differences between meadow soils with different degrees of
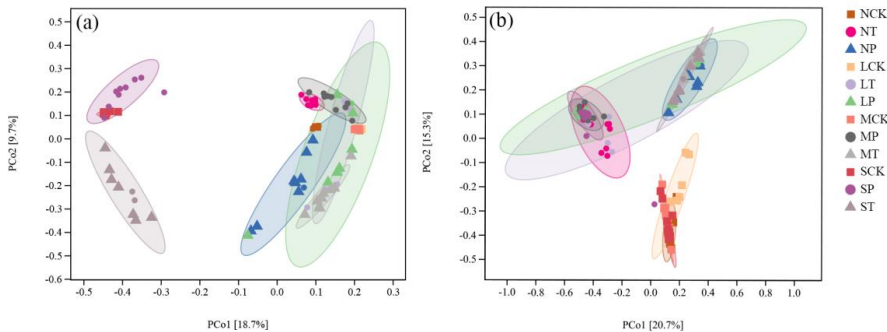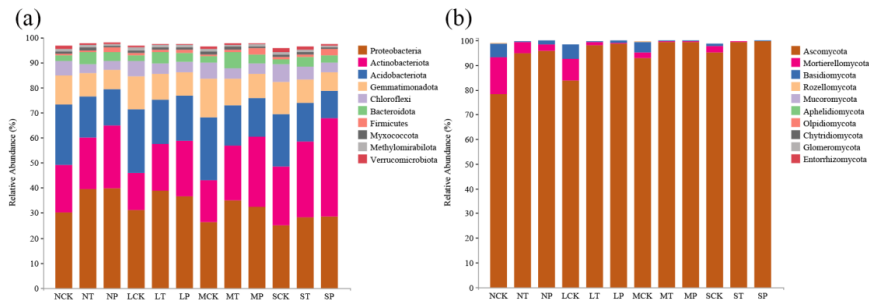
749 degradation ($P < 0.05$). The same below.

24

Figure 3 Alpha diversity (including Chao1 index and Shannon index) of soil bacteria (a, b) and
fungi (e, d) in different degradation degrees without addition (CK) and the addition of two types
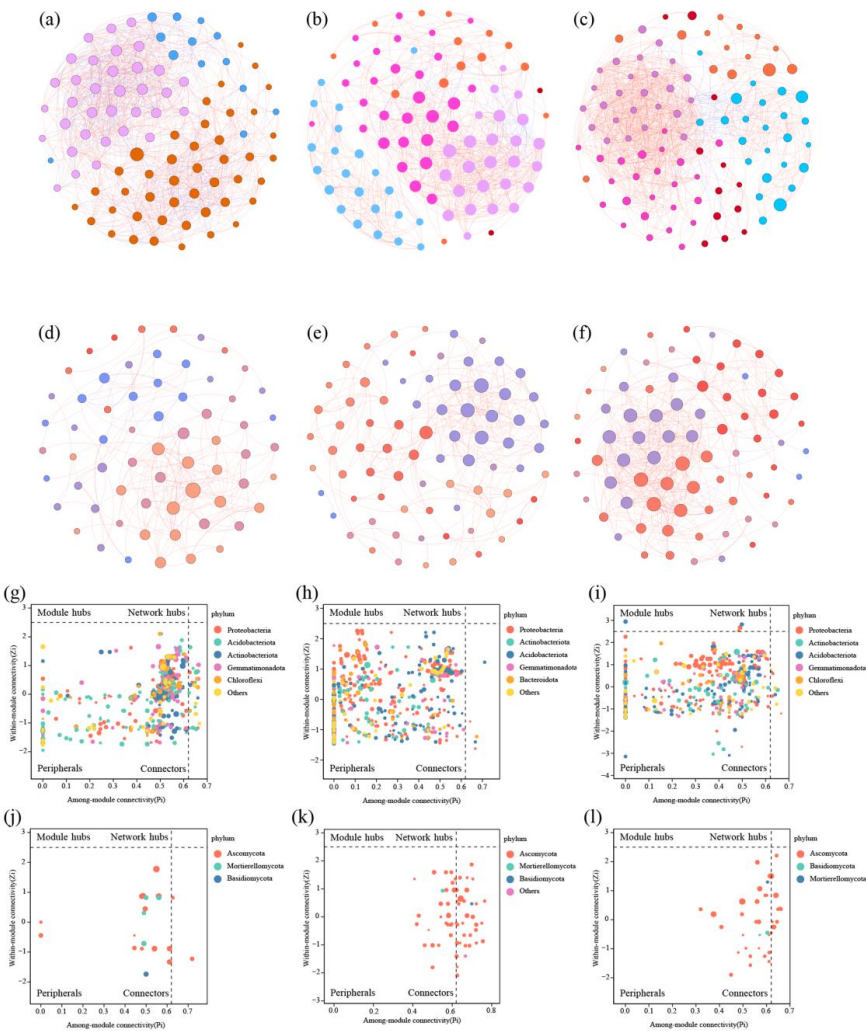of excrement.

25

754

Figure 4 β diversity of soil bacteria (a) and fungi (b) in alpine meadows with different

degradation degrees without addition and the addition of two types of excrement.

757

Figure 5 Taxonomic composition of soil bacteria (a) and fungi (b) in soils with different

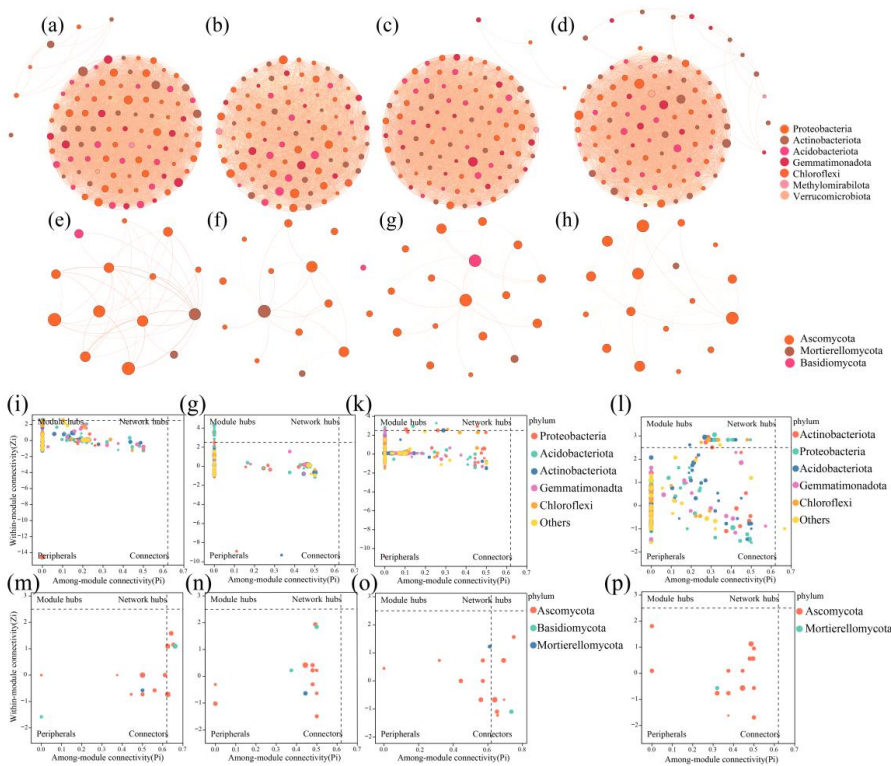degradation degrees after the addition of two types of excrement.

26

Figure 6 Co-occurrence networks and ZiPi plots of soil bacterial (a-c, g-i) and fungal (d-f, j-l)
communities without addition (CK) and with the addition of two types of excrement (CK, a,
d, g, j; Tibetan sheep excrement, b, e, h, k; Plateau pika excrement, c, f, i, l). Different colors
represent different modules (a-f), and different phyla (g-l).

27
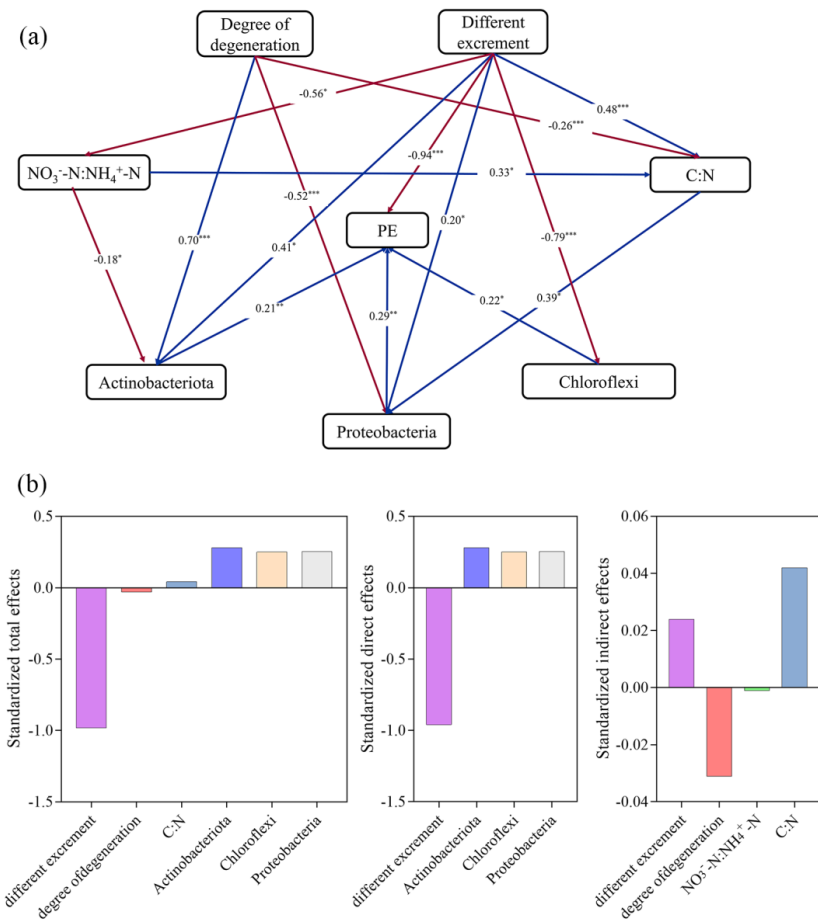

Figure 7 Co-occurrence networks and ZiPi plots of soil bacterial (a-d, i-l) and fungal (e-h, m-p) communities in non-degraded (a, e, i, m), lightly degraded (b, f, j, n), moderately degraded (c, g, k, o), and severely degraded alpine meadows (d, h, l, p). Different colors represent different phyla.

28

Figure 8 Structural Equation Model (a) depicting the relationships among soil stoichiometric ratios, key

microbial species, and soil organic carbon priming effects after the addition of two types of excrement

(CMIN/df = 1.019 (P=0.422); RMSEA = 0.023; CFI = 0.999; GFI = 0.945; NFI = 0.975), along with the

direct and indirect effects (b). In Fig. a, red indicates negative effects, blue indicates positive effects, *

represents $P < 0.05$, ** represents $P < 0.01$, and *** represents $P < 0.001$.

Table 1

Physicochemical properties of the collected excrements and soil samples.

	SOC (g·kg^{-1})	TN (g·kg^{-1})	C:N ratio	NO$_3^-$-N (mg·kg^{-1})	NH$_4^+$-N (mg·kg^{-1})	NO$_3^-$-N: NH$_4^+$-N ratio
T	336.42±3.29	13.03±0.24	25.84±0.42	85.03±30.23	85.6±0.61	0.99±0.35

29

P	285.24±13.01	44.11±0.36	6.47±0.3	517.99±14.38	5.46±0.72	97.6±9.91
N	83.53±1.17	3.22±0.09	25.99±0.42	40.99±0.56	3.2±0.15	12.88±0.66
L	66.44±0.95	2.63±0.04	25.23±0.63	34.94±0.55	3.15±0.03	11.11±0.24
M	70.26±0.53	2.98±0.01	23.53±0.12	45.01±0.22	3.12±0.08	14.44±0.32
S	80.22±0.89	3.27±0.05	24.57±0.42	58.21±1.33	3.33±0.14	17.52±0.63

778 Values are expressed as mean ± standard error (n = 3). T, Tibetan sheep excrements, P, plateau pika

779 excrements, N, non-degraded, L, lightly degraded, M, moderately degraded, S, severely degraded,

780 SOC, soil organic carbon, TN, total nitrogen, NO_3^--N, nitrate nitrogen, NH_4^+-N, ammonium

781 nitrogen, the same below.

30

782 Table 2

783 Topological parameters of soil bacterial and fungal co-occurrence networks without addition (CK)

784 and with the addition of two types of excrement.

	Bacteria			Fungi		
	CK	T	P	CK	T	P
Number of nodes	91.00	92.00	98.00	64.00	77.00	75.00
Number of edges	1024.00	697.00	946.00	169.00	248.00	394.00
Average degree	22.51	15.15	19.11	5.28	6.44	10.51
Network diameter	5.00	6.00	5.00	11.00	12.00	9.00
Graph density	0.25	0.17	0.20	0.08	0.09	0.14
Modularity	0.36	0.47	0.36	0.56	0.57	0.33
Average clustering coefficient	0.65	0.64	0.66	0.56	0.63	0.67
Edges(+)/Total edges	0.62	0.90	0.93	0.89	0.99	0.98

785

31

786   Table 3

787   Topological parameters of symbiotic network of soil bacteria and fungi in alpine meadows degraded

788   to different degrees.

	Bacteria				Fungi			
	N	L	M	S	N	L	M	S
Number of nodes	97.00	91.00	96.00	97.00	14.00	15.00	17.00	17.00
Number of edges	4108.00	4095.00	4281.00	3581.00	37.00	39.00	51.00	61.00
Average degree	84.70	90.00	89.60	73.84	5.29	5.20	6.71	7.18
Network diameter	2.00	1.00	1.00	2.00	3.00	3.00	3.00	3.00
Graph density	0.82	1.00	0.94	0.77	0.41	0.37	0.42	0.45
Modularity	0.01	0.02	0.00	0.01	0.12	0.13	0.19	0.15
Average clustering coefficient	0.99	1.00	1.00	0.99	0.56	0.70	0.47	0.68
Edges(+)/Total edges	0.96	0.74	1.00	0.93	0.59	0.69	0.53	0.49

789