

1 **Short-term effects of snow cover manipulation on soil bacterial**
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3 **diversity and community composition**
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1 **Abstract**

2 Winter snow cover is a major driver of soil microbial processes in high-latitude and high-altitude
3 ecosystems. Warming-induced reduction in snow cover as predicted under future climate scenarios
4 may shift soil bacterial communities with consequences for soil carbon and nutrient cycling. The
5 underlying mechanisms, however, remain elusive. In the present study, we conducted a snow
6 manipulation experiment in a Tibetan spruce forest to explore the immediate and intra-annual legacy
7 effects of snow exclusion on soil bacterial communities. We analyzed bacterial diversity and
8 community composition in the winter (i.e., the deep snow season), in the transitional thawing period,
9 and in the middle of the growing season. Proteobacteria, Acidobacteria, and Actinobacteria were
10 dominant phyla across the seasons and snow regimes. Bacterial diversity was generally not
11 particularly sensitive to the absence of snow cover. However, snow exclusion positively affected
12 Simpson diversity in the winter but not in the thawing period and the growing season. Bacterial
13 diversity further tended to be higher in winter than in the growing season. In the winter, the
14 taxonomic composition shifted in response to snow exclusion, while composition did not differ
15 between exclusion and control plots in the thawing period and the growing season. Soil bacterial
16 communities strongly varied across seasons, and the variations differed in specific groups. Both soil
17 climatic factors (i.e., temperature and moisture) and soil biochemical variables partly accounted for
18 the seasonal dynamics of bacterial communities. Taken together, our study indicates that soil bacterial
19 communities in Tibetan forests are rather resilient to change in snow cover, at least at an intra-annual
20 scale.

21

22 **Keywords:** Winter climate change; Snow cover; Bacteria; Community diversity; Community
23 composition; Illumina sequencing.

24

25 **1. Introduction**

26 Reduced snow cover is one of the most dramatic changes under projected climate change scenarios
27 for many cold-climate regions in the world (Stielstra et al., 2015). Snow cover change has major
28 impacts on soil temperature, moisture, and the frequency of freeze-thaw cycles, during winter but
29 especially also during the early growing season (Isobe et al., 2018; Potopová et al., 2016). Soil
30 temperature and freeze-thaw cycles play critical roles in regulating soil microbial communities in
31 cold-climate ecosystems (Groffman et al., 2011; Sharma et al., 2006). Hence, the lack of an insulating
32 snow cover can have profound effects on the composition, diversity, and functioning of soil microbial
33 communities (Gavazov et al., 2017; Robroek et al., 2013).

34

35 Soil bacteria play key roles in biogeochemical cycles in cold-climate ecosystems (Isobe et al., 2018;
36 Zhang et al., 2014b). Previous studies have shown that some key drivers of soil bacterial communities,
37 including soil temperature, moisture, and nutrient availability, vary in different ways due to changes
38 in snow (Aanderud et al., 2013; Edwards et al., 2007; Lipson and Schmidt, 2004). Snowpack can
39 prevent soil temperatures from falling much below freezing (Edwards et al., 2007). Conversely, the
40 absence of snow cover may result in deep soil freezing and reduced soil moisture content (Groffman
41 et al., 2011). Thus, changes in soil temperature and moisture associated with snow cover change may
42 shift the structure of soil bacterial communities (Stark and Firestone, 1995; Lipson, 2007). In addition,
43 snow cover change may also induce significant changes in soil biochemical properties (e.g., pH and N
44 availability), and thereby mediate climate-induced shifts in soil bacterial communities (Kim et al.,
45 2014; Ricketts et al., 2016).

46

47 The responses of soil microbes to environmental change may differ among seasons (Aanderud et al.,
48 2013; Schimel et al., 2007; Zinger et al., 2009). Some studies have shown that snow cover change has
49 immediate impacts on soil bacterial community composition in winter (Ricketts et al., 2016; Robroek

50 et al., 2013). Recent studies have also found that microbial response induced by snow cover change
51 can carry over into the following snow-free growing season (Aanderud et al., 2013; Wubs et al.,
52 2018). However, soil bacterial communities may also adapt and respond quickly to changing soil
53 conditions, even in the short-term (Männistö et al., 2018; Schimel et al., 2007). Therefore, the
54 post-winter legacy effects of changes in snow cover on soil bacterial communities may be limited. To
55 our knowledge, soil bacterial responses to snow cover change have rarely been studied both in the
56 winter and the following growing season (Aanderud et al. 2013). However, investigating the
57 immediate and legacy effects of snow cover change on soil bacterial communities is important for
58 better understanding microbial ecological processes in cold-climate areas.

59

60 The Tibetan Plateau has seen a significant warming trend over the last few decades (Wang et al.,
61 2016). In this region, the temperature has been increasing at a rate of about 0.2°C/decade with the
62 most pronounced warming occurring in winter (You et al., 2017). Climate warming may strongly
63 impact on winter snowfall, thereby further affecting the seasonal snow accumulation and snowmelt
64 time (Kapnick and Delworth, 2013). Our previous studies have shown that snow exclusion led to
65 intensified soil freezing and increased soil nutrient availability, whereas snow exclusion suppressed
66 winter soil respiration (Li et al., 2017; Yang et al., 2019). Future snow-free winters will alter winter
67 soil conditions and, in turn, may have strong impacts on microbial communities in Tibetan forest soils.
68 To test this, we conducted a snow-manipulation experiment in a Tibetan spruce forest to examine the
69 effects of snow exclusion on the diversity and structure of soil bacterial communities in the winter,
70 the transitional thawing period, and the subsequent growing season. Specifically, we tested the
71 following hypotheses: (i) snow exclusion decreases the abundance and diversity of soil bacterial
72 communities, and thereby changes the bacterial community composition; (ii) snow exclusion will
73 cause both immediate effects (during winter) and legacy effects (during the thawing period and the
74 following growing season) on soil bacterial communities; (iii) snow exclusion will impact soil

75 bacterial communities through changes in environmental and/or soil biochemical conditions.

76

77 **2. Materials and methods**

78 2.1. Site description

79 The study was conducted in a dragon spruce (*Picea asperata*) stand at the Long-term Research
80 Station of Alpine Forest Ecosystems of Sichuan Agricultural University, which is located at the
81 eastern Tibetan Plateau of China (31°15'N, 102°53'E; 3021 m a.s.l.). The mean annual temperature is
82 3.0 °C, with maximum and minimum temperatures of 23.0 °C (July) and -18.0 °C (January),
83 respectively. Annual precipitation is about 850 mm. In general, snow begins to accumulate in late
84 November and melts in late March of the following year. The soil is classified as a Cambic Umbrisol
85 (IUSS Working Group WRB, 2007). The organic carbon (C), nitrogen (N), and pH at the soil depth of
86 0-15 cm were 88.5 g C kg⁻¹, 5.4 g N kg⁻¹, and 6.4, respectively (Li et al., 2017). The understory is
87 dominated by *Salix paraplesia*, *Rhododendron lapponicum*, *Cacalia* sp., *Carex* sp., and *Cyperus* sp.
88 (Li et al., 2017).

89

90 2.2. Experimental design

91 Winter snowfall was excluded using shelters with the aim to manipulate the depth and intensity of soil
92 frost. This shelter method is a useful tool for studying the responses of soil processes to winter
93 climate change, as it effectively reduces snow cover and minimize unwanted environmental side
94 effects (Li et al., 2016). Six wooden roofs (3 m × 3 m ground area) were installed in November 2015
95 to prevent the accumulation of snow on the ground. One control plot was randomly set up in the
96 vicinity of each snow-exclusion plot. The snow removal manipulation began in late November 2015
97 and ended in early April 2016. See, Li et al. (2017) for further details.

98

99 2.3. Microclimate

100 Air temperature (2 m height) and soil temperature (5 cm depth) were measured using ThermoChron
101 DS1923-F5 iButtons (Maxim Dallas Semiconductor Corp, USA) every 1 h during the experimental
102 period. Snow depth of the control plots was measured approximately every 2 weeks with a ruler. The
103 minimum and maximum air temperatures were -14.1 °C and 18.1 °C, respectively. The minimum
104 daily mean soil temperatures were -2.2 °C and -0.5 °C in the snow exclusion plots and control plots,
105 respectively (Fig. S1). Seasonal snow began to accumulate in late November 2015 and melted in late
106 March 2016, with the maximum depth in the control plots of 40 cm in late February (Fig. S1). The
107 number of freeze-thaw cycles over the winter period was 25 and 13 in the snow exclusion and control
108 plots, respectively (Li et al., 2017). Snow exclusion resulted in more severe soil frost in the winter of
109 2015/2016 but did not affect winter soil moisture content. Additionally, soil temperature and moisture
110 did not differ between control and snow exclusion plots during the growing season (Fig. S1).

111

112 2.4. Soil sampling

113 Three paired plots (snow exclusion vs. control) were randomly selected. Soils were sampled in the
114 deep snow period (DSP, mid-February 2016), early thawing period (ETP, early April 2016) and in the
115 middle of the growing season (MGS, mid-August 2016). At each sampling event, three soil cores
116 were collected from each plot using an auger (15 cm long and 5 cm diameter) and were mixed into
117 one composite sample per plot. The composite samples were passed through a 2 mm sieve, and any
118 visible living plant material was removed from the sieved soil. Subsamples of the sieved soils were
119 stored at -70°C and 4°C for molecular and biochemical analyses, respectively.

120

121 2.5. Soil physiochemical and biological analyses

122 Soil pH value was measured by a pH meter using a soil to water ratio of 1:2.5 (*m/v*). Soil ammonium
123 (NH_4^+ -N) and nitrate (NO_3^- -N) were extracted with 2 M KCl and then measured by colorimetry (Xu et
124 al., 2010). Soil microbial biomass carbon (MBC) was measured by the fumigation-extraction method

125 (Vance et al., 1987).
126 We assessed the activities of seven enzymes which are involved in soil C, N, and P cycling:
127 β -glucosidase (BG), cellobiohydrolase (CBH), polyphenol oxidase (PPO), peroxidase (POD),
128 β -N-acetyl-glucosaminidase (NAG), leucine aminopeptidase (LAP), and acid phosphatase (AP). The
129 soil enzyme activities were assayed using the method described by Allison and Jastrow (2006).
130 Substrate solutions were 5 mM pNP- β -glucopyranoside for BG, 2 mM pNP-cellobioside for CBH, 50
131 mM pyrogallol and 50 mM EDTA for PPO, 5 mM L-DOPA and 10 μ L of 0.3% H₂O₂ for POD, 2 mM
132 pNP- β -N-acetylglucosaminide for NAG, 5 mM leucine p-nitroanilide for LAP, and 5 mM
133 pNP-phosphate for AP. PPO and POD were incubated for 2 h at 20 °C, BG and AP were incubated for
134 1 h at 30 °C, and CBH, NAG, and LAP were incubated for 4 h at 30 °C. The absorbance of products
135 was read using a microplate spectrophotometer at 405 nm for the hydrolytic enzymes and 450 nm for
136 the oxidative enzymes. Enzyme activities were expressed in units of nmol of substrate and converted
137 per hour per gram of dry soil ($\text{nmol h}^{-1} \text{g}^{-1}$) (Yang et al., 2019).

138

139 2.6. DNA extraction, PCR amplification and Illumina MiSeq sequencing

140 Microbial DNA was extracted from 18 samples using the E.Z.N.A.[®] Soil DNA Kit (Omega Bio Inc.
141 Norcross, GA, USA) according to manufacturer's protocols. The final DNA concentration and
142 purification were determined by Nanodrop[®] ND-1000 UV-Vis spectrophotometer (Nano-Drop
143 Technologies, Wilmington, DE, USA), and DNA quality was checked by 1% agarose gel
144 electrophoresis. The bacterial 16S rDNA gene was amplified with the primers 338F_806R (Dennis et
145 al., 2013) by a thermocycler PCR system (GeneAmp 9700, ABI, USA). The PCR reactions were
146 conducted using the following program: 3 min of denaturation at 95°C, 35 cycles of 30 s at 95°C, 30 s
147 for annealing at 55°C, and 45 s for elongation at 72°C, and a final extension at 72°C for 10 min. PCR
148 reactions were performed in triplicate using a 20 μ L mixture containing 4 μ L of 5 \times FastPfu Buffer, 2
149 μ L of 2.5 mM dNTPs, 0.8 μ L of each primer (5 μ M), 0.4 μ L of FastPfu Polymerase and 10 ng of

150 template DNA.

151 The PCR products were extracted from a 2% agarose gel and further purified using the AxyPrep DNA
152 Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and quantified using
153 QuantiFluor™-ST (Promega, USA) according to the manufacturer's protocol. The purified amplicons
154 were pooled in equimolar and then paired-end sequenced (2×300) on an Illumina MiSeq platform
155 (Illumina, San Diego, USA) according to the standard protocols by Majorbio Bio-Pharm Technology
156 Co. Ltd. (Shanghai, China). The raw reads were deposited in the NCBI Sequence Read Archive (SRA)
157 database with accession number PRJNA633722.

158

159 2.7. Processing of sequencing data

160 Raw FASTQ files were demultiplexed and quality-filtered by Trimmomatic and merged by FLASH
161 with the following criteria: (1) The reads were truncated at any site receiving an average quality score
162 <20 over a 50 bp sliding window. (2) Primers were matched allowing two-nucleotide mismatching,
163 and reads containing ambiguous bases were removed. (3) Sequences whose overlap was longer than
164 10 bp were merged. Operational taxonomic units (OTUs) were clustered based on a 97% similarity
165 cutoff using UPARSE (version 7.1 <http://drive5.com/uparse/>), and chimeric sequences were identified
166 and removed using UCHIME (Edgar et al., 2011). The taxonomy of each 16S rDNA gene sequence
167 was analyzed by the RDP Classifier algorithm (<http://rdp.cme.msu.edu/>) against the Silva
168 132/16S_bacteria database using a confidence threshold of 70%. Alpha-diversity indices were
169 calculated by Mothur 1.30.1 to test differences in bacterial diversity among samples (Schloss et al.,
170 2009).

171

172 2.8. Statistical analysis

173 Alpha diversity metrics, including the Shannon-Wiener, Simpson, and Chao indices, were calculated
174 using the “diversity” and “richness” functions of the bacterial community. Repeated measures

175 ANOVAs were performed to test the effects of snow exclusion, sampling date (deep snow period,
176 thawing period, growing season), and their interactions on the bacterial indices. One-way ANOVA
177 was performed to test the effects of sampling date on bacterial community structure within the same
178 treatment. For individual sampling dates, Wilcoxon rank-sum test was used to compare the effects of
179 snow exclusion on bacterial community structure. Effects of snow exclusion on bacterial community
180 structure was further tested by Principal Coordinate Analysis (PCoA) using OTUs. Spearman
181 correlation analysis was used to assess the relationships between the relative abundance of bacterial
182 taxa and biochemical properties (i.e., soil physicochemical properties and enzyme activities). Both
183 PCoA and Spearman analyses were performed using the VEGAN package (Oksanen et al., 2013) in R
184 (R Development Core Team, 2015). Other statistical analyses were performed using SPSS 20.0 (IBM
185 Corporation, Armonk, NY, USA). The statistical tests were considered significant at the $P < 0.05$
186 level.

187

188

189 3. Results

190 3.1. Sequence data characteristics and bacterial community diversity

191 Across all soil samples, a total of 664576 high-quality sequences were identified. Each library had
192 18127 reads and 1512 OTUs were obtained in all. All rarefaction curves tended to approach the
193 saturation plateau, indicating that the data volumes of the sequenced reads were reasonable (Fig. S2).
194 There were significant differences in diversity (Shannon and Simpson) and richness (Chao) indices
195 among different sampling dates (Fig. 1). In the growing season, Shannon and Chao indices were
196 lower, while the Simpson index was higher, compared to the deep snow period and the thawing period.
197 Importantly, while the Shannon and Chao indices were unaffected by snow exclusion, we found a
198 significant interactive effect between snow exclusion and sampling date for the Simpson index with
199 positive effects of snow exclusion on bacterial diversity during the deep snow period and negative
200 effects during the thawing period and the growing season (Fig. 1b).

201

202 3.2. Taxonomic composition of bacteria

203 Sequences that could not be classified into any known group were assigned as unclassified and groups
204 with an average relative abundance of less than 1% were classified as 'others' (Fig. 2). At the phylum
205 level, the classified sequences were affiliated to 28 groups. Proteobacteria (29.21-48.60%),
206 Acidobacteria (19.50-25.59%), Actinobacteria (5.92-13.16%), and Chloroflexi (6.31-10.36%) were
207 the most dominant bacterial phyla. The relative abundances of the Bacteroidetes (0.91-5.72%),
208 Firmicutes (1.43-5.22%), Gemmatimonadetes (1.84-4.67%), Rokubacteria (1.27-6.68%), Nitrospirae
209 (0.86-4.36%), Verrucomicrobia (1.36-2.54%), Latescibacteria (0.81-1.89%), Planctomycetes
210 (0.74-1.29%), and Patescibacteria (0.31-1.00%) were relatively low across seasons (Fig. 2a).
211 Bacterial community composition varied among seasons (Fig. S3) but was largely unaffected by snow
212 exclusion (Fig. S5). For instance, regardless of snow manipulation treatment, the relative abundance
213 of Nitrospirae was significantly increased in the growing season, whereas the opposite was found for

214 Bacteroidetes. The relative abundance of Firmicutes was higher in the thawing period than in the
215 other two periods, while the opposite patterns was found for Gemmatimonadetes. The relative
216 abundance of Actinobacteria was lowest in the deep snow period (Fig. S3). However, no significant
217 differences were found in bacterial phyla between snow regimes for all sampling events (Fig. S5).

218

219 At the class level, 20 bacterial classes (>1%) were observed across seasons (Fig. 2b). There were also
220 significant seasonal dynamics in the classes of soil bacteria (Fig. S4), while snow exclusion had little
221 effect (Fig. S6). For example, across the snow manipulation treatments, Bacilli had higher relative
222 abundance in the thawing period than in the deep snow period and the growing season. However,
223 Deltaproteobacteria, Acidobacteriia, and Gemmatimonadetes showed opposite trends. For NC10 and
224 Nitrospira, the relative abundances were highest in the growing season whereas the relative
225 abundances of Bacteroidia and Alphaproteobacteria were lowest in the growing season. For
226 Gammaproteobacteria, the relative abundance was highest in the deep snow period whereas the
227 relative abundance of Actinobacteria was lowest in the deep snow period. Consistent with the phylum
228 level, no significant differences were detected between control and snow exclusion plots at the class
229 level (Fig. S6).

230

231 The bacterial composition was further analyzed with principal coordinate analysis (PCoA) at the OTU
232 level (Fig. 3). The first and second principle component axes together explained as much as 64.71%
233 of the variance in bacterial communities. The results of PCoA showed that soil bacterial communities
234 were different among the deep snow period, the early thawing period, and the growing season. In the
235 deep snow period, bacterial communities were clearly different between control and snow exclusion
236 plots, while for the other periods snow exclusion did not have any effects.

237

238 3.3. Relationships between bacterial communities and biochemical or environmental factors

239 Spearman correlation heatmap analysis was performed to explore the relationships between bacterial
240 communities and biochemical or environmental factors (Fig. 4). At the phylum level, soil moisture,
241 ammonium nitrogen, and activities of polyphenol oxidase, leucine aminopeptidase, cellobiohydrolase,
242 β -N-acetyl-glucosaminidase, acid phosphatase, and β -glucosidase were positively correlated with
243 Firmicutes, Verrucomicrobia, Bacteroidetes, Actinobacteria, and Patescibacteria but negatively
244 correlated with Chloroflexi, Rokubacteria, Latescibacteria, Gemmatimonadetes, Nitrospirae, and
245 Planctomycetes (Fig. 4a). However, microbial biomass C, pH, nitrate, temperature, and peroxidase
246 were negatively correlated with Firmicutes, Verrucomicrobia, and Bacteroidetes, but positively
247 correlated with Chloroflexi, Rokubacteria, Latescibacteria, Gemmatimonadetes, Nitrospirae, and
248 Planctomycetes (Fig. 4a). At the class level, Bacteroidia and Verrucomicrobiae were positively
249 correlated with ammonium, moisture, polyphenol oxidase, leucine aminopeptidase, acid phosphatase,
250 β -glucosidase, β -N-acetyl-glucosaminidase, and cellobiohydrolase, but negatively correlated with soil
251 temperature, nitrate, pH, microbial biomass C, and peroxidase (Fig. 4b). Conversely, Nitrospira,
252 Gemmatimonadetes, NC10, AD3, Latescibacteria, and Anaerolineae were negatively correlated with
253 ammonium, soil moisture, polyphenol oxidase, leucine aminopeptidase, acid phosphatase,
254 β -glucosidase, β -N-acetyl-glucosaminidase, and cellobiohydrolase, but positively correlated with soil
255 temperature, nitrate, pH, microbial biomass C, and peroxidase (Fig. 4b). In addition, some classes
256 belonging to the Proteobacteria (i.e., Alphaproteobacteria and Gammaproteobacteria) showed
257 significant negative correlations with soil temperature and peroxidase, while the opposite was found
258 for Deltaproteobacteria (Fig. 4b). Among the phylum of Firmicutes, the class of Clostridia was
259 negatively correlated with soil temperature and peroxidase, while Bacilli was positively correlated
260 with soil temperature (Fig. 4b).

261

262

263 4. Discussion

264 Across treatments and seasons, the predominant bacterial phyla in our experimental site in a Tibetan
265 spruce forest were Proteobacteria, Acidobacteria, and Actinobacteria, which is consistent with
266 observations in the Swiss and Australian Alps (Wunderlin et al., 2016) and the Qinghai-Tibetan
267 Plateau (Wu et al., 2017). Alphaproteobacteria and Gammaproteobacteria were the most dominant
268 classes of Proteobacteria, which agrees well with observations in shrub-dominated tundra heaths of
269 northern Finland (Männistö et al., 2018) and subarctic tundra in Alaska (Kim et al., 2014). These
270 findings together indicate that soil bacteria share common dominant higher taxa in high-altitude and
271 high-latitude ecosystems. Previous studies have reported that Chloroflexi, Bacteroidetes, Firmicutes,
272 Gemmatimonadetes, Nitrospirae, Verrucomicrobia, and Planctomycetes are often found in
273 snow-covered ecosystems (Lipson, 2007; Lipson and Schmidt, 2004; Männistö et al., 2013; Ricketts
274 et al., 2016; Wunderlin et al., 2016). However, some bacterial phyla found in our experimental site,
275 such as Rokubacteria, Latescibacteria, and Patescibacteria, has rarely been reported in other snowy
276 soils (Ricketts et al., 2016). This may be partially attributable to technological differences among
277 studies (e.g., DNA extraction, primer specificities, and taxonomic classification of downstream
278 analysis). In addition, it is important to note that the detected Chloroflexi is not a dominant phylum in
279 alpine ecosystems, but its active role in biogeochemical cycles of cold soils has been long established
280 (Costello and Schmidt, 2006; Zhang et al., 2014a).

281

282 Contrary to our hypothesis, snow exclusion had weak effects on bacterial diversity and snow
283 exclusion only affected community composition in wintertime. This is inconsistent with previous

284 findings in tundra and temperate deciduous forest (Aanderud et al., 2013; Ricketts et al., 2016). The
285 following reasons may account for the differences found. Firstly, the magnitude of soil freezing
286 caused by snow exclusion in our study is smaller than those reported in temperate and arctic
287 ecosystems (Aanderud et al., 2013; Ricketts et al., 2016). This could be due to differences winter
288 conditions between low-latitude alpine ecosystems and high-latitude boreal ecosystems (e.g., snow
289 density, depth, and albedo). Short-term mild frost, therefore, may not be severe enough to cause
290 lasting changes in the diversity and composition of soil bacterial communities. In addition, most of
291 the dominant bacterial taxa in frozen soils may have strong adaptive capabilities and resistance,
292 thereby maintaining the stability of community structure via diverse ecological strategies (Männistö
293 et al., 2018; Ricketts et al., 2016). For example, some specific traits (e.g., mycelium structures and
294 sporing formations) of Actinobacteria help them to survive in the extreme cold and low-nutrient
295 alpine conditions (Embley and Stackebrandt, 1994; Zhang et al., 2016). Further, high substrate
296 affinities and extracellular enzyme production favor the effective establishment of defense structure
297 of Alphaproteobacteria to resist extreme conditions, such as soil freezing (Fontaine et al., 2003). On
298 the other hand, the measured biotic and abiotic soil properties did not differ between snow regimes in
299 the following growing season (Yang et al., 2019). This can explain why we did not find legacy effects
300 of snow exclusion on soil bacterial communities.

301

302 The diversity of soil bacterial community varied with seasons largely independent of snow regimes.
303 Both the diversity indices of Shannon and Chao were lower but Simpson index was higher in the
304 growing season than in the winter season, indicating a detectable decrease in bacterial diversity and
305 richness from winter to growing season. The relatively high diversity in the winter may be because of
306 bacterial adaptation to and survival in frozen conditions. Winter bacterial communities may mainly
307 utilize complex and recalcitrant substrates (i.e., cellulose and salicylate), to resist the frost stress. Thus,
308 different functional responses to environmental stress may result in the separation of bacterial

309 communities (Schimel et al., 2007). Low-resource winter conditions may favor oligotrophic
310 K-strategists, such as Alphaproteobacteria and Acidobacteria (Fierer et al., 2007; Lauro et al., 2009).
311 These members usually have high functional diversity to break down complex C substrates (Lladó et
312 al., 2016; Männistö et al., 2013). In contrast, rich-resource growing season conditions, may favor
313 copiotrophic r-strategists, such as Gammaproteobacteria and Actinobacteria. These R-strategists
314 reproduce fast, thereby gaining a quantitative advantage and suppressing other groups, which in turn
315 leads to a decrease in diversity (Fierer et al., 2007).

316

317 In general, different bacterial populations have different preference for specific conditions, which can
318 induce the shifts in community structure among seasons (McMahon et al., 2011). Previous studies
319 have shown that the relative abundances of some specific groups of bacteria (e.g., Actinobacteria and
320 Bacteroidetes) differed among seasons in snowy ecosystems (Lipson, 2007; Lipson and Schmidt,
321 2004; Männistö et al., 2018; Zinger et al., 2009). Some studies have found that season-related
322 changes in environmental variables profoundly affected microbial communities, in contrast to the
323 transient and mild influences of change in snow cover (Lipson and Schmidt, 2004; Männistö et al.,
324 2018). In our study, we found that shifts in bacterial community composition across seasons. Several
325 possible mechanisms may account for the phenomenon.

326

327 Firstly, bacterial community composition is often driven by the thermal adaptability of the total
328 community in a specific season (Aanderud et al., 2013; Lipson, 2007). Winter communities may be
329 driven by physiological stress associated with freeze-thaw cycles, which could select for
330 frost-resistant taxonomic units (Sharma et al., 2006). In our study, Bacteroidetes, Alphaproteobacteria,
331 and Gammaproteobacteria had negative correlations with soil temperature. Although mineralization
332 rate were low in winter due to low temperature, some bacteria can still survive by utilizing
333 recalcitrant substrates (Zhang et al., 2014b; Straza et al., 2010; Tada et al., 2013). Secondly, soil

334 moisture is also an important factor affecting bacterial communities. Microbial communities may
335 respond to changes in soil moisture through their capability of inherent acclimation for the reasonable
336 allocation of limited resources (Schimel et al., 2007). In our study, Nitrospirae, Gemmatimonadetes,
337 and Latescibacteria were negatively correlated with soil moisture. It has previously been
338 demonstrated that Gemmatimonadetes can survive at low humidity conditions (DeBruyn et al., 2011).
339 Besides, since substrate diffusion mainly relies on the presence of a water film, the access of bacteria
340 to nutrients may increase during the (warm) growing season. As a result, bacterial taxa grow and
341 reproduce rapidly in warm and humid conditions (Stark and Firestone, 1995). On the other hand,
342 intense hydrological activity during the early thawing period can cause considerable leaching losses
343 of nutrients (Edwards et al., 2007), which may further shift microbial composition due to nutrient
344 competition (Walker et al., 1999).

345
346 Additionally, biochemical variables can further mediate soil bacterial communities. Firstly, some
347 biogeochemical reactions catalyzed by microbial enzymes may be closely related certain bacterial
348 taxa (Langenheder et al., 2006). For example, it is well known that some members of Bacteroidetes
349 secrete enzymes (e.g. acid phosphatase) to decompose complex organic matter, such as chitin,
350 cellulose, and other high-molecular organic compounds (Wolińska et al., 2017). Likewise, some
351 cellulose-degrading bacteria phylotypes affiliated to Firmicutes, such as the classes of Bacilli and
352 Clostridia (Rastogi et al., 2009), may be positively correlated with some hydrolytic enzymes (e.g.,
353 β -glucosidase, β -N-acetyl-glucosaminidase, and acid phosphatase) (Menon et al., 2013). Similar
354 findings were also revealed in our study, suggesting that the production of soil enzymes can partially
355 reflect seasonal changes in the diversity and composition of bacterial communities. Secondly, nutrient
356 availability is one of most the important factors that regulate soil bacterial communities (Gavazov et
357 al., 2017). For example, soil bacterial community diversity is largely affected by ammonium
358 availability in some forest ecosystems (Zeng et al., 2016). Previous studies have suggested that

359 ammonium concentration in soil is usually high in the thawing period due to increased nitrate
360 leaching (Li et al., 2017). Thus, higher ammonium availability may partly account for richer bacterial
361 communities in the early thawing period. Lastly, it has been well recognized that pH is a key driver of
362 soil bacterial community composition (Lauber et al., 2009; Ricketts et al., 2016). In cold regions, soil
363 thawing-induced rapid changes in nitrogen availability can significantly influence soil pH during the
364 early thawing period (Edwards et al., 2007). In our study, some dominant bacterial phyla (e.g.,
365 Gemmatimonadetes, and Nitrospirea) showed strong dependence on soil pH. This result is consistent
366 with observations in other cold snowy ecosystems (DeBruyn et al., 2011; Lauber et al., 2009).

367

368

369 **5. Conclusions**

370 We examined the immediate and legacy effects of a short-term change in snow cover on the diversity
371 and composition of bacterial communities in an alpine spruce forest on the Tibetan Plateau of China.
372 Our results showed that snow exclusion had some effects on soil bacterial communities in
373 snow-covered winter season, but not during the thawing period and the following growing season.
374 This suggests that soil bacterial communities in Tibetan forests are resilient to the short-term change
375 of snow cover. In addition, soil bacterial communities strongly varied with seasons, showing a
376 significant shift from winter to the growing season. Season-related changes in environmental factors
377 (e.g., temperature and moisture) and biochemical variables (e.g., soil N availability and enzyme
378 activities) accounted to some extent for the seasonal variation in bacterial communities. Considering
379 the already-observed strong winter warming in the Tibetan region, the ecological responses of soil
380 microorganisms to winter climate change require long-term experimental studies.

381

382 **Declaration of Competing Interest**

383 The authors declare that they have no competing financial interests or personal relationships that
384 could have influenced the work reported in this paper.

385

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574

575 **Figure legends**

576

577 Fig. 1. Shannon diversity index(a), Simpson index (b) and Chao index(c) of soil bacterial community
578 in control (C) and snow exclusion (SE) plots in the deep snow period (DSP), early thawing period
579 (ETP), and in the middle of the growing season (MGS). Data shown are mean \pm s.e.

580

581 Fig. 2. Taxonomic profiles of bacterial community composition at the phylum level (a) and the class
582 level (b) in control (C) and snow exclusion (SE) plots in the deep snow period (DSP), early thawing
583 period (ETP), and in the middle of the growing season (MGS). Shown are group accounting for >1%
584 of the relative abundance, while groups accounting for <1% are integrated into 'others'.

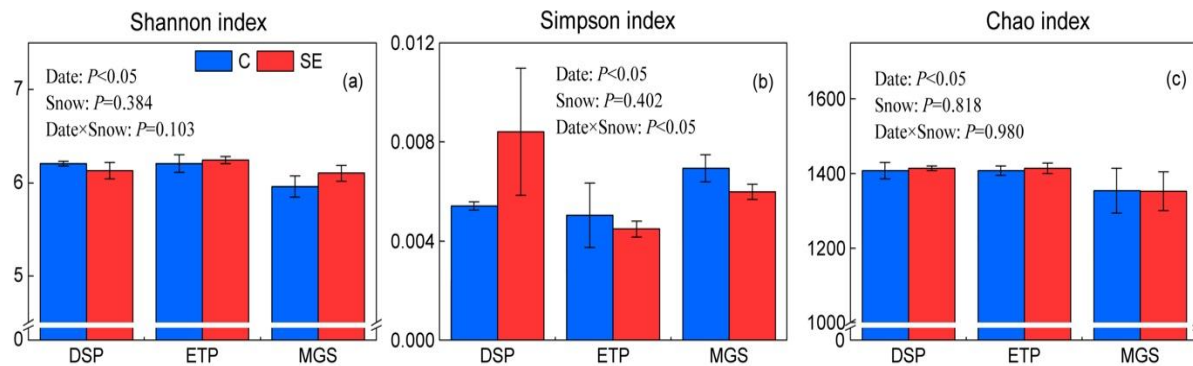
585

586 Fig. 3. Principal Coordinate Analysis (PCoA) of bacterial community composition at the OTU level
587 in control (C) and snow exclusion (SE) plots in the deep snow period (DSP), early thawing period
588 (ETP), and in the middle of the growing season (MGS).

589

590 Fig. 4. Correlation heatmap of environment factors, biochemical properties and bacterial gene read
591 numbers at the phylum (a) and class (b) levels. Tm: temperature, MBC: microbial biomass carbon,
592 BG: β -glucosidase, CBH: cellobiohydrolase, PPO: polyphenol oxidase, POD: peroxidase, NAG:
593 β -N-acetyl-glucosaminidase, LAP: leucine aminopeptidase, AP: acid phosphatase. The color intensity
594 in each panel indicates the relative correlation between soil properties and read numbers of each
595 group. * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$.

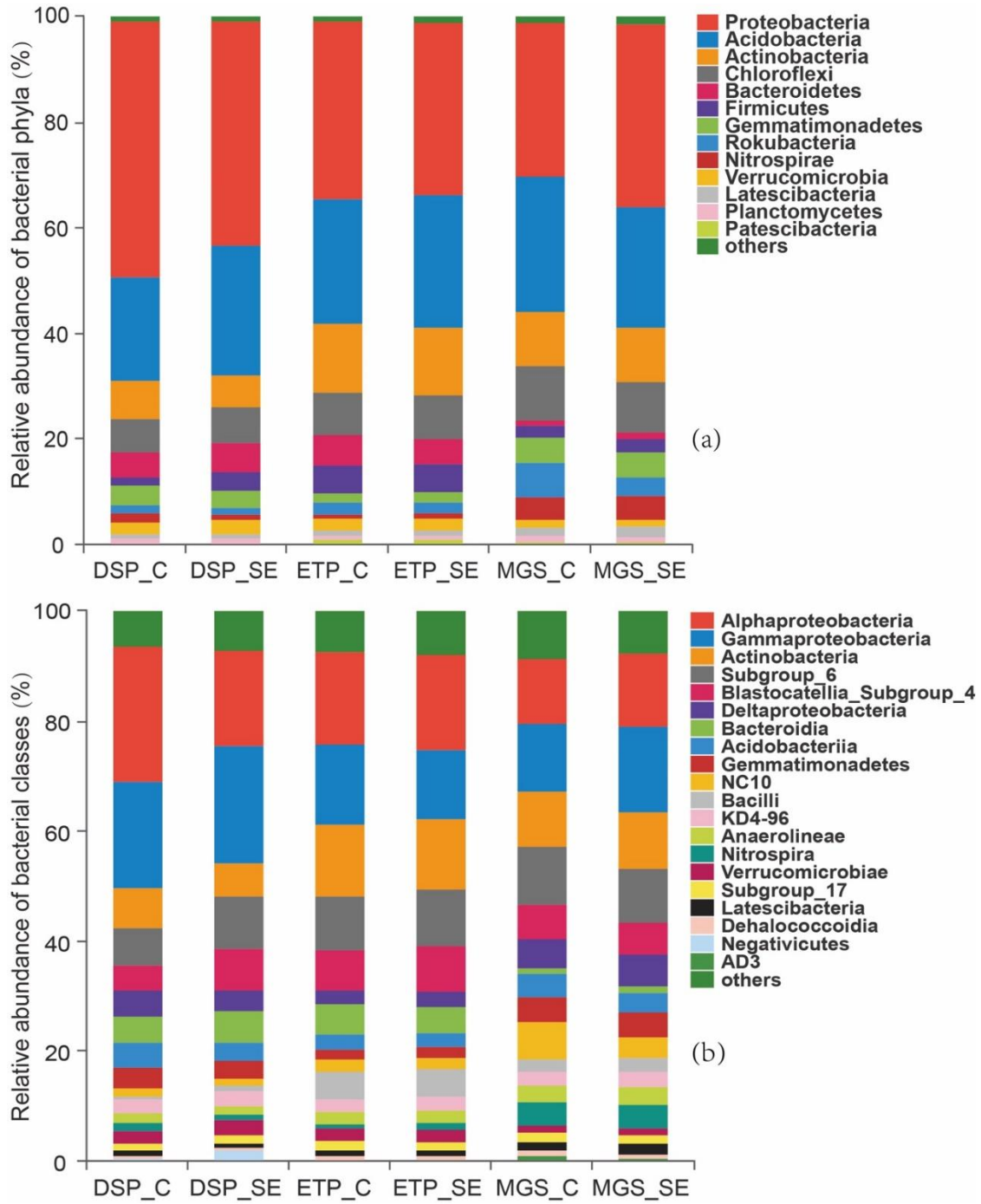
596 **Figure 1**



597

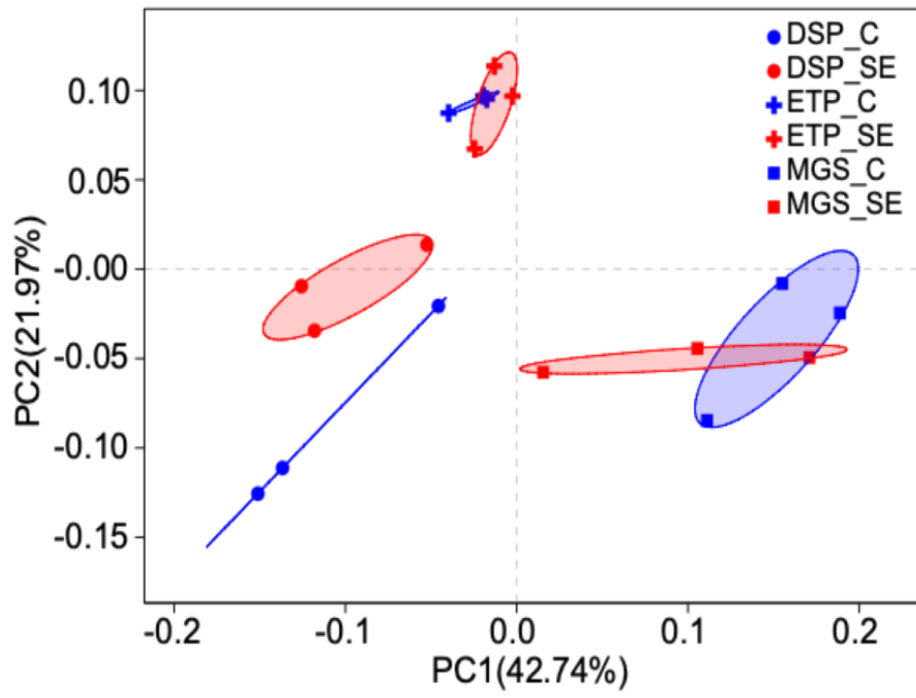
598 **Figure 2**

599



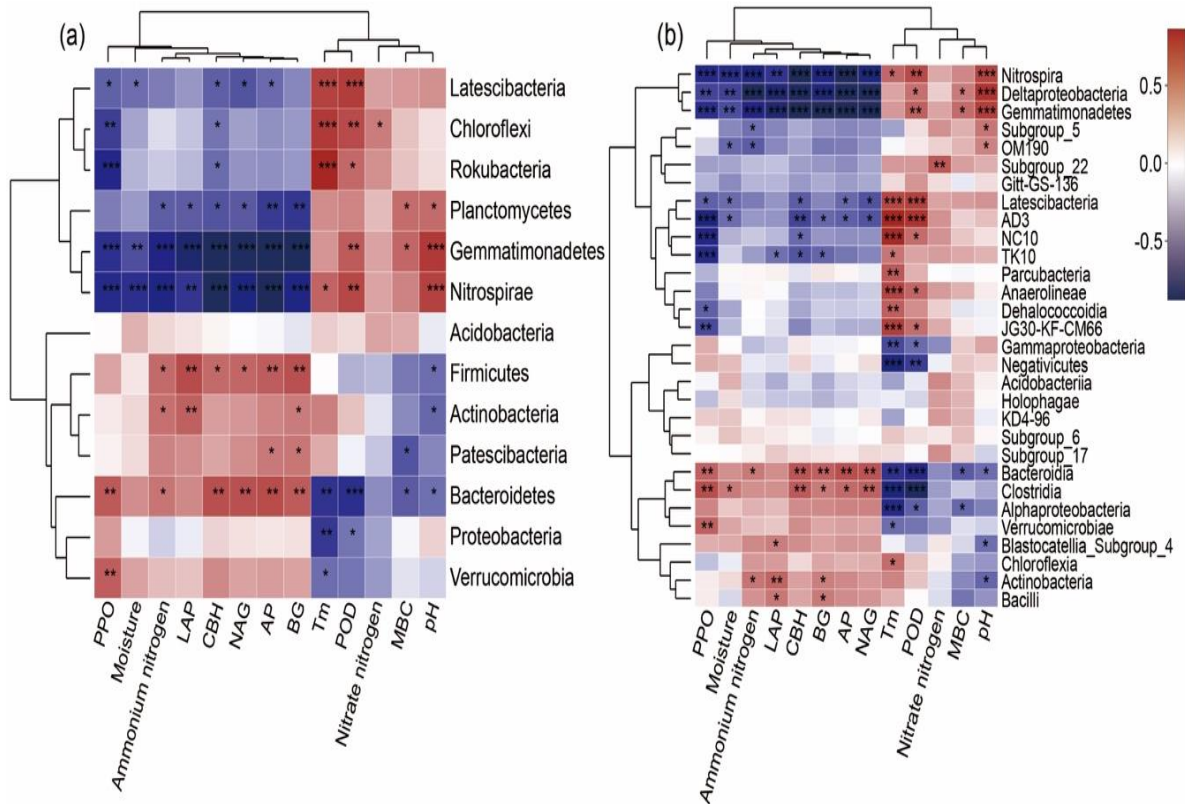
600 **Figure 3**

601



602 **Figure 4**

603



604