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

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11 **ABSTRACT:**

12 Tibetan sheep (*Ovis aries*) and plateau pika (*Ochotona curzoniae*) excrements are important
13 organic materials that influence soil carbon cycling in the Qinghai-Tibetan Plateau. However, their
14 exact priming effects (PE) on soil and mechanisms of influence in alpine meadows are subject to
15 their health status. To fill this knowledge gap, we carried out a 45-day incubation experiment using
16 alpine meadow soils with differing degrees of degradation that had been enriched with these two
17 types of excrement. Soil PE was assessed via the natural abundance method, while soil microbial
18 communities and their compositions were examined through high-throughput sequencing. **The**
19 **findings indicated that severely degraded alpine meadow soils exhibit significantly stronger positive**
20 **priming effects (PE) from Tibetan sheep and plateau pika excrements than non-degraded soils ($P <$**
21 **0.05).** These excrements restructure soil microbial communities, reducing bacterial α -diversity
22 while increasing β -diversity and microbial biomass C:N ratios. Key microbial drivers of PE include
23 Actinobacteriota (positive correlation, $r = 0.70$) and Proteobacteria (negative correlation, $r = -0.52$),
24 whose abundances are modulated by soil properties: the C:N ratio promotes Proteobacteria ($r =$
25 0.39), while a lower $\text{NO}_3\text{-N}:\text{NH}_4^+\text{-N}$ ratio suppresses Actinobacteriota ($r = -0.18$). Meadow
26 degradation thus indirectly intensifies PE by altering these microbial phylum and stoichiometric
27 balances. These findings support Qinghai-Tibetan Plateau grassland management through: (1)
28 restricting grazing/pika activity in degraded zones to reduce carbon loss, (2) optimizing soil C:N



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29 ratios to suppress priming effect (PE)-enhancing microbes, and (3) conserving intact meadows for
30 carbon sequestration—collectively enabling sustainable grassland management.

31 Keywords: Soil microbes phylum, degree of meadow degradation, soil priming effects,
32 excrement supplements

33 **1. Introduction**

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

48 In an effort to restore the compromised meadow ecosystem functions, various
49 measures have been attempted, such as supplementation of exogenous organic matter.
50 After its application to the soil, it may promote or inhibit soil organic carbon
51 mineralization temporally, a phenomenon termed the priming effect (PE) (Chen et al.,
52 2014; Fontaine et al., 2004; Fontaine et al., 2003; Kuzyakov et al., 2000; Smith et al.,
53 2014). Previous studies have indicated that the nature of exogenous organic matter, the
54 existing soil nutrient status, and microbial composition are all factors influential to the
55 magnitude and duration of PE (Blagodatskaya and Kuzyakov, 2008). Moreover,
56 alterations in soil microbial composition exert a significant impact on ecosystem carbon
57 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).

140 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,



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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 CO₂ 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 BaCl₂. 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 δ¹³C
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 CO₂ 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 CO₂-C (α) from soil respiration was calculated using the two-
173 component mixing model (Pausch et al., 2013):

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

175 In this context, δ_t represents the $\delta^{13}\text{C}$ value of the total CO₂-C released from the
176 soil after the addition of excrements. δ_s represents the $\delta^{13}\text{C}$ value of the excrement itself,
177 while δ_{ck} represents the $\delta^{13}\text{C}$ value of the total CO₂-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:

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

181 In this context, R_t represents the total CO₂-C released from respiration in the soil
182 after the addition of excrements, while R_{ck} represents the total CO₂-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 HClO₄-H₂SO₄. Total potassium
190 (TK) was measured spectrophotometrically using the flame photometric method,
191 following digestion with HF-HClO₄. Concentrations of NH₄⁺-N and NO₃⁻-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 NaHCO₃. Available potassium was measured spectrophotometrically
195 after extraction with NH₄OAc (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 ITS1
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.

243 **3. Results**

244 *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 indices (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 ($P < 0.05$).



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 $\text{N}:\text{NH}_4^+\text{-N}$ 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).

327 **4. Discussion**

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^- - $\text{N}:\text{NH}_4^+\text{-N}$ ratio. After the addition of excrement,
338 compared to non-degraded soil, severely degraded soil had a higher NO_3^- - $\text{N}:\text{NH}_4^+\text{-N}$
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



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 succeed 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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401 mechanisms of PEs include "cometabolism," "N-mining," and "stoichiometric
402 decomposition" (Blagodatskaya and Kuzyakov, 2008; Chen et al., 2014; Craine et al.,
403 2007; Fang et al., 2018; Hessen et al., 2004). In this study, the excrement supplements
404 were the main influencer of soil PEs in the alpine meadows degraded to various degrees
405 ($r = -0.94$) (Fig. 7). The microbial community activities associated with different
406 stoichiometries in the excrements were consistent across the alpine meadows regardless
407 of their degree of degradation. The degree of degradation affected only key microbial
408 phylum such as Actinobacteriota ($r = 0.70$), Proteobacteria ($r = -0.52$), and C:N ratio(r
409 $= -0.26$), indirectly influencing PEs.

410 Based on the analysis of soil symbiotic networks, the key phylum were identified
411 at different phylum levels. These key microorganisms, including Actinobacteriota ($r =$
412 0.21), Proteobacteria ($r = 0.29$), and Chloroflexi ($r = 0.22$), directly and positively
413 influenced PEs. Actinobacteria participate in nitrogen and organic matter
414 decomposition and cycling processes (Sunagawa et al., 2015), which aligns with our
415 findings. The soil NO_3^- -N: NH_4^+ -N ratio negatively affected the key microbial phylum
416 Actinobacteriota ($r = -0.18$), thereby influencing soil PE. Proteobacteria promote the
417 dissolution of organophosphates and soil nitrogen fixation (Pascault et al., 2013), which
418 is consistent with our results. Soil C:N ratio positively influenced the key microbial
419 phylum Proteobacteria ($r = 0.39$), thereby affecting PEs. Chloroflexi can survive in
420 extreme environments, performs anaerobic photosynthesis (Zeng et al., 2021; Zeng et
421 al., 2015), possesses carbon fixation capabilities (Vavourakis et al., 2019; Zorz et al.,
422 2019), and can reduce N_2O in the nitrogen cycle (Park et al., 2017).

423 Previously, this paper has demonstrated that the supplementation of both Tibetan
424 sheep excrement and plateau pika excrement elevated the relative abundance of crucial
425 soil microorganisms and bolstered microbial community stability. Consequently, the
426 added excrements modulated PEs through alterations in the composition of key
427 microbial communities. This research further reveals that, in contrast to plateau pika
428 excrement, Tibetan sheep excrement boasted carbon stability more and a lower carbon-
429 to-nitrogen ratio (C:N ratio). Based on the N mining hypothesis, the lower C:N ratio of
430 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.

435 **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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459 **Data availability**

460 The data will be made available upon reasonable request.

461 **Declaration of competing interest**

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

465 **CRedit authorship contribution statement**

466 Qinyao Li: Data curation, Investigation, Software, Writing—original draft;
467 Wenquan Yang: Conceptualization, Supervision, Writing—review & editing; Jiancun
468 Kou: Conceptualization, Project administration, Funding acquisition, Supervision,
469 Writing—review & editing; Qian Liu: Investigation; Yangcan Zhang: Investigation;
470 Weiliang Kou: Investigation; Jiaqing Liu: Investigation; Xilai Li: Conceptualization,
471 Formal analysis, Methodology; Jing Zhang: Conceptualization, Formal analysis,
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478 **References**

- 479 Ai, C., Zhang, S., Zhang, X., Guo, D., Zhou, W., Huang, S., 2018. Distinct responses of soil bacterial
480 and fungal communities to changes in fertilization regime and crop rotation. *Geoderma* 319:156-
481 66. <http://doi.org/10.1016/j.geoderma.2018.01.010>.
- 482 Banerjee, S., Walder, F., Buchi, L., Meyer, M., Held, A.Y., Gattinger, A., Keller, T., Charles, R., van der
483 Heijden, M.G.A., 2019. Agricultural intensification reduces microbial network complexity and the
484 abundance of keystone taxa in roots. *Isme J.* 13:1722-36. [http://doi.org/10.1038/s41396-019-0383-](http://doi.org/10.1038/s41396-019-0383-2)
485 [2](http://doi.org/10.1038/s41396-019-0383-2).
- 486 Bastian, F., Bouziri, L., Nicolardot, B., Ranjard, L., 2009. Impact of wheat straw decomposition on
487 successional patterns of soil microbial community structure. *Soil Biol. Biochem.* 41:262-75.
488 <http://doi.org/10.1016/j.soilbio.2008.10.024>.



- 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](http://doi.org/10.1111/1462-2920.15333).
- 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](http://doi.org/10.1038/s41467-018-06232-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., Nikkila, 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](http://doi.org/10.1016/s0038-0717(03)00123-8).



- 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](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 CO₂ 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](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](http://doi.org/10.1016/s1002-0160(11)60194-x).



- 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-](http://doi.org/10.1007/s10021-013-9650-7)
613 [013-9650-7](http://doi.org/10.1007/s10021-013-9650-7).
- 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 CO₂ 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., Balsler, 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-](http://doi.org/10.1111/j.1469-1795.1999.tb00069.x)
650 [1795.1999.tb00069.x](http://doi.org/10.1111/j.1469-1795.1999.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 American 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.

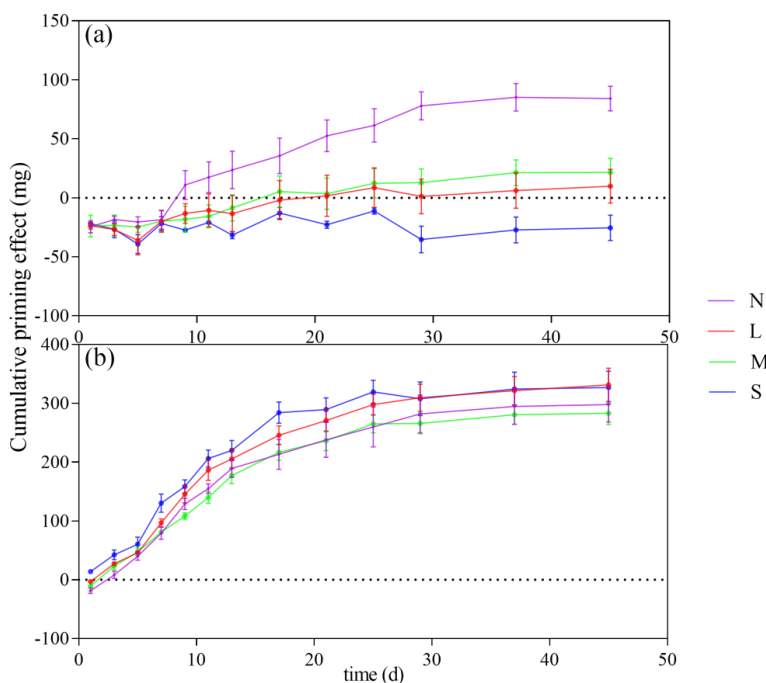


- 660 Sullivan, B.W., Hart, S.C., 2013. Evaluation of mechanisms controlling the priming of soil carbon
661 along a substrate age gradient. *Soil Biol. Biochem.* 58:293-301.
662 <http://doi.org/10.1016/j.soilbio.2012.12.007>.
- 663 Sunagawa, S., Coelho, L.P., Chaffron, S., Kultima, J.R., Labadie, K., 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/s12915-019-0688-7>.
- 680
- 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](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>.

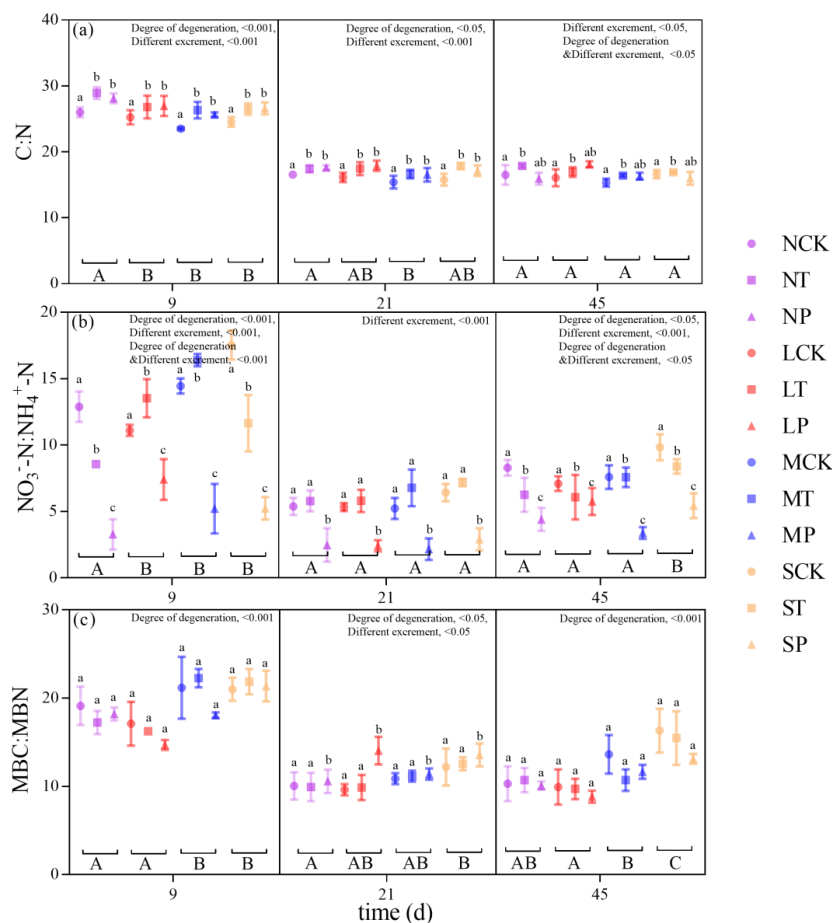


738

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.



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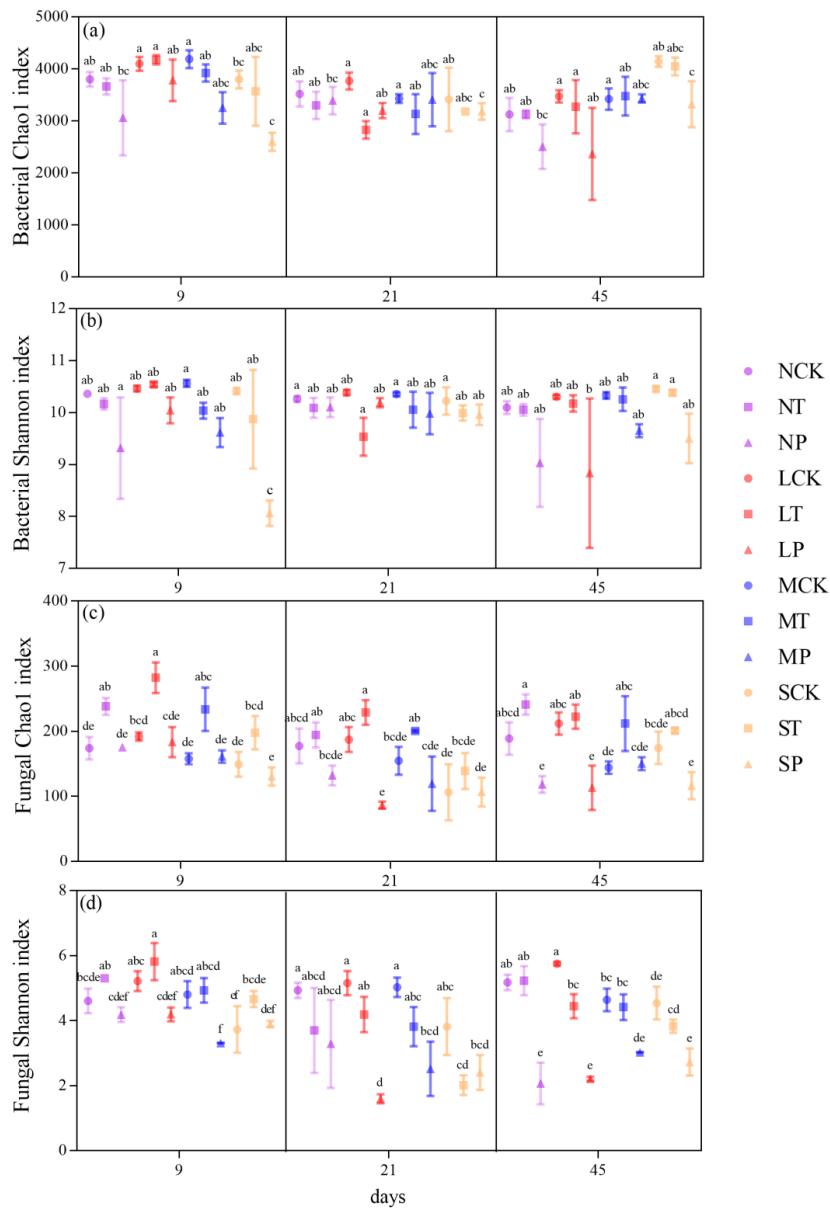


743

744 Figure 2 C:N ratio (a), NO₃-N:NH₄⁺-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.



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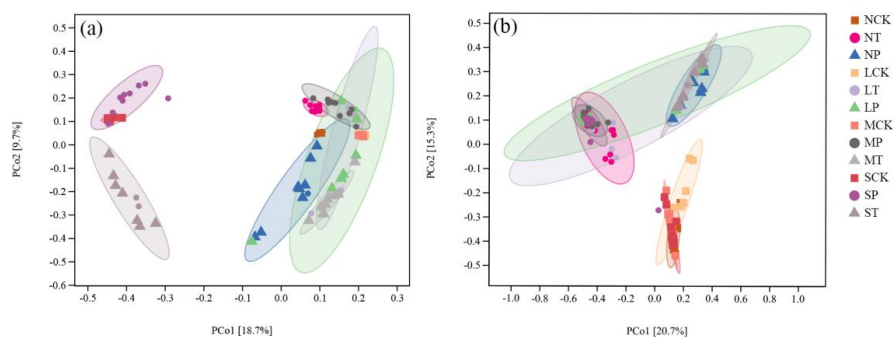


750

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



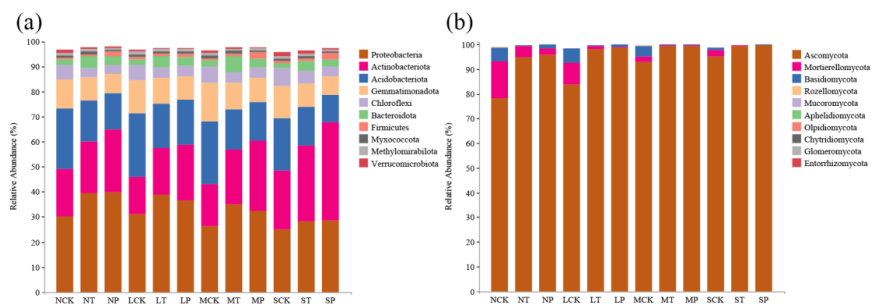
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754

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

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



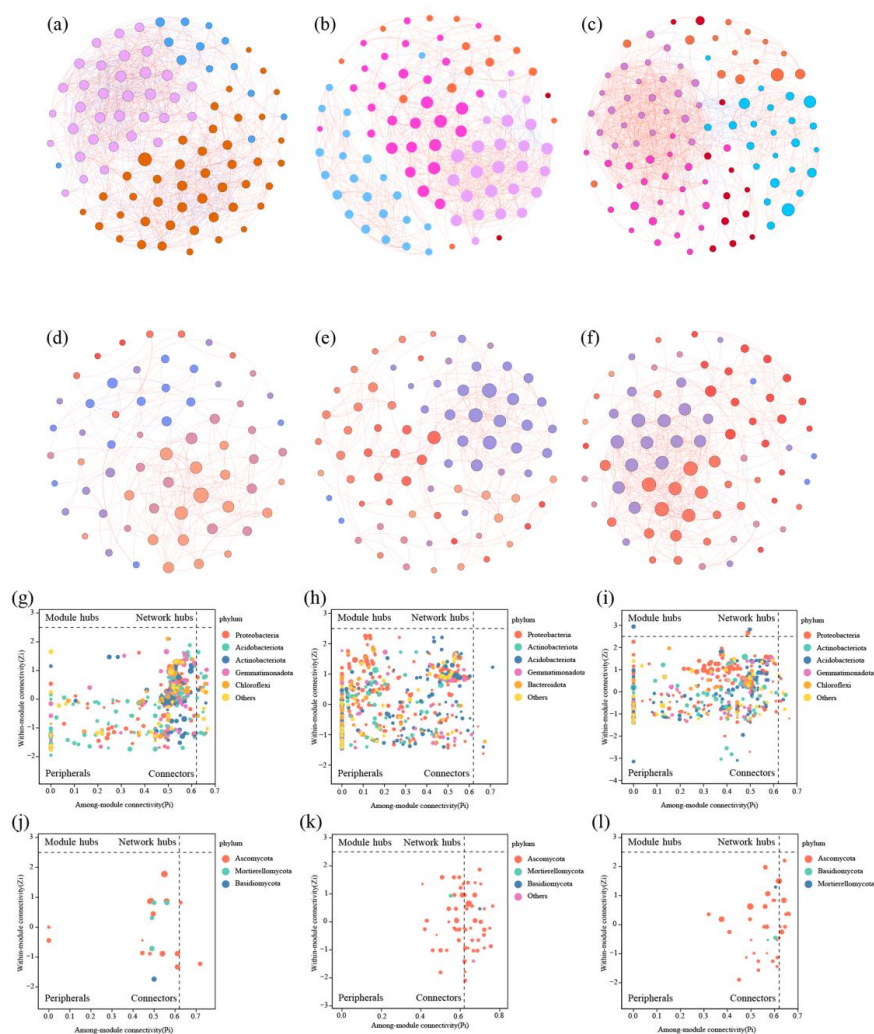
757

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

759 degradation degrees after the addition of two types of excrement.



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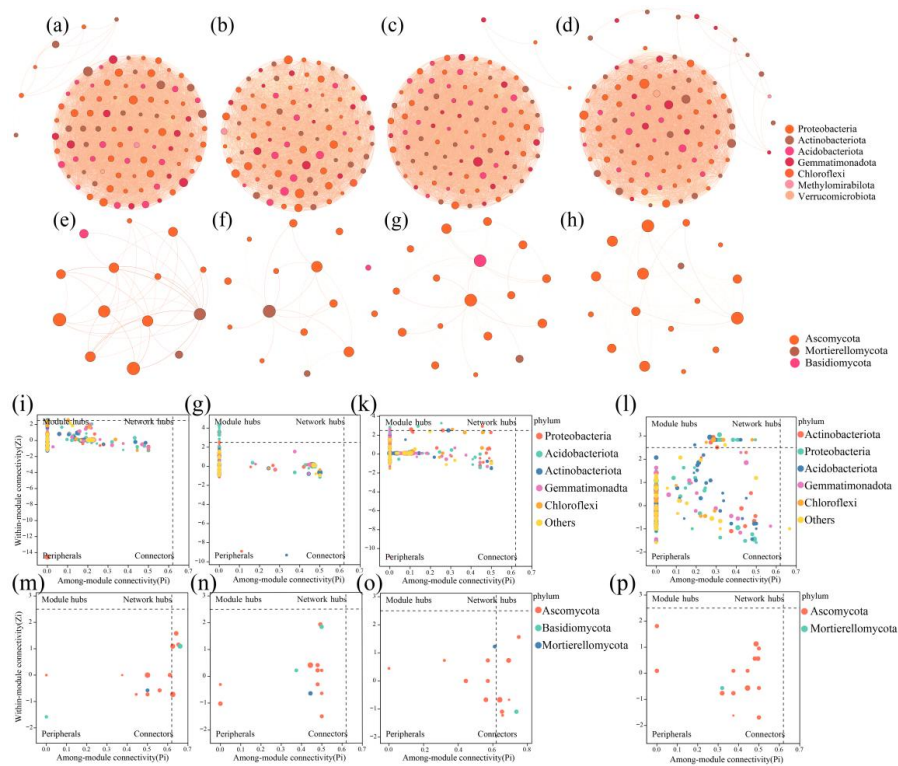


760

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



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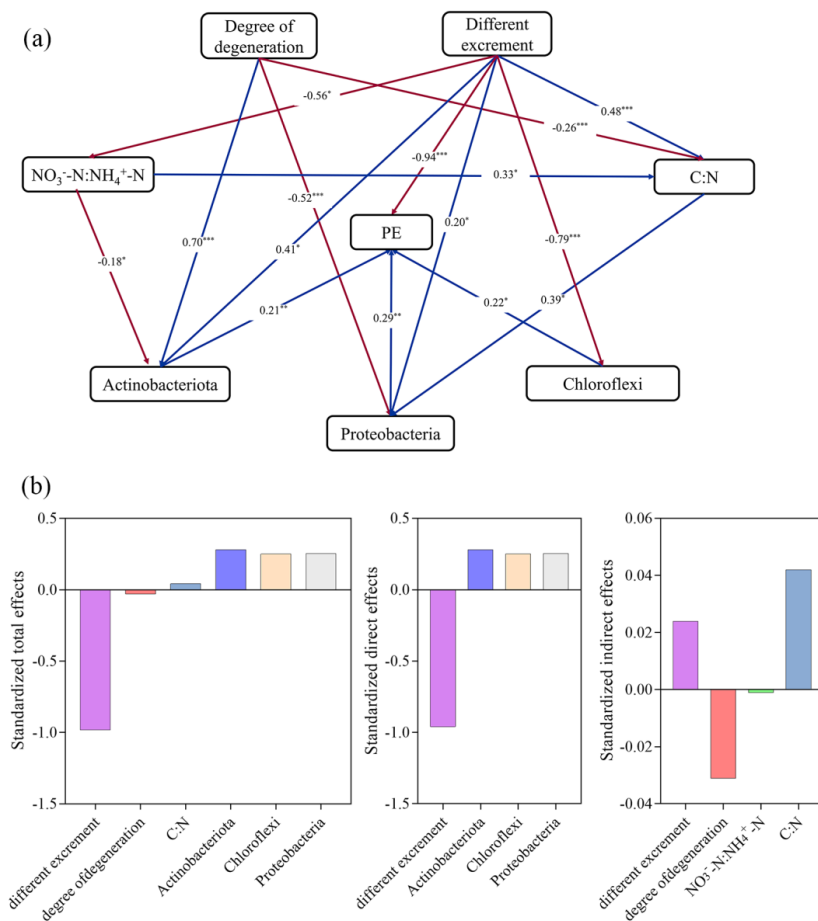


765

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



28



770

771 Figure 8 Structural Equation Model (a) depicting the relationships among soil stoichiometric ratios, key
 772 microbial species, and soil organic carbon priming effects after the addition of two types of excrement
 773 (CMIN/df = 1.019 (P=0.422); RMSEA = 0.023; CFI = 0.999; GFI = 0.945; NFI = 0.975), along with the
 774 direct and indirect effects (b). In Fig. a, red indicates negative effects, blue indicates positive effects, *
 775 represents $P < 0.05$, ** represents $P < 0.01$, and *** represents $P < 0.001$.

776 Table 1

777 Physicochemical properties of the collected excrements and soil samples.

	SOC ($\text{g} \cdot \text{kg}^{-1}$)	TN ($\text{g} \cdot \text{kg}^{-1}$)	C:N ratio	$\text{NO}_3^- \text{N}$ ($\text{mg} \cdot \text{kg}^{-1}$)	$\text{NH}_4^+ \text{N}$ ($\text{mg} \cdot \text{kg}^{-1}$)	$\text{NO}_3^- \text{N} :$ $\text{NH}_4^+ \text{N}$ 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, NO₃⁻-N, nitrate nitrogen, NH₄⁺-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