

Soil Biological Community and Chemical Characteristics of Evergreen and Deciduous Shrub Islands in an Alpine Shrub Meadow

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1 **Title page**

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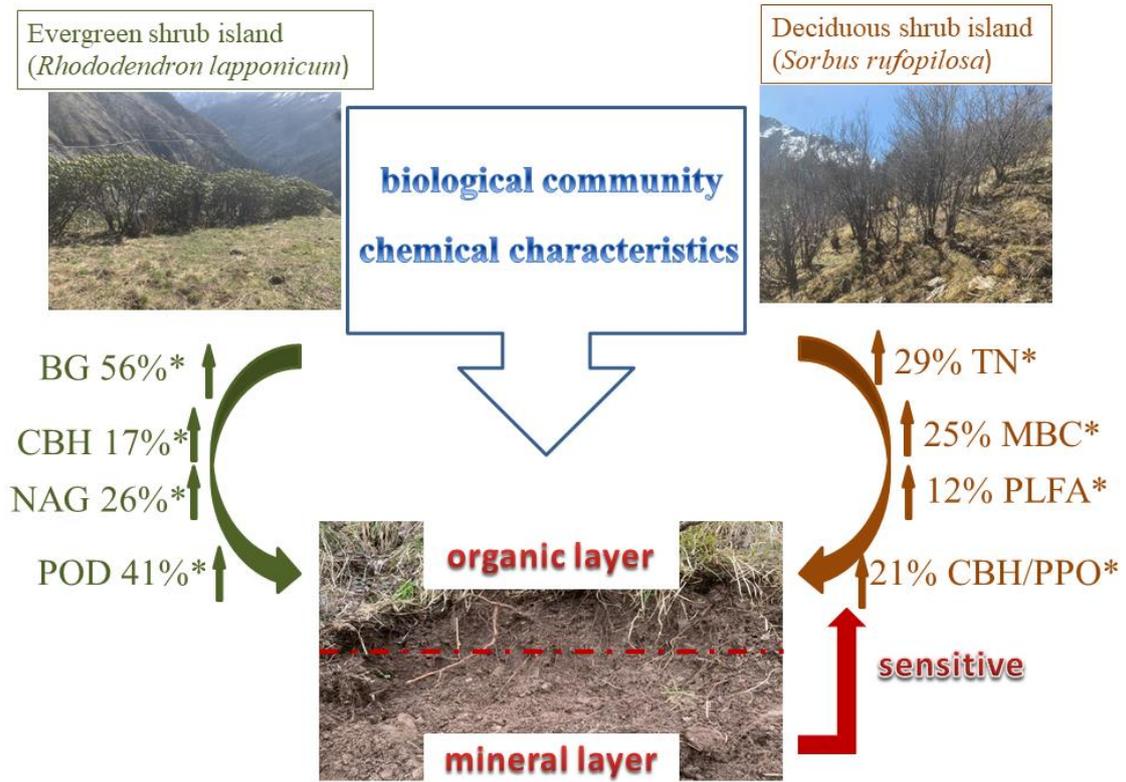
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17

18 **Graphical abstract**



20 **Abstract**

21 *Aim* Global change is driving shrub expansion in alpine and Arctic tundra, increasing
22 the size and number of shrub islands. This phenomenon can directly affect the
23 vegetation microclimate and indirectly affect biogeochemical cycling. However, it is
24 unclear how shrub island formation, with a shift in dominant vegetation from
25 graminoids to deciduous or evergreen shrub islands, affects the alpine biochemical
26 cycling of carbon, nitrogen, phosphorus and decomposer diversity and activity.

27 *Methods* Two dominant shrub species in the alpine shrub meadow in the eastern
28 Tibetan Plateau were selected. *Rhododendron lapponicum* (RL) represents evergreen
29 shrubs, and *Sorbus rufopilosa* (SR) represents deciduous shrubs. Soil fauna
30 community diversity, soil microbial biomass, extracellular enzyme activity and
31 enzyme stoichiometry of the organic soil layer (OL) and mineral soil layer (ML) were
32 determined.

33 *Results* Presence of shrub islands significantly affected nutrient cycling and microbial
34 processes. Compared with the SR shrub island, the RL shrub island featured lower
35 soil total nitrogen and microbial biomass carbon; lower total microbial phospholipid
36 fatty acid, Gram-negative bacteria and total bacteria contents; and higher enzyme
37 activities of β -glucosidase, cellobiohydrolase, β -N-acetylglucosaminidase, peroxidase
38 and polyphenol oxidase. The OL response was greater than the ML response,
39 especially in terms of enzyme activities.

40 *Conclusion* Evergreen and deciduous shrub islands in an alpine shrub meadow had
41 differences in soil biological communities and nutrient cycling, we suspect the OL
42 was more sensitive than the ML to the shrub expansion in the alpine tundra.

43 **Keywords:** shrub island; alpine shrub meadow; evergreen shrub; deciduous shrub;
44 Soil biological community; enzyme activity

45

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55 **Authors' contributions:**

56 **Moyu Sha:** Formal analysis, Investigation, Data Curation, Writing - Original Draft,
57 Writing - Review & Editing, Visualization

58 **Huiqin Jin:** Formal analysis, Investigation, Data Curation

59 **Lifeng Wang:** Conceptualization, Methodology, Formal analysis, Investigation, Data
60 Curation, Writing - Review & Editing, Supervision

61 **Yu Zhou:** Formal analysis, Investigation, Data Curation

62 **Chengming You:** Resources

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70 administration, Funding acquisition

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77 **Introduction**

78 Global climate change is reshaping the distribution of many terrestrial organisms and
79 ecosystems, especially at high latitudes and high elevations (IPCC 2014; Dial et al.,
80 2016; Hagedorn et al., 2019). The upward invasion and area expansion of shrubs are
81 the two most obvious effects of global climate change (Myers-Smith et al., 2018;
82 Parker et al., 2021). The increase in temperature and subsequent input of more
83 nutrients may be driving the increase in shrub islands at high elevations (Aguirre et al.,
84 2021). Shrub islands are composed primarily of deciduous species, such as *Betula*
85 *pubescens* and *Sorbus rufopilosa* (Jump et al., 2001; Wardle et al., 2012), but an
86 increasing number of recent studies have reported that shrub islands composed of
87 evergreen species, such as *Rhododendron lapponicum*, are also expanding (Zamin et
88 al., 2014; Vowles et al., 2019). Shrub islands feature dwarfed growth and island-like
89 shrub flora distributed in meadow or tundra matrices (Benedict et al., 1984; Seastedt
90 et al., 2001; Wang et al., 2007). This phenomenon can directly or indirectly affect the
91 function of soil ecosystems (Blok et al., 2010; Ward et al., 2018; Aguirre et al., 2021).
92 Understanding the potential effects of these shrub islands on the soil biological
93 community and chemical characteristics is essential for predicting future alpine shrub
94 meadow functions.

95 The shrub island effect is widely recognized in many ecosystems, and this
96 phenomenon can directly affect the soil microclimate, soil nutrients and decomposers
97 (Blok et al., 2010; Ward et al., 2018). In winter, taller and richer shrubs can
98 accumulate deeper snow than less shrubby tundra areas, and snow, as an insulator, can
99 increase the soil temperature (i.e., the “snow-shrub hypothesis”, Sturm et al., 2001). In
100 summer, the leaves of shrubs form a sheltered area that reduces the soil temperature
101 (Myers-Smith et al., 2013). Shrubs affect nutrients because of the large amount of
102 aboveground biomass and high nutrient requirements, driving the direct absorption of
103 nutrients and increasing the flow of nutrients from the soil. Organic carbon (TC), total
104 nitrogen (TN) and total phosphorus (TP), available nitrogen and available phosphorus
105 in shrub islands have been shown to be significantly higher than those in a control site
106 (Zhou et al., 2019; Parker et al., 2021). Shrub islands can affect the soil characteristics

107 (such as soil moisture, pH or light) and heterogeneity of the soil (Darrouzet-Nardi et
108 al., 2006; Kappes et al., 2007; Wang et al., 2011) and affect the decomposition process
109 (Moore et al., 2004). Shrubs increase the quantity of litter, providing more available
110 carbon and other resources for the soil biological community than tundra, resulting in
111 increased decomposer abundance and activity (Prescott & Zekker, 2016; Jennie et
112 al., 2014). Shrub islands have been shown to increase bacterial biomass by 16% and
113 microbial abundance by 89% via the litter pathway (Zhang et al., 2018). Mixed litter
114 under shrub islands also affects microbial biomass and enzyme activity by altering
115 substrate quality and substrate availability (Burns et al., 2002; McLaren et al., 2017).
116 In the summer, shrub islands have been directly linked to increased microbial biomass
117 (Buckeridge et al., 2010). Since the presence of shrubs increases nitrogen and thus
118 inhibits carbon-related enzymes, it is also possible that microorganisms do not need to
119 invest a large amount of energy in oxidized lignin and other refractory organics, and
120 the activities of enzymes related to nitrogen and phosphorus release under shrub
121 islands are significantly higher than those under control treatments, with inconsistent
122 effects on carbon-decomposing enzymes (Jian et al., 2016).

123 Similarly, the soil biological community is one of the key factors affecting the soil
124 ecology of shrub islands (Wagg et al., 2014; Jing et al., 2015). Underground fauna and
125 microbial communities release nutrients from organic matter or dead organisms
126 (Burns et al., 1982; Bonkowski et al., 2004) and can affect shrub island growth and
127 nutrient cycling through symbiosis (Clay et al., 1999; Redman et al., 2002) and direct
128 uptake (Bardgett et al., 2014). Since vegetation is the source of microbial uptake, the
129 microbial community structure reflects differences in vegetation types. For example,
130 bacteria prefer moderate hospitable environments and easy-to-decompose organic
131 matter, whereas fungi can tolerate broader environmental conditions and increase in
132 abundance with increasing refractory organic matter (Lundquist et al., 1999; Treseder
133 et al., 2015). Deciduous shrub litter easily decomposes, while evergreen shrub litter is
134 difficult to decompose (Vowles et al., 2019). For example, *Sorbus rufopilosa* (SR), a
135 deciduous shrub, is easily decomposed due to the high quality of the litter (Demarco
136 et al., 2014). However, *Rhododendron lapponicum* (RL) is an evergreen shrub with a

137 poor litter quality (low N and P contents and high lignin content), and the tannin and
138 phenol substances in the litter hinder decomposition (Zheng et al., 2020).

139 The type and functional composition of vegetation have an important feedback effect
140 on soil characteristics (Seastedt et al., 2001). The vegetation type with different
141 biological characteristics determines the ecosystem functional mechanism in the
142 region where it is located. Wardle et al. (2004) defined living space as two ecosystems
143 according to the biological characteristics of vegetation: fertile and productive
144 ecosystems are formed through leaky nutrient cycling and limited net accumulation of
145 soil carbon; infertile and unproductive ecosystems are formed through slow nutrient
146 cycling, nutrient conservation and greater carbon sequestration. The different
147 biological characteristics of shrubs determine the different ecological characteristics.
148 In recent studies on alpine shrub expansion, deciduous shrubs have received much
149 attention due to their sensitivity to temperature changes, high growth speed, high
150 specific leaf area and high leaf nitrogen content, and easy decomposition (Chapin et
151 al., 1980; Demarco et al., 2014). However, the utilization of nutrients by evergreen
152 shrubs is relatively conservative, the relative growth rate is low, the litter quality is
153 poor (lower leaf N and P contents and higher lignin contents), and the production of
154 tannins and phenols makes the litter difficult to decompose; consequently, there is a
155 lack of research on the ecological effects of evergreen shrubs (Zamin et al., 2014;
156 Vowles et al., 2019). Our team found a large number of evergreen RL shrub islands
157 and deciduous SR shrub islands in a preliminary plant diversity survey of an alpine
158 shrub meadow region (Liu et al., 2010; 2019). These findings are consistent with
159 recent findings demonstrating that evergreen shrubs are rapidly expanding (Adamczyk
160 et al., 2016; Vowles et al., 2019). Evergreen shrub islands have also become an
161 important part of climate change, and research on shrub islands will play a vital role
162 in predicting ecosystem functions of alpine shrub meadow environments in the future.
163 In this study, we propose the following scientific questions: What are the impacts of
164 different shrub islands on soil biological community biomass and diversity and on C,
165 N, and P cycling in alpine shrub meadow areas? What are the differences between
166 organic and mineral layers in the soil? Therefore, we investigated the organic and

167 mineral soil layers in dominantly evergreen and deciduous shrub islands
168 (*Rhododendron lapponicum* and *Sorbus rufopilosa*, respectively) in an alpine shrub
169 meadow.

170 **Materials and methods**

171 **Study site**

172 This study was carried out above the Long-term Research Station of Alpine Forest
173 Ecosystems (31°51'428" N, 102°41'230" E), in an alpine shrub meadow region on
174 Zhegu Mountain, Miyaluo Nature Reserve in Li County, Sichuan Province,
175 southwestern China. Moving from the coniferous forest to the alpine shrub meadow,
176 the light intensity increases gradually, the evaporation capacity increases gradually,
177 and the thicknesses of the litter layer and ground cover decrease gradually. This region
178 includes typical alpine shrub islands formed under natural conditions, which can
179 respond sensitively to the aboveground and underground ecological processes
180 influenced by climate change. The region has a typical alpine climate (Zhou et al.,
181 2020; Wang et al., 2021). The woody plants of this alpine shrub meadow include
182 *Rhododendron lapponicum*, *Sorbus rufopilosa*, *Sibiraea angustata*, *Dosiphora*
183 *fruticosa*, and *Salix cupularis*. The dominant herbal species include *Festuca*
184 *wallichanica*, *Saussurea woodiana*, *Polygonum sphaerostachum*, *Pedicularis roylei*,
185 and *Chamaenerion angustifolium* (Liu et al., 2010). Based on the United States
186 Department of Agriculture Soil Taxonomy, the soils in the alpine shrub land are
187 classified as Cryumbrepts, and the soils in the alpine meadows are Histosols (Zheng et
188 al., 2019). The experimental plot is located in an alpine shrub meadow area with
189 elevations ranging from 4150 to 4200 m (31°51'27" N, 102°41'12" E), a slope of 28, a
190 slope oriented to the southeast, and two types of shrub islands dominated by RL and
191 SR.

192 **Experimental design and sample collection**

193 Two dominant shrubs, RL and SR, were selected as the focus of this research on the
194 alpine shrub meadow. RL is an evergreen broad-leaved shrub with a height of 1.2-2.1
195 m. Its leaves are often scattered on the top of the branches; are dark grey green to
196 green, leathery, long, round, and oval; and have a specific leaf area of 49.87 cm² g⁻¹.

197 This plant has a relatively conservative nutrient utilization and low relative growth
198 rate and the following nutrient compositions: leaf N 8.3 g kg⁻¹, leaf P 1.03 g kg⁻¹,
199 cellulose 191.8 g kg⁻¹, and lignin 353 g kg⁻¹. The organic layer is 4-5 cm. SR is a
200 deciduous shrub with a height of 2.7-4 m. It has odd pinnate compound leaves that are
201 oval or oblong, 10-20 mm long and 5-7 mm wide. This plant has a high growth rate
202 and the following nutrient compositions: leaf N 17.0 g kg⁻¹, leaf P 2.17 g kg⁻¹,
203 cellulose 111.6 g kg⁻¹, and lignin 316 g kg⁻¹. The organic layer is 2-3 cm. The organic
204 layer in meadow areas is approximately 1 cm thick (Liu et al., 2010; Zheng et al.,
205 2020; Vowles et al., 2019).

206 The effects of island size (Peay et al., 2007), isolation (Peay et al., 2010), and
207 elevation (Jump et al., 2012) on the soil biological community in shrub islands should
208 be considered. We selected three sample plots of uniform size (5 × 5 m) for each
209 shrub type at the same elevation. Similar to the isolation distance of the shrub
210 ecosystem, six sampling points were set up in each sampling area to study soil fauna
211 and microorganisms. Tundra soil that was not covered by shrubs and was
212 approximately 10 m away from a shrub island was selected as a control. Samples of
213 the organic layer and mineral soil layer were taken from each sampling point, and a
214 total of 108 samples (3 types × 3 sample plots × 6 sampling points × 2 soil layers)
215 were collected.

216 We collected soil samples from the organic and mineral soil layers under the three
217 types of cover. For the soil nutrient and microbial analyses, the organic soil layer was
218 collected as intact 20 × 20 cm blocks to the depth of the mineral soil, and the
219 underlying mineral soil was sampled to 10 cm depth in the same locations using a
220 steel auger (2.5 cm diameter). The samples were collected, stored in a 4 °C cooler and
221 shipped to the laboratory immediately (Zheng et al., 2019). For the soil fauna
222 community, samples of the organic and mineral soil layers with a 5 cm radius and
223 volume 100 cm³ were collected at each sampling point with a cutting ring and then
224 stored in a sealed and ventilated black sack at low temperature and transported to the
225 laboratory immediately (Wu et al., 2014).

226 **Soil chemical analyses**

227 All of the samples from the RL and SR shrub islands in each transect were
228 characterized in terms of physicochemical properties. The samples were dried in an
229 oven at 105°C for 48 h and weighed to calculate the measured soil moisture content.
230 The pH of the mineral layer and organic layer was measured by using ratios of soil to
231 deionized water of 1:2.5 and 1:5 (g/v). soil total C was determined by dichromate
232 oxidation-ferrous sulfate titration, total N content was determined using the
233 semi-micro-Kjeldahl method, and the total P content was determined by
234 molybdenum-blue colorimetric method (Wang et al., 2021).

235 **Soil fauna community structure**

236 The residual soil debris on the black sack was removed, and the soil in the sack was
237 placed in a modified Tullgren dry funnel to separate and extract small and
238 medium-sized soil fauna for a separation period of 48 h. The fauna were classified and
239 counted using a pose dissector and a biological microscope with reference to Retrieval
240 Illustration of Soil Animals in China (Yi et al., 1998; Liu et al, 2019) for identification
241 to the family level.

242 **Phospholipid fatty acid analysis**

243 We investigated the soil phospholipid fatty acid (PLFA) composition to examine
244 changes in the microbial biomass in the soil in the three cover types and as an index of
245 the viability in the microbial community structure. A 1 g fresh subsample was
246 extracted with chloroform, methanol, and potassium phosphate buffer (1:2:0.8).
247 Phospholipids in the concentrated solution were sequentially eluted with organic
248 solvents with increasing polarity on a silica gel column and then saponified and
249 methylated to form fatty acid methyl ester (Bossio & Scow, 1998). The peak areas
250 were converted to nanomoles per gram of dry soil (nmol g^{-1} DM) using an
251 internal standard (19:0 methyl nonanoate). The following PLFAs were used as
252 markers for a specific group: total bacteria (TB): i15:0, a15:0, 16:1 ω 7c, i17:0, a17:0,
253 cy17:0 and cy19:0; total fungi (TF): 18:1 ω 9c and 18:2 ω 6c; Gram-positive (G^+)
254 bacteria: i15:0, a15:0, i17:0 and a17:0; and Gram-negative (G^-) bacteria: 16:1 ω 7c,
255 cy17:0 and cy19:0:0. The sum of all PLFAs and nonspecific PLFAs (15:0, 16:0, 16:1 ω

256 5T, 17:0, and 18:0) was used to determine microbial community composition and
257 indicate microbial biomass and to calculate the fungal to bacterial (F:B) and G⁻
258 bacteria to G⁺ bacteria (G⁻:G⁺) ratios (Zelles et al., 1999).

259 **Microbial biomass carbon and nitrogen**

260 Microbial C and microbial N were estimated by chloroform fumigation–extraction
261 (Ran & Liu, 2009). The microbial biomass in the soil was determined according to the
262 difference between fumigated and non-fumigated samples after extraction with 0.5
263 mol L⁻¹ K₂SO₄. After shaking for 20 min, the extract was filtered, and microbial
264 biomass carbon (MBC) and microbial biomass nitrogen (MBN) were determined by
265 K₂Cr₂O₇ oxidation-FeSO₄ titration and the semi-micro Kjeldahl N method,
266 respectively. The conversion factors for MBC and MBN were $k_{MBC} = 0.38$ (Vance et
267 al., 1987) and $k_{MBN} = 0.45$ (Tan et al., 2014).

268 **Enzyme extraction and assays**

269 We determined the activities of six extracellular enzymes associated with carbon,
270 nitrogen, and phosphorus nutrient cycling in soil using a modified measurement
271 technique (Allison et al., 2004; Li et al., 2020). The absorbance values of hydrolase
272 acid phosphatase (AP), β -glucosidase (BG), cellobiohydrolase (CBH),
273 β -N-acetylglucosaminidase (NAG), leucine aminopeptidase (LAP), peroxidase (POD)
274 and polyphenol oxidase (PPO) were measured by a microspectrophotometer. Two
275 grams of wet soil was weighed and dissolved in 60 ml of acetic acid buffer and mixed
276 through stirring for 1 min to obtain a crude enzyme solution. The different substrates
277 were dissolved in 50 ml of acetic acid buffer. Then, 150 μ l of substrate and 50 μ l of
278 crude enzyme solution were mixed on the ELISA plate. A control site (crude enzyme
279 + buffer) and a test group (crude enzyme + substrate) were set for each sample. For
280 POD, 3% H₂O₂ was added to the substrate control group and sample group. The
281 incubation times were as follows: AP 45 min, POD 1~2 h, NAG 3 h, CBH 4 h, BG 1 h,
282 and LAP 4~6 h. After incubation, 5 μ l of 1 M sodium hydroxide was added to each
283 well to stop the reaction. AP, BG, NAG, CBH, and LAP were determined at 405 nm.
284 The POD was determined at 450 nm. Activity is expressed as the micromoles of
285 hydrolysed substrate per hour per gram of dry organic matter (μ mol h⁻¹ g DOM⁻¹).

286 **Statistical analysis**

287 The Margalef richness index, Simpson dominance index, Shannon-Wiener diversity
288 index, and Pielou evenness index were calculated based on the species and number of
289 soil fauna individuals observed in the experiment (Tan et al., 2012):

290 (1) Margalef richness index = $(S - 1)/\ln N$

291 (2) Simpson dominance index = $1 - \sum P_i^2$

292 (3) Shannon-Wiener diversity index = $-\sum P_i \ln P_i$

293 (4) Pielou evenness index = $(-\sum P_i \ln P_i)/\ln S$

294 In the formula, P_i is the relative importance value, S is the number of species
295 appearing in the quadrat, and N is the total number of individuals of all species in the
296 quadrat.

297 The division of dominance of each type of group: the dominant group was the one in
298 which the number of individuals accounted for more than 10% of the total catch,
299 groups with 1%-10% were common, and groups with less than 1% were rare (Liu et
300 al., 2019).

301 The stoichiometric ratios of enzyme activity were determined using the methods of Li
302 (Li et al., 2020):

303 (5) $EE_{A_{C:N}} = \ln BG / \ln (LAP + NAG)$

304 (6) $EE_{A_{C:P}} = \ln BG / \ln AP$

305 (7) $EE_{A_{C:P}} = \ln (LAP + NAG) / \ln AP$

306 (8) $CBH/POD = \ln CBH / \ln POD$

307 The carbon-nitrogen ratio of the enzyme was calculated by formula (5), the
308 carbon-phosphorus ratio of the enzyme was calculated by formula (6), the
309 nitrogen-phosphorus ratio of the enzyme was calculated by formula (7) (Sinsabaugh et
310 al., 2012), and the relative abundance of refractory carbon was calculated by formula
311 (8) (Sinsabaugh et al., 2011).

312 (9) Vector $L = \sqrt{(\ln BG / \ln [NAG + LAP]) + (\ln BG / \ln AP)^2}$

313 (10) Vector A = Degrees($ATAN2((\ln BG/\ln AP), (\ln bg/\ln [NAG+LAP]))$)

314 Resource limitation was analysed by vector analysis (length, L; angle, A) of the
315 enzyme stoichiometry. In this analysis, a relatively longer vector length represents a
316 larger microbial relative carbon limit. An angle of the vector $< 45^\circ$ indicates the
317 degree of relative nitrogen limitation of the microorganism, whereas an angle of the
318 vector $> 45^\circ$ indicates the degree of relative phosphorus limitation of the
319 microorganism (Moorhead et al., 2016).

320 First, the values of extracellular enzyme activities were log transformed to satisfy the
321 tests of homogeneity of variance and normal distribution. Two-way analysis of
322 variance (two-way ANOVA) was used to test the effects of soil layers and shrub
323 species on soil chemistry analysis, enzyme activity, microbial biomass, microbial
324 community and soil animal diversity index. One-way ANOVA and the
325 Student-Newman-Keuls (S-N-K) method were used to determine the variability in
326 each index. The relationship among soil physicochemical properties, microbial
327 community structure, microbial biomass, enzyme activity, and enzyme stoichiometry
328 was evaluated using Pearson correlation analysis. The relationships between microbial
329 community structure and soil fauna diversity indices and soil physical and chemical
330 properties were analysed by redundancy analysis (RDA). The significance of the RDA
331 results was tested by a Monte Carlo permutation test ($P < 0.001$)

332 **Results**

333 **Soil C/N/P and stoichiometry**

334 The shrub species had a significant effect on soil TN ($P < 0.05$), while the soil layer
335 had a very significant effect on soil organic C, TN, TP, C/N, and N/P ($P < 0.01$, Fig. 1).
336 The TN in the organic layer of the SR shrub island was the highest (mean value: SR:
337 22.40 g kg^{-1} , RL: 15.96 g kg^{-1} , and CK: 11.48 g kg^{-1}), followed by that in the RL shrub
338 island and the control site. There was no significant difference in the TC content
339 between the RL and SR shrub island soil layers, while the TN, TC and TP in the
340 organic layer soil of the control site were higher than those in the mineral layer. The
341 C/P of the organic layer in the RL shrub island was higher than that in the control

342 group and SR shrub island (Fig. 1).

343 **Soil fauna community structure and diversity**

344 A total of 222 soil fauna individuals belonging to 1 phylum, 5 classes, 10 orders, and
345 33 families were collected from two soil layers in three shrub islands (Table 1). A total
346 of 89 soil fauna individuals belonging to 20 families were obtained under the RL
347 shrub island, of which 62 fauna individuals belonging to 16 families were recovered
348 from the organic layer and 27 fauna individuals belonging to 12 families were
349 recovered from the mineral layer. A total of 69 soil fauna individuals belonging to 22
350 families were obtained under the SR shrub island, of which 48 fauna individuals
351 belonging to 21 families were recovered from the organic layer and 21 fauna
352 individuals belonging to 12 families were recovered from the mineral layer. From the
353 control site, 64 soil fauna individuals belonging to 18 families were obtained,
354 including 42 fauna individuals belonging to 17 families from the organic layer and 22
355 animals belonging to 10 families from the mineral layer.

356 The families Phthiraeoridae, Galumnidae, Lepidopsocidae, Notodontidae, Geophilidae,
357 and Lithobiidae appeared only in RL. The families Trombididae, Parasitidae,
358 Sciaridae, Ptiliidae, and Brentidae appeared only in SR. The families Lycidae,
359 Elateridae, and Parajapygidae appeared only in the control site. In the humus layer of
360 the RL shrub island, Pygmephoridae, Onychiuridae, and Eremulidae were dominant
361 groups, while Poduridae and Isotomidae were common groups. In the humus layer of
362 the SR shrub island, Onychiuridae, Poduridae, and Liacaridae were dominant groups,
363 while Nothridae were common groups. In the control site, Onychiuridae and
364 Poduridae were the dominant groups, while Cryptotaeniaceae and Uropodidae were
365 common groups.

366 The soil layer had a very significant effect on the number of individuals and number
367 of groups of soil fauna (Table. 2). The Shannon-Wiener diversity index, Pielou
368 evenness index, and Margalef richness index of the organic layer at the control site
369 were higher than those of the mineral layer, while the Shannon-Wiener diversity index,
370 Simpson dominance index, Pielou evenness index, and Margalef richness index

371 values did not differ significantly between the SR and RL soil layers (Table. 2)

372 **Soil microbial community biomass**

373 The shrub species and soil layer had significant effects on the bacteria content, G^+ and
374 G^- proportions, and microbial biomass ($P < 0.05$, Fig. 2) The bacteria, G^+ , G^- , and
375 total PLFA contents in the soil layers of the RL and SR shrub islands were higher than
376 those of the control site, and the bacteria, G^+ , G^- , $G^+:G^-$ and total PLFA values in the
377 soil layers of the SR shrub island were higher than those of the RL shrub island. The
378 bacteria, G^- , and total PLFA values were the highest in the SR shrub island and the
379 lowest in the control group. The fungi, bacteria, F:B, G^+ , G^- , and total PLFA values in
380 the organic layer of the shrub islands were higher than those in the mineral layer and
381 showed a downward trend from the organic layer to the mineral layer (Fig. 2).

382 **Microbial biomass carbon and nitrogen**

383 Tree species and soil layer had extremely significant effects on MBC and MBN ($P <$
384 0.01 , Fig. 4). The MBC and MBN in the organic layer under RL shrub island and SR
385 shrub island were higher than those in the control site, but there was no difference in
386 the mineral layer. The MBN and MBC in the organic layer of shrub species were more
387 abundant than those in the mineral layer and showed a downward trend (Fig. 3).

388 **Enzyme activity and enzyme stoichiometry**

389 The BG, CBH, NAG, and PPO activities in the soil layers in the RL shrub island were
390 higher than those in the SR shrub island, while the AP activity in the soil layers in the
391 SR shrub island (mean value: soil organic layer: 23.91; mineral soil layer: 4.30 μmol
392 $\text{h}^{-1} \text{g DOM}^{-1}$) was higher than that in the RL shrub island (mean value: OL: 18.92 ML:
393 1.16 $\mu\text{mol h}^{-1} \text{g DOM}^{-1}$). The PPO activity in the soil layers was the highest in the RL
394 shrub island and the lowest in the SR shrub island, while the POD activity in the
395 mineral layer was the highest in the control site and the lowest in the SR shrub island.
396 The AP, BG, LAP, and NAG activities in the organic layer under the RL and SR shrub
397 islands were higher than those in the mineral layer, but the PPO activity showed the
398 opposite pattern. In addition, the AP, BG, CBH and LAP activities in the organic layer
399 and mineral layer in the control site were not significantly different (Fig. 4).

400 The shrub species and soil layer had extremely significant effects on $EEA_{N:P}$, $EEA_{C:N}$,
401 $EEA_{C:P}$, and vector angle ($P < 0.01$), and the interaction of shrub species and soil layer
402 had significant effects on $EEA_{C:N}$ and $EEA_{C:P}$ ($P < 0.05$, Fig. 6). $EEA_{N:P}$ in the organic
403 layer was the highest in the RL shrub island, followed by the SR shrub island, and the
404 lowest in the control site. The $EEA_{N:P}$ in the organic layer in the RL and SR shrub
405 islands was higher than that in the mineral layer, but there was no significant
406 difference in the control group (Fig. 5).

407 **Heat map of Pearson correlation**

408 Enzyme activities, $EEA_{C:P}$, $EEA_{N:P}$, MBC, MBN and microbial community structure
409 were all positively correlated with soil TC and TN. AP, BG, CBH and NAG were
410 positively correlated with MBC and MBN. $EEA_{C:P}$ was negatively correlated with soil
411 TP, while $EEA_{N:P}$ was positively correlated with bacteria, fungi, G^+ , G^- , F/B, and total
412 PLFA concentration (Fig. 6).

413 **Redundancy analysis**

414 Based on RDA, the first two axes captured 74.63% of the variability in the microbial
415 community structure, with RDA_1 (the x-axis) and RDA_2 (the y-axis) accounting for
416 74.55% and 0.08% of the variation, respectively. Soil moisture content (SMC), TP,
417 TN, TC, N/P and C/N were significantly correlated with the first two axes (SMC TP,
418 TN, TC and N/P, $p < 0.01$; C/N, $P < 0.05$; Fig. 7). The SMC, N/P and TC were
419 positively correlated with total PLFA, G^+ , TB and TF and negatively correlated with
420 TC. The C/N ratio was negatively correlated with J, D, H, total PLFA, G^+ , TB and TF
421 (Fig. 7).

422 **Discussion**

423 **Effect of shrub islands on the soil biological community and chemical** 424 **characteristics**

425 Shrubs increase soil TN and microbial biomass and affect the soil microbial
426 community structure and enzyme activities, exerting a shrub island effect similar to
427 the results of previous studies (Marr 1977; Wang et al., 2017; David et al., 2018).
428 Although the effect on soil fauna diversity was not significant, this might be due to

429 the proximity of sampling areas for different species. The shrub island effect occurs
430 because shrubs provide more available carbon and other resources for soil biological
431 communities than alpine tundra (Prescott & Zekker, 2016). Shrubs can also alter the
432 microclimate. By increasing the soil moisture content through the crown and litter, in
433 winter, shrubs can accumulate thicker snow and maintain higher soil temperatures,
434 which leads to an increase in decomposer abundance and activity, in turn affecting the
435 soil environment and material circulation (Bonkowski et al., 2004; Demarco et al.,
436 2014).

437 This effect also affects deeper soils. There was no significant difference in soil
438 nutrient and soil fauna diversity, richness, or evenness between the organic layer and
439 the mineral layer under the shrub islands, but the difference was significant in the
440 tundra control site. There were significant differences in enzyme activities between
441 the organic and mineral layers under the shrub islands, but no significant differences
442 in the control site. Shrub islands affect soil nutrients at deeper levels by affecting the
443 soil environment and soil biological community (Kašák et al., 2015). Additionally, it
444 is possible that the existence of shrubs affected the habitat environment of soil fauna
445 and thus affected the soil fauna. Our team found in a previous study (HE et al., 2016;
446 Huang et al., 2010) that the number of individuals and number of species of soil fauna
447 are the highest under shrubs in the alpine region of western Sichuan, while the
448 diversity of soil fauna is the lowest under tundra. This result is also similar to the
449 conclusion of Koehler (Koehler, 1998) that the community distribution of soil fauna is
450 obviously affected by vegetation species and environmental factors. There are great
451 differences in light, temperature, humidity, and soil physical and chemical properties
452 (soil type, pH, and organic matter) among different species, resulting in different
453 dominant groups. The high temperature difference, low humidity, and high light in the
454 tundra are not conducive to the survival of soil fauna, while the low temperature, high
455 humidity, and low light under the shrub island can serve more soil fauna individuals
456 (Connell 1977; Wu et al., 2014). The difference in soil enzyme activities between the
457 organic layer and the mineral layer under shrub islands showed that the shrub effect
458 significantly affected the decomposition of organic matter in topsoil, while the lack of

459 a difference in control site might be due to the lack of enzyme from the organic layer
460 to the mineral layer due to the difference in soil environment and the fewer number
461 and species of underground biological communities, so that there was no significant
462 difference (Zheng et al., 2019).

463 **Differences in evergreen and deciduous shrub island effects**

464 Wardle et al., (2004) defined fertile-productive and infertile-unproductive ecosystems
465 formed by species with different biological characteristics. The SR shrub island has
466 low carbon sequestration, a high nitrogen content, bacterial-based energy channels,
467 and greater soil microbial biomass. The enzyme activity data indicate a fast material
468 cycle. The leaves grow fast, have shorter lives, and have higher litter quality (Chapin,
469 1980; Wardle et al., 2004; 2012). This forms represents a fertile-productive ecosystem.
470 In contrast, the RL shrub island represents an infertile-unproductive ecosystem
471 (Vowles et al., 2019; Wardle et al., 2004). The significant difference in soil TN
472 between the two shrub islands may be caused by two factors. First, RL adapts to the
473 extreme environment of alpine ecosystems and maintains a relatively low growth rate.
474 Second, the leaf nitrogen content of RL shrubs was also lower than that of SR shrubs,
475 resulting in a significant difference in soil nitrogen (Damczyk et al., 2016; Yin et al.,
476 2017). Correlation analysis and RDA also showed that the TN content had an
477 extremely significant correlation with microbial community structure, microbial
478 biomass, and multiple enzyme activities, and the differences in soil physical and
479 chemical properties might have different effects on underground ecosystems (David et
480 al., 2018; Aguirre et al., 2021). The results of soil fauna diversity were consistent with
481 those of previous investigations conducted by He and Huang (HE et al., 2016;
482 Huang et al., 2010). The dominant species of soil fauna were different under different
483 shrub islands. Poduridae was the dominant group under the SR island, while
484 Pygmephoridae was the dominant group under the RL island; this difference was
485 regulated by the physical and chemical properties of soil and species (Koehler, 1998;
486 Dai et al., 2021).

487 Compared with the deciduous shrub (SR) island, the evergreen shrub (RL) island
488 featured a lower MBC, which was due to the poor substrate quality of the litter of RL

489 (lower leaf N and P contents and higher lignin content and lignin/N ratio), which the
490 microorganisms had difficulty utilizing. This finding is consistent with the results of
491 previous studies (Horwath et al., 2017; Jiang et al., 2021). Higher-quality litter tends
492 to have higher microbial respiration rates and soil MBC, while lower-quality litter
493 tends to have the opposite (Li et al., 2015). The total PLFA content of the microbial
494 biomass under the RL island was lower than that under the SR island. This may be
495 due to the phenolic substances and tannins produced by many low evergreen shrubs,
496 which form organic compounds that are difficult to degrade and slow the nutrient
497 cycle by reducing the soil pH, which is not conducive to microbial access to nutrients
498 (Zamin et al., 2014; Vowles et al., 2019). While most studies have demonstrated that
499 fungi tend to be responsible for long cycles and more difficult decomposition of
500 substances and that fungi have a wider range of pH adaptation than bacteria, this is
501 not demonstrated by comparison between the RL shrub island and the SR shrub island
502 (Redman et al., 2002; Feng et al., 2014). However, the dominance of bacteria in the
503 SR shrub island was most likely caused by the higher quality of litter in the SR shrub
504 island (Lundquist et al., 1999).

505 The litter of species with more refractory substances limits the C supply to the
506 microbial community and results in higher potential activities of enzymes involved in
507 cellulose and protein degradation and phenol oxidation, which might be the reason for
508 the higher activities of PPO, POD, BG and CBH in the RL shrub island soil (Burns et
509 al., 1982; 2013; Baldrian et al., 2009). Our results were similar to those reported by
510 Phuyal, in which the AP increased with increasing nitrogen. The AP in the soil under
511 the RL shrub island was lower, which corresponded to the low content of TN and
512 MBN in the soil. In contrast, the rapid nutrient cycle of SR shrubs increases the
513 demand for phosphorus and thus AP (Phuyal et al., 2008).

514 **Stoichiometry of evergreen and deciduous shrub island**

515 The RL shrub island had a higher C/P ratio, while the SR shrub island and the control
516 site had lower C/P ratios, showing that the RL shrub island had a higher carbon
517 sequestration capacity. The lower litter quality, the unique soil biocommunity
518 structure, and the lower microbial biomass in the RL shrub island make the material

519 cycle slower, so nutrient conservation promotes carbon sequestration (Coleman, 1983).
520 Some studies have shown that G^- bacteria are associated with simple C compounds
521 (alkyl), while G^+ bacteria are more associated with the more complex form C
522 (carbonyl) and that the G^-/G^+ ratio has the potential to serve as a useful indicator of
523 the relative C availability of soil bacterial communities in organic soils and as a rough
524 indicator of energy limitation in natural ecosystems (Fanin et al., 2016; 2019). Thus,
525 the G^-/G^+ in the SR shrub island was higher than that in the RL shrub island, showing
526 that there were more abundant decomposable substrates or higher carbon availability
527 in the SR shrub island.

528 The enzyme stoichiometry, i.e., $EE_{AC:P}$ and $EE_{N:P}$, of the RL shrub island was higher
529 than that of SR shrub island, which showed that the microorganisms in the soil in the
530 RL shrub island were more limited by carbon and nitrogen than phosphorus. The
531 correlation analysis also showed that $EE_{AC:P}$ and $EE_{N:P}$ had a positive correlation
532 with carbon, nitrogen and phosphorus. The carbon and nitrogen limitations of RL
533 were mostly related to litter quality. Lower litter N and P, higher lignin content, and
534 the production of phenols would reduce the material circulation speed and finally
535 make the microorganisms subject to carbon and nitrogen limitations (Sinsabaugh et
536 al., 2008; Mori et al., 2020). The included angles of the vectors of the alpine shrub
537 island and the tundra control site were higher than 45° , showing that the soil in the
538 alpine region of western Sichuan was limited by phosphorus, which was consistent
539 with the previous research results of our team (Liu et al., 2019; Zheng et al., 2019).
540 Vector A of the control site was higher than the values of the two shrub islands,
541 showing that the phosphorus limitation pressure in the control site was greater than
542 that in the soil under the shrub islands, which is closely related to the regulation of
543 soil formation and fixation of nutrients by soil animals and microorganisms
544 (Moorhead et al., 2016; Almeida et al., 2020). The CBH/PPO ratio in the RL shrub
545 island was higher than that in the SR shrub island, according to the definition of
546 Sinsabaugh (Sinsabaugh et al., 2011; Takriti et al., 2018). The role of oxidase is rather
547 non-specific and can catalyse the decomposition of complex irregular substrates. Soil
548 oxidative activity represents a soil characteristic that depends on a combination of

549 biological and non-biological factors. Therefore, the ratio of CBH/PPO activity is
550 defined as negatively correlated with the relative abundance of recalcitrant carbon.
551 The stoichiometric ratio of the CBH/PPO enzyme shows that the soil under the RL
552 island contains more recalcitrant carbon.

553 **Conclusions**

554 Roughly as we expected, the results showed that the shrub islands affected the soil
555 microbial community structure and enzyme activity, impacted soil nutrient cycling
556 and microbial processes, and increased soil TN, MBC and MBN. The enzyme activity
557 response was more significant in the organic layer, especially AP, BG, LAP and POD
558 activities; however, there was no significant difference in soil fauna diversity index,
559 evenness index, or richness index under the shrub islands. The evergreen and
560 deciduous shrub islands had different effects on the soil biological community and
561 chemical characteristics. Compared with the SR shrub island, the RL shrub island had
562 lower soil TN, MBC and total microbial PLFA content and higher enzyme activities.
563 The SR shrub island had higher TF, G⁻ and total PLFA. The families Phthiraeoridae,
564 Galumnidae, Lepidopsocidae, Geophilidae, and Lithobiidae were observed only in the
565 RL island. The families Trombidiidae, Parasitidae, Sciaridae, Ptiliidae, and Brentidae
566 appeared only in the SR island. The OL response was greater than the ML response,
567 especially in terms of enzyme activities, including AP, BG, LAP and POD. There is an
568 inseparable relationship between environmental factors and decomposers. In the
569 context of global climate change, the mechanism of shrub island response in alpine
570 ecosystems may become a key factor in the global carbon cycle.

571

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801

802 **Table legends**803 **Table 1** Number and species composition of soil fauna in shrub islands and soil layers

804 (total of all samples)

Layer	Organic layer			Mineral layer		
	RL	SR	CK	RL	SR	CK
Onychiuridae	10	5	10	8	5	5
Poduridae	5	5	5	0	0	4
Entomobryidae	1	0	2	1	0	0
Isotomidae	6	4	1	2	1	2
Pygmephoridae	12	2	4	5	1	1
Rhagidiidae	3	1	0	0	0	0
Tarsonemidae	0	0	0	2	0	0
Trombidiidae	0	1	0	0	1	0
Erythraeidae	0	1	1	0	0	0
Caeculidae	0	1	3	0	2	3
Nothridae	0	4	1	0	0	0
Cryptognathidae	0	1	3	0	0	1
Uropodidae	1	1	3	0	0	0
Parasitidae	0	1	0	0	0	0
Phthiraeidae	2	0	0	1	0	0
Damaeidae	2	1	0	1	1	0
Galumnidae	1	0	0	1	0	0
Liacaridae	3	7	1	3	1	3
Eremulidae	9	2	1	0	4	0
Amphientomidae	2	0	0	0	1	1
Lepidopsocidae	0	0	0	1	0	0
Notodontidae	1	0	0	0	0	0
Sciaridae	0	1	0	0	2	0
Kalotermitidae	1	1	0	0	1	0
Cantharidae	0	2	1	0	1	1
Ptiliidae	0	1	0	0	0	0
Brentidae	0	3	0	0	0	0
Lycidae	0	0	1	0	0	1
Elateridae	0	0	3	0	0	0
Parajapygidae	0	0	1	0	0	0
Geophilidae	0	0	0	1	0	0
Lithobiidae	3	0	0	1	0	0
Group number	16	21	17	12	12	10
Ind. number	62	48	42	27	21	22

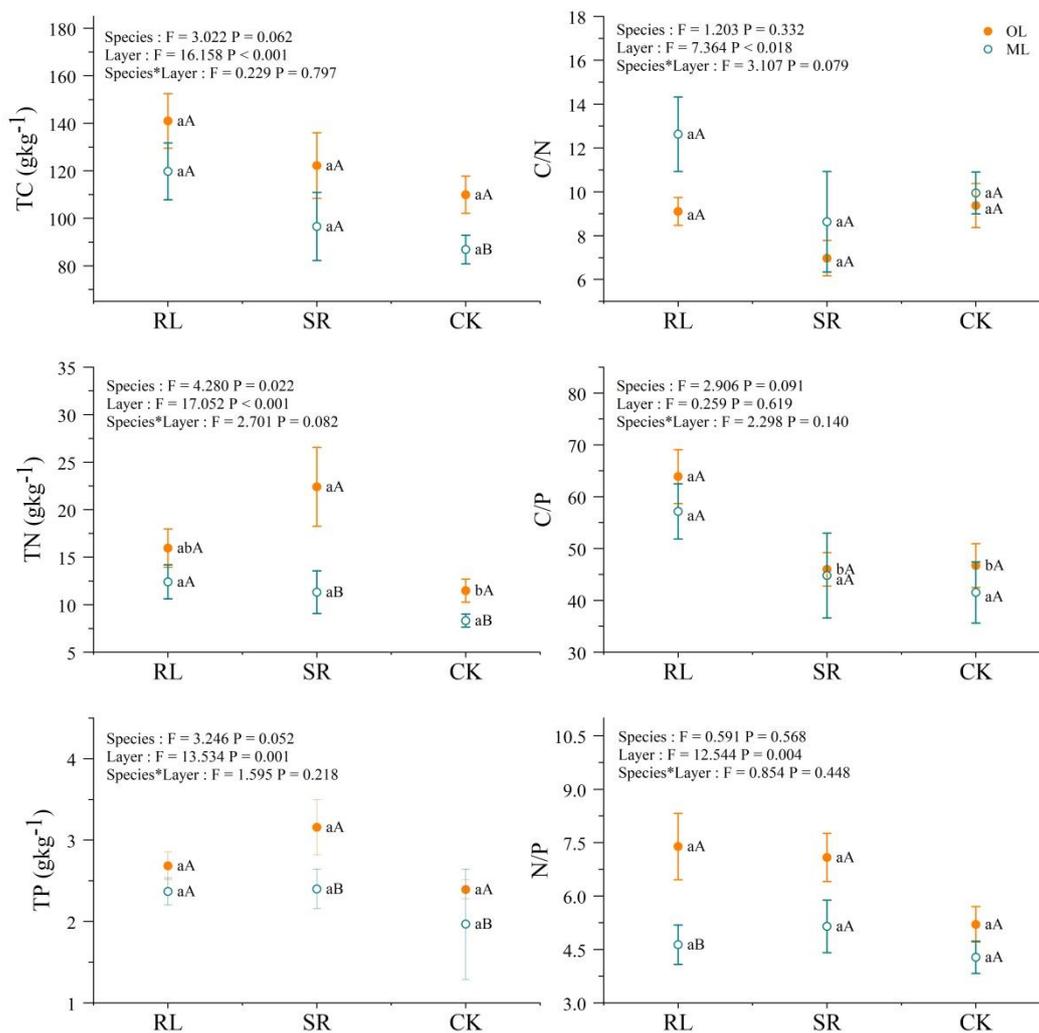
805 **Table 2** Soil fauna diversity in the organic and mineral soil layers of the evergreen and deciduous
 806 shrub islands.

Shrub species Layer	RL		SR		CK	
	OL	ML	OL	ML	OL	ML
H	1.12±0.18 aA	0.78±0.20 aA	1.17±0.21 aA	0.56±0.16 aB	1.19±0.12 aA	0.57±0.24 aB
C	0.4±0.08 aA	0.43±0.09 aA	0.39±0.09 aA	0.42±0.11 aA	0.33±0.04 aA	0.36±0.12 aA
J	0.84±0.11 aA	0.75±0.14 aA	0.86±0.11 aA	0.63±0.16 aA	0.98±0.01 aA	0.43±0.17 aB
D	1.50±0.23 aA	1.26±0.28 aA	1.72±0.32 aA	0.90±0.26 aA	1.78±0.09 aA	0.85±0.35 aB

807 Different lowercase letters indicate significant differences among different shrub islands within the same soil
 808 layer ($p < 0.05$). Different uppercase letters indicate significant differences between soil layers within the same
 809 shrub island ($p < 0.05$). H, Shannon-Wiener diversity index; C, Simpson dominance index; J, Pielou evenness
 810 index; D, Margalef richness index.

811

812 **Figure legends**

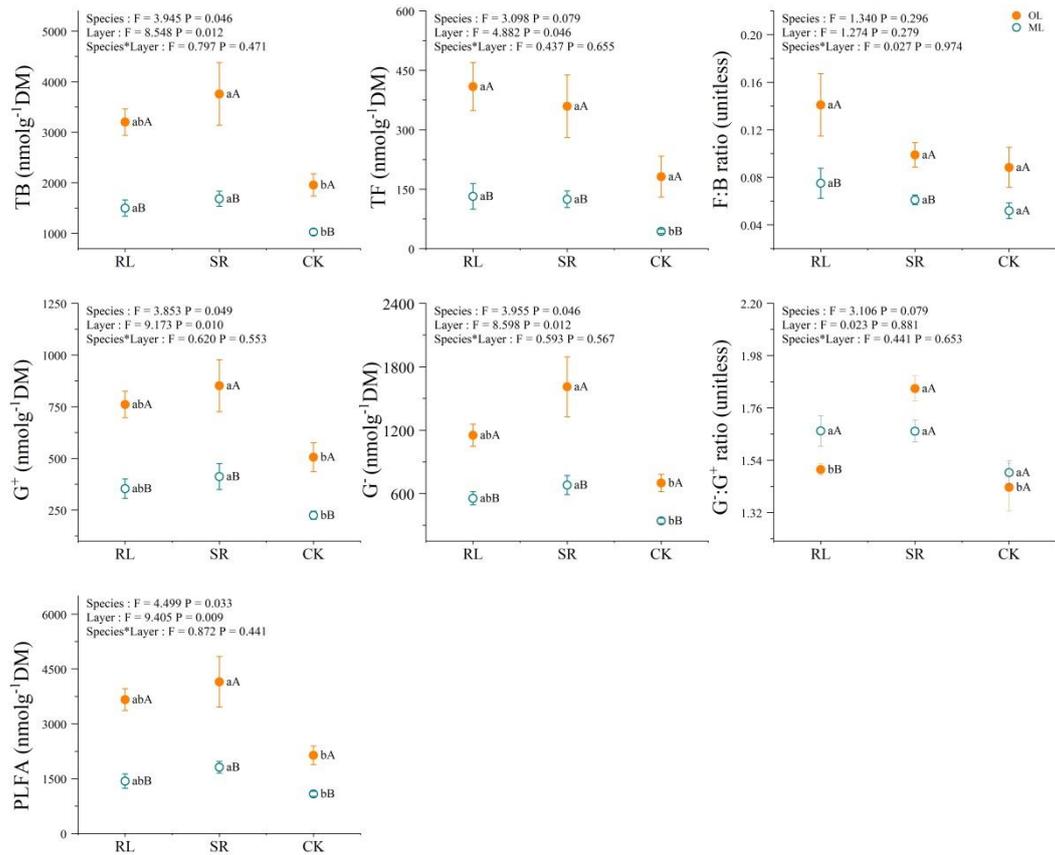


813

814 **Fig. 1** Total C, N, P, C/N, C/P and N/P in the organic and mineral soil layers of the evergreen and deciduous
 815 shrub islands. Different lowercase letters indicate significant differences among different shrub islands within
 816 the same soil layer ($p < 0.05$). Different uppercase letters indicate significant differences between soil layers
 817 within the same shrub island ($p < 0.05$). RL, *Rhododendron lapponicum*; SR, *Sorbus rufopilosa*; CK, control
 818 site; OL, organic soil layer; ML, mineral soil layer; TC, Total carbon; TN, Total nitrogen; TP, Total
 819 phosphorus. The same scheme is used in other figures.

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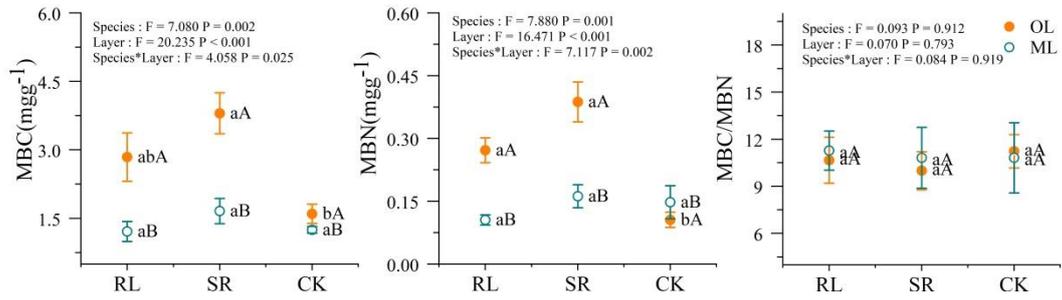
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823 **Fig. 2** Microbial community structures in the organic and mineral soil layers of the evergreen and deciduous
 824 shrub islands. TB, total bacteria; TF, total fungi; G⁺, Gram-positive bacteria; G⁻, Gram-negative bacteria; F:B,
 825 total fungi:total bacteria; G⁻:G⁺, Gram-negative bacteria: Gram-positive bacteria; PLFA, PLFA microbial
 826 biomass.

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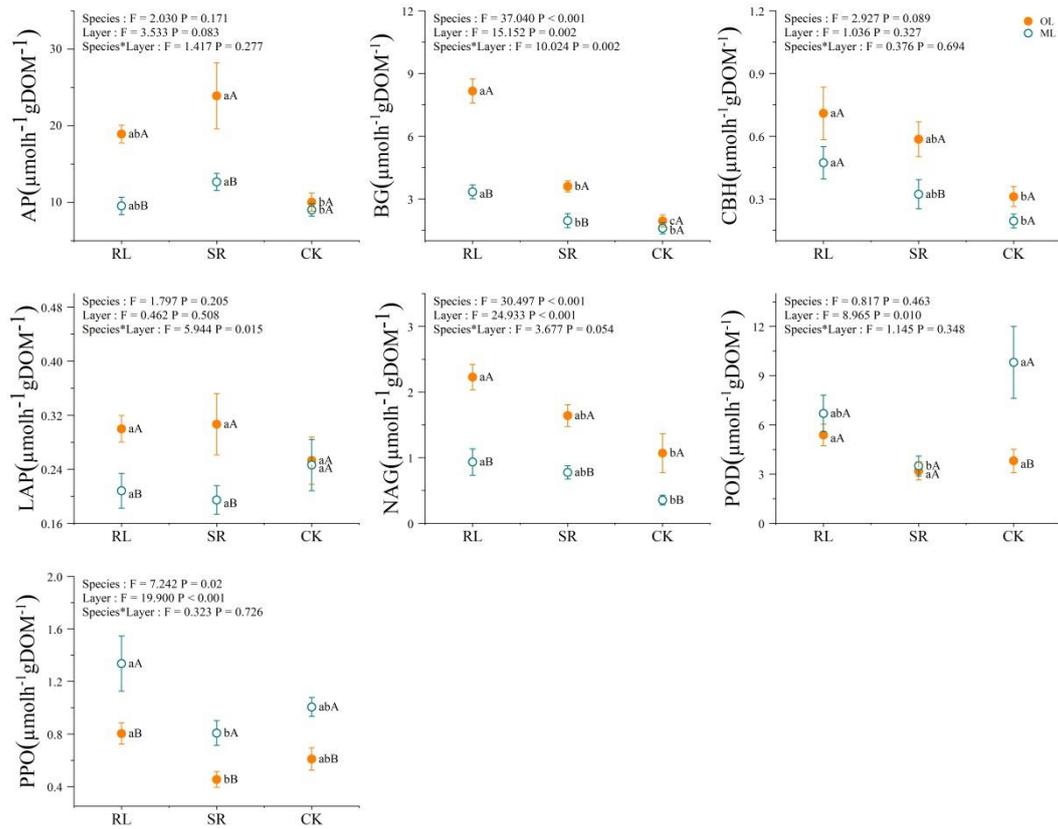
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829 **Fig. 3** Microbial biomass in the organic and mineral soil layers of the evergreen and deciduous shrub islands.

830 MBC, Microbial biomass carbon; MBN, Microbial biomass nitrogen.

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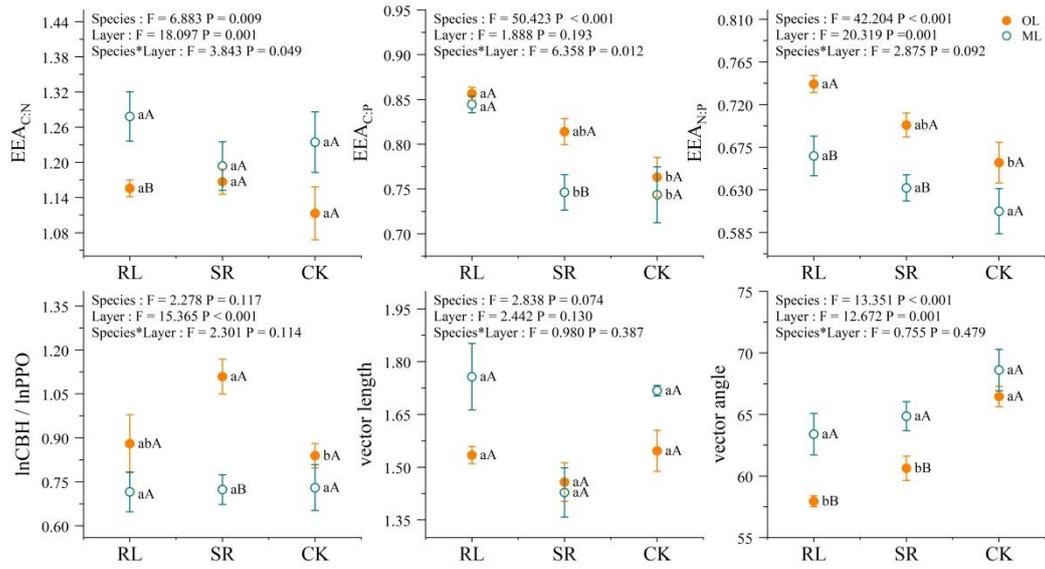
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834 **Fig. 4** Enzyme activity for AP (acid phosphatase), BG (β -glucosidase), CBH (cellobiohydrolase), LAP
 835 (leucine aminopeptidase), NAG (β -N-acetylglucosaminidase), POD (peroxidase) and PPO (polyphenol
 836 oxidase) in the organic and mineral soil layers of the evergreen and deciduous shrub islands.

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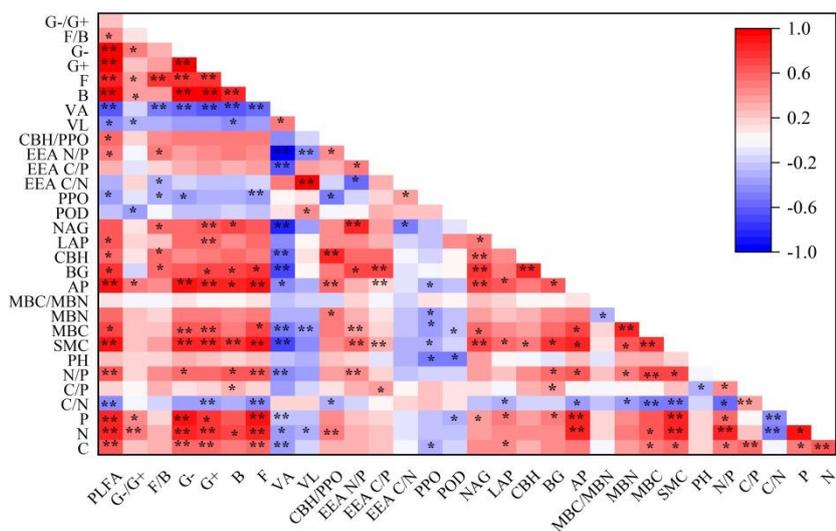
839 **Fig. 5** Enzyme stoichiometry in the organic and mineral soil layers of the evergreen and deciduous shrub

840 islands.

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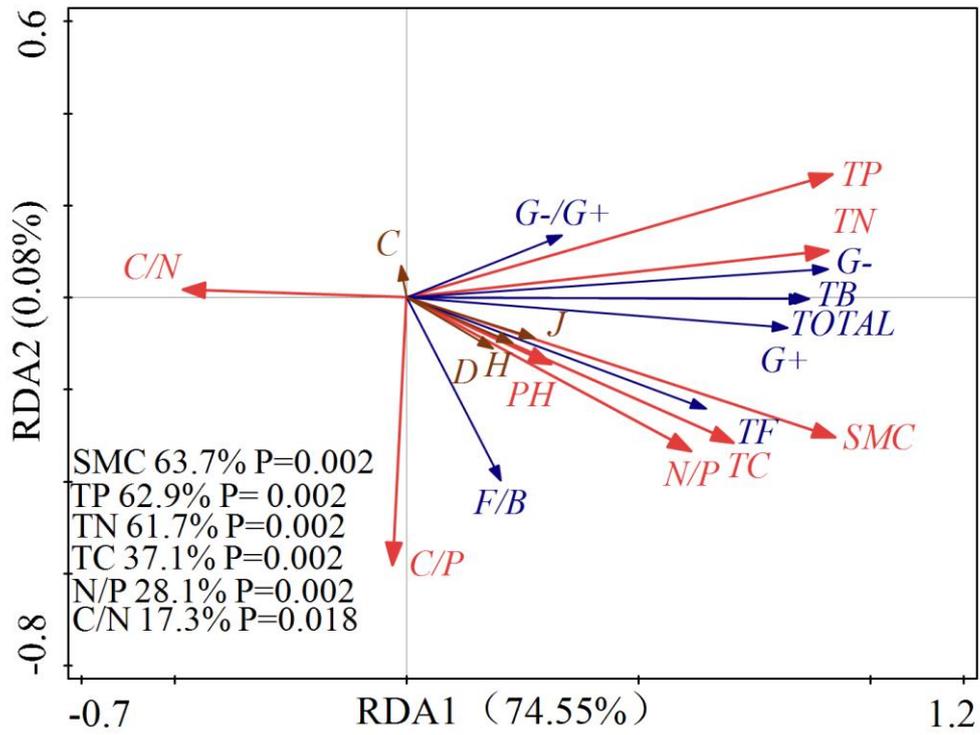
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845 **Fig. 6** Pearson correlation heat map of environmental factors and soil nutrients, microbial community
846 structure, microbial biomass, enzyme activity and enzyme stoichiometry. The correlation coefficient values
847 are represented by the corresponding colour level, and the higher the correlation coefficient is, the darker the
848 colour. * indicates significant correlations (*: $p < 0.05$; **: $p < 0.01$). SMC, soil moisture content; VL, vector
849 length; VA, vector angle.

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854 **Fig. 7** Redundancy analysis (RDA) of enzyme activities, microbial community structure and environmental
 855 variables in the organic and mineral soil layers of the evergreen and deciduous shrub islands.

856

Supplementary Files

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