

1 Understory vegetation plays the key role on sustaining soil microbial biomass 2 and extracellular enzyme activities

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11 **Abstract:**

12 While we know that understory vegetation affects the soil microbial biomass and extracellular enzyme activities in
13 subtropical Chinese fir (*Cunninghamia lanceolata*) forests, we are less certain about the degree of its influence. We
14 determined the degree to which the soil abiotic and biotic properties, such as PLFAs and extracellular enzyme activities,
15 were controlled by understory vegetation. We established a paired treatment in a subtropical Chinese fir plantation,
16 which comprised one plot from which the understory vegetation and litter were removed (None) and another from
17 which the litter was removed but the understory vegetation was left intact (Understory). We evaluated how the
18 understory vegetation influenced the soil abiotic properties; the bacterial, fungal, and actinobacterial PLFAs, and the
19 activities of five hydrolases and two oxidative enzymes. The dissolved organic carbon (DOC), particulate organic
20 carbon, soil organic carbon, ammonia nitrogen (NH₄⁺-N), and total nitrogen contents and soil moisture were 18%, 25%,
21 12%, 34%, 8%, and 4% lower in the None treatments than in the Understory treatments, respectively ($P < 0.05$). Soil
22 bacterial, fungal, and total PLFAs, and the potential activities of β -1,4-glucosidase (β G), β -1,4-N-acetylglucosaminidase,
23 phenol oxidase, and peroxidase were as much as 24% lower in None treatments than in the Understory treatments ($P <$
24 0.05). The specific activities of C-acquiring enzymes were as much as 41% higher ($P < 0.05$), and the ratio of C- to
25 N-acquiring enzymes was also higher, in the None treatments than in the Understory treatments. This suggests that the
26 microbes invested more in C acquisition than N acquisition because the carbon (C) inputs were less labile. The negative
27 relationship between DOC and AP shows that DOC is consumed when P-acquiring enzymes are produced. The positive
28 correlation between NH₄⁺-N and β G suggested the increased availability of N promoted the decomposition of C. More
29 extracellular enzymes that degrade soil organic matter are produced when there is understory vegetation, which leads to

30 losses of soil C. On the other hand, the soil C sink is maintained by increased inputs of C. We can therefore conclude
31 that understory vegetation contributes to C sequestration in Chinese fir forests and suggest that understory should be
32 maintained to sustain soil quality in subtropical Chinese fir plantations.

33 **Keywords:** Chinese fir forest; Red soil; Enzyme activities; Phospholipid fatty acids; Understory vegetation

34

35 1. Introduction

36 The interactions that occur between above-ground vegetation functional groups and soil microbial communities are
37 thought to be important drivers of carbon (C) and nutrient cycling in terrestrial ecosystems (Murugan et al., 2014).
38 When the understory vegetation is removed from forest ecosystems, soil processes are influenced, such that the
39 above-ground plant diversity and biomass decrease (Lamb et al., 2011; Fu et al., 2015) and the characteristics of the
40 below-ground rhizodeposits change (Li et al., 2013). The understory vegetation absorbs water and nutrients from soil
41 (Wang et al., 2014), and also releases carbohydrates back to the soil as discarded root cap and border cells; mucilage
42 and exudates from roots (McNear Jr, 2013), and cellulose, hemicelluloses, and lignin from leaf litter (Loeppmann et al.,
43 2016a, b). The net effect of understory vegetation on soil nutrients is determined by the balance between its nutrient
44 demand and capacity to release carbohydrates to soil via the decomposition of understory-derived litter and
45 rhizodeposits.

46 Soil extracellular enzymes produced by microorganisms or plant roots catalyze the cycling of soil C, nitrogen (N),
47 and phosphorus (P) (Burns et al., 2013; Nannipieri et al., 2018). Because they respond rapidly to soil environmental
48 changes, soil enzyme activities are often used as indicators of soil quality (Trasar-Cepeda et al., 2008; Burns et al., 2013).
49 Individual enzyme activities can reflect the substrate availability, the nutrient requirements of microorganisms and
50 plants, and the strategies used by microbes and plants to maintain the nutrient balance when the soil environment
51 changes (Burns et al., 2013; Nannipieri et al., 2018). Because it is difficult to know whether changes in the enzymatic
52 activities reflect changes in the soil microbial biomass or differences in the actual activities (Trasar-Cepeda et al., 2008),
53 we need to study the specific enzyme activities, i.e., the activity normalized to the total PLFA contents (Zhang et al.,
54 2015; Zhang et al., 2017). The enzyme ratio is used to examine the relative allocation of energy versus nutrient
55 acquisition, since it intersects the metabolic theory of microbial ecology and the theory of ecological stoichiometry
56 (Stone et al., 2014; Loeppmann et al., 2016a; Xu et al., 2017). By studying how the enzyme activities and ratios change
57 when the understory vegetation is removed, we hope to improve our understanding of how the storage of C in soil is
58 influenced by the understory vegetation, and how microbial nutrient acquisition is affected by microbial biomass and
59 soil nutrients.

60 Studies have shown that understory vegetation-induced changes in soil properties are closely related to climate,
61 soil type, plant species, and time (Li et al., 2013; Nilsson and Wardle, 2005; Zhang et al., 2014). There is however, no
62 consensus about how understory vegetation impacts the physical, chemical, and biological properties of forest soils. For
63 example, some studies have reported decreases in the litter decomposition rate, soil organic matter (SOM) content, and
64 the soil respiration rate (Wang et al., 2011; Liu et al., 2012; Wang et al., 2014), while others have reported little change
65 in the soil properties, after understory vegetation was removed (Xiong et al., 2008; Zhao et al., 2011). Wu et al. (2011)
66 and Zhao et al. (2013) found that the fungal biomass and the fungi to bacteria ratio decreased, but Murugan et al. (2014)
67 found that the bacterial and saprophytic fungal biomass increased, and ectomycorrhizal fungi and arbuscular
68 mycorrhizal fungi reduced after understory vegetation was removed from eucalyptus plantations. In an alpine shrubland,
69 the soil arbuscular mycorrhizal fungal biomass decreased 5 months after plant functional groups were removed, but this
70 effect disappeared after 17 months (Urcelay et al., 2009). The effects of understory vegetation on soil microbial biomass
71 vary by ecosystem-type. Huang et al. (2014) reported that soil enzyme activities decreased in a subtropical alpine
72 coniferous forest, while Lin et al. (2012) found that they did not change in a *Pinus sylvestris* var. *mongolica* plantation,
73 when understory vegetation was removed. The current information about the responses of soil enzyme activities to
74 understory vegetation removal is therefore inconsistent.

75 Yu et al. (2014) reported that the average net ecosystem productivity of Chinese subtropical forests (362 ± 39 g C
76 $m^{-2} yr^{-1}$) was approximately 82.6% and 64.9% higher than that of tropical and temperate forests. To maintain the soil
77 fertility, it is important that C sinks and tree growths are sustained in these forests. A valuable economic resource,
78 Chinese fir (*Cunninghamia lanceolata*) plantations are widespread throughout southern China. They cover an area of
79 9.11×10^6 ha, and account for approximately 18% of the total plantation area in China (Huang et al., 2013). Understory
80 vegetation and litter are commonly removed from the forest floor in southern China and elsewhere to facilitate seed
81 germination; ensure survival of seedlings; avoid the intense competition between understory vegetation and trees for
82 water, nutrients, and light, and for fuel for rural inhabitants (Xiong et al., 2008; Wu et al., 2011; Liu et al., 2012).

83 Therefore, we established a long-term field experiment to assess how the soil abiotic properties, PLFAs, and
84 enzyme activities in a Chinese fir plantation changed when the understory vegetation was removed. We hypothesized
85 that rhizodeposition, and therefore microbial biomass and activity, would decrease when the understory vegetation was
86 removed.

87 2. Material and Methods

88 2.1 Experimental treatments

89 Our study site was in the Shixi forest plantation in Taihe County, Jiangxi Province, China (115°03'29.9" E,

90 26°44'29.1" N). The area has a subtropical monsoon climate with a mean annual temperature of 18.8 °C and a mean
91 annual precipitation of 1340 mm. According to the USDA-NRCS soil taxonomy (Soil Survey Staff, 1996), the soil in
92 this area is dominated by Udults, which forms from red sandstone and sandy conglomerate and has moist and dry
93 Munsell values of 7.5 YR 5/6 and 7.5 YR 6/6, respectively.

94 The study site is a second-generation Chinese fir plantation that was planted in 1998. The average tree height and
95 diameter at breast height (measured at 1.3 m above ground level) were about 18 m and 17 cm, respectively. The
96 understory vegetation, including shrubs and herbs, is dominated by Old World forked fern (*Dicranopteris dichotoma*
97 *Berth*), gambir (*Uncaria*), oriental blueberry (*Vaccinium bracteatum*), nutgall tree (*Rhus chinensis*), Chinese witch hazel
98 (*Loropetalum chinense*), short shank robe oak (*Quercus glandulifera* Bl.), root of mayflower glorybower (*Clerodendron*
99 *cyrtophyllum Turcz*), and azalea (*Rhododendron*).

100 Three plots, measuring 30 × 30 m and separated by a buffer of a least 10 m to avoid any between-plot influence,
101 were established in the plantation in January 2013. One paired treatment with three replicates was established within
102 each of the three plots. Each plot was divided into 4 subplots (15 × 15 m each) and contained 2 treatments, namely
103 None, from which both the understory vegetation and litter were removed, and Understory, from which the litter was
104 removed but the understory vegetation was left. The two subplots in a plot with the same treatment were distributed
105 across each plot to avoid the effects of slope (Fig. 1) and their results were averaged. The litter and understory were
106 managed monthly. The amount of litter and understory vegetation at the study site amounted to about 1020 and 6236 kg
107 ha⁻¹ year⁻¹, respectively, under natural conditions.

108 2.2 Soil sampling and analysis

109 Bulk soil samples were collected in the wet (April and November) and dry (July) seasons in 2015. Five soil cores
110 with an inner diameter of 5 cm were collected randomly from between the surface and a depth of 10 cm from each
111 subplot and then mixed as one composite sample. All fresh soil samples were sieved to 2 mm and stored at 4 °C until
112 analysis.

113 Soil physical and chemical properties were determined as outlined by Bao (2008). Soil temperature (ST) was
114 determined at a depth of 10 cm with a soil thermometer (TP101) during sampling. The soil moisture content (SMC) was
115 measured by drying aliquots of soil at 105 °C to constant weight. Soil pH was measured at a soil to water ratio of 1:2.5
116 by a pH digital meter. The contents of nitrate N (NO₃⁻-N) and ammonia N (NH₄⁺-N) were measured with a continuous
117 flow analyzer (Bran Luebbe, AA3) after extraction with a 2 M KCl solution (soil: solution ratio of 1: 10). Dissolved
118 organic carbon (DOC) contents were measured with a TOC analyzer (Elementar, Liquid II) after extraction with
119 ultra-pure water (soil: solution ratio of 1: 5) (Jones and Willett, 2006). Particulate organic carbon (POC) was determined

120 as outlined by Garten et al. (1999). The contents of soil organic C (SOC) and total nitrogen (TN) were measured with an
121 elemental analyzer (Vario Max CN).

122 Soil phospholipid fatty acids (PLFAs) were extracted following the procedure outlined by Bossio and Scow (1998),
123 and were determined with a gas chromatograph (Agilent 6890N). Soil total PLFAs were represented by various PLFA
124 biomarkers; gram positive bacteria (G^+) were represented by i14:0, i15:0, a15:0, i16:0, i17:0, and a17:0, and gram
125 negative bacteria (G^-) were represented by 16:1 ω 7c, cy17:0, 16:1 ω 9c, and cy19:0. The total bacterial PLFAs were
126 represented by biomarkers of G^+ and G^- . The total fungi PLFAs were represented by arbuscular mycorrhizal fungi
127 (AMF) biomarkers 16:1 ω 5, as well as 18:1 ω 9c, 18:2 ω 6c, and 18:3 ω 6c, and the actinobacterial PLFAs were represented
128 by 10Me16:0, 10Me17:0, and 10Me18:0 (Bradley et al., 2007; Deneff et al., 2009).

129 Soil enzyme activities were measured following the methods of Saiya-Cork et al. (2002). The specific substrates
130 and functions of the enzymes assayed are listed in Table A1. Five hydrolase activities (α -1,4-glucosidase (α G),
131 β -1,4-glucosidase (β G), β -1,4-N-acetylglucosaminidase (NAG), β -1,4-xylosidase (β X) and acid phosphatase (AP)) were
132 assayed using fluorogenically-labeled substrates. Briefly, a soil suspension was prepared by adding 1 g of fresh soil to
133 125 mL of 50 mM acetate buffer. We added 200 μ L of the soil suspension and 50 μ L of the substrate solution (200 μ M)
134 to 96 microplates, making a total of 8 analytical replicates. Methylumbelliferone (MUB) was used to calibrate the
135 hydrolase activities. The microplates were incubated in the dark at 20 $^{\circ}$ C for up to 4 h. After incubation, 10 μ L of 1 M
136 NaOH was added to each well to terminate the enzymatic reaction. When the reactions had ended, the fluorescence was
137 measured using a microplate fluorometer (SynergyH4, BioTek) with excitation and emission filters of 365 and 450 nm,
138 respectively. We calculated the specific enzyme activities by dividing the individual potential hydrolase activities by the
139 total PLFA contents (Zhang et al., 2015; Zhang et al., 2017). The total C-acquiring enzyme activity (C_{enz}) was
140 operationally defined as the sum of the α G, β G, and β X activities (Stone et al., 2014) (Table A2).

141 The soil oxidase activities (polyphenol oxidase (PPO) and peroxidase (PER)) were assayed spectrophotometrically.
142 We added 600 μ L of the soil suspension and 150 μ L of the substrate solution to deep-well plates. We also added 30 μ L
143 of 0.3% H_2O_2 solution before determining PER. After incubation in the dark at 20 $^{\circ}$ C for up to 5 h, the deep-well plates
144 were centrifuged for 3 minutes at 3000 $r\ h^{-1}$. We then transferred 250 μ L of the supernatant to the microplates and
145 measured the absorbance at 450 nm with a microplate fluorometer (SynergyH4, BioTek) (DeForest, 2009).

146 2.3 Statistical Analysis

147 Data are presented as the means \pm standard errors. By applying the one-sample Kolmogorov-Smirnov test within
148 SPSS 17.0, we found that the data satisfied the normal distribution criteria. We assessed the differences between the soil
149 abiotic properties, PLFA contents, and enzyme activities for the understory treatments with a paired-sample t -test (SPSS

150 17.0). Where two subplots within the same plot had the same treatment, we averaged the data before analysis. We
151 investigated the relationships between the soil abiotic properties, PLFA contents, and enzyme activities for the two
152 treatments using redundancy analysis (RDA, CANOCO, version 4.5) and Pearson correlation analysis (SPSS 17.0). We
153 tested the significance of the variables with the Monte Carlo Permutation Test before applying RDA. Figures were
154 generated using SigmaPlot (Version 10.0). A significance level of $P < 0.05$ was applied throughout.

155 3. Results

156 3.1 Soil abiotic properties

157 The results suggest that the soil abiotic properties were influenced by the understory vegetation management
158 (Table 1). The contents of DOC, POC, SOC, NH_4^+ -N, and TN were 18%, 25%, 12%, 34%, and 8% lower in the None
159 treatments than in the Understory treatments ($P < 0.05$), respectively. The SMC and POC/SOC were also 4% and 15%
160 lower in the None treatments than in the Understory treatments, respectively ($P < 0.05$). There were no significant
161 differences between the contents of NO_3^- -N, ST, pH, and the SOC/TN ratios in the None and the Understory treatments
162 ($P > 0.05$).

163 3.2 Soil PLFAs

164 The soil total PLFAs were 27% lower in the None treatments than in the Understory treatments (Fig. 2).
165 Specifically, the bacterial and fungal PLFAs were 26% and 20% lower ($P < 0.05$) in the None treatments than in the
166 Understory treatments, respectively, but there were no significant differences between the G^+ , G^- , or actinobacterial
167 PLFAs in the two treatments ($P > 0.05$). The fungi/bacteria ratios did not change because the bacterial and fungal
168 PLFAs were both lower in the None treatments.

169 3.3 Soil enzyme activities

170 The soil enzyme activities varied as the understory vegetation management varied. The potential activities of βG ,
171 NAG, PPO, and PER were 13%, 24%, 21%, and 20% lower in the None treatments than in the Understory treatments
172 (Fig. 3a and b) ($P < 0.05$), respectively, but the potential activities of acid phosphatases did not differ significantly ($P >$
173 0.05) between the two treatments. The ratio of $\ln C_{\text{enz}}/\ln \text{NAG}$ was 6% higher in the None treatments than in the
174 Understory treatments, but the ratios of $\ln C_{\text{enz}}/\ln \text{AP}$ were similar for the different treatments. The trends were
175 enzyme-specific when normalized by the total PLFAs (Fig. 3d and e). The specific activities of the C-acquiring
176 enzymes, i.e., $\alpha\text{G}_{\text{PLFAs}}$, $\beta\text{G}_{\text{PLFAs}}$ and $\beta\text{X}_{\text{PLFAs}}$, were 40%, 22%, and 41% higher, respectively, in the None treatments than
177 in the Understory treatments ($P < 0.05$), but the specific activities of N- ($\text{NAG}_{\text{PLFAs}}$) and P-acquiring enzymes (AP_{PLFAs})
178 were not significantly different between the two treatments ($P > 0.05$).

179 3.4 Correlations between soil enzyme activities, soil PLFAs, and soil abiotic properties

180 The first (RD1) and second (RD2) ordination axes explained 62.0% and 15.5% of the total variability in the
181 different PLFAs, respectively. Soil temperature, SMC, NO_3^- -N, NH_4^+ -N, DOC, SOC, and SOC/TN were mainly
182 correlated with RD1 (Fig. 4a). Ammonia nitrogen and DOC were positively correlated with bacterial, actinobacterial,
183 and total PLFAs, and SOC was positively correlated with G^- , bacterial, fungal, and total PLFAs ($P < 0.05$) (Table A3).

184 The first (RD1) and second (RD2) ordination axes explained 50.1% and 19.9% of the total variability in the
185 potential enzyme activities, respectively. The contents of DOC, NO_3^- -N, and NH_4^+ -N were mainly related to RD2 (Fig.
186 4b). Dissolved organic carbon was positively correlated with αG and negatively correlated with βX and AP, and NH_4^+ -N
187 was positively correlated with αG and βG ($P < 0.05$; Table A3). Bacterial and total PLFAs were positively correlated
188 with αG , βG , NAG, PPO, and PER, and fungal PLFAs were positively correlated with αG , βG , and NAG ($P < 0.05$;
189 Table A4).

190 4. Discussion

191 Consistent with our hypothesis, the contents of organic C (including DOC, POC, and SOC) and N (including
192 NH_4^+ -N and TN) were lower in the plots from which the understory vegetation was removed than in those with intact
193 understory vegetation (Table 1), which suggests that understory vegetation promotes C and N cycling in soil. Other
194 researchers reported minimal changes in the soil physical and chemical properties when the understory vegetation was
195 removed (Xiong et al., 2008; Zhao et al., 2011), and the different results may reflect the variable composition of the
196 understory vegetation (Nilsson and Wardle, 2005). In our study, we removed the litter from all the treatments to avoid
197 any effects of above-ground litter. The roots of the Chinese fir trees may take over the space previously occupied by the
198 understory vegetation and may increase their exudation to compensate for the reduced C inputs (Li et al., 2016), and the
199 residues from the roots of understory vegetation may also decompose in the soil (Li et al., 2013). However, the
200 increased quantities of liable C from Chinese fir roots and understory vegetation root residues may not fully compensate
201 for the C loss when the understory vegetation is removed. Additionally, soil C tends to be higher when the plant
202 functional diversity is high (Zhou et al., 2016). When the understory vegetation is removed, the plant diversity
203 decreases, and the soil C content also decreases. Previously, researchers found that the soil N contents increased when
204 the amount of N taken up by plants decreased during tree girdling experiments (Kaiser et al., 2010) and in soils without
205 live roots (Loeppmann et al., 2016a). However, we found that the soil N increased when the understory vegetation
206 remained intact, which suggests that the amount of available N released from plant roots and SOM degradation
207 exceeded the amount taken up by plants. The POC/SOC ratios were lower for the understory vegetation removal plots
208 than for the plots with intact understory vegetation (Table 1), which suggests that POC declined stronger than SOC

209 when the understory vegetation was removed. Since POC is related to aggregate stability, the soil in Chinese fir
210 plantations will be more productive when the understory vegetation remains intact (Bouajila and Gallali, 2010). As also
211 reported by Wang et al. (2014), the SMC decreased when the understory vegetation was removed (Table 1), which
212 shows that the understory vegetation has benefits for soil moisture.

213 Consistent with our hypothesis, total PLFAs declined when the understory vegetation was removed (Fig. 2). It is
214 known that fungal biomass decreases when understory vegetation was removed (Wu et al., 2011; Liu et al., 2012; Zhao
215 et al., 2013). The PLFAs in AMF were lower in the plots with no understory vegetation (Fig. A1), which reflects the
216 reduced plant diversity. Since certain AMF may only grow when specific plants are present, changes in the plant
217 communities over time will result in changes in their mycorrhizal partners (Hart et al., 2001). Other studies have
218 suggested that decreases in the fungal PLFAs were mainly related to a reduction in mycorrhizal fungi, as mycorrhizal
219 fungi are more dependent on below-ground C allocation by plants than other fungi (Kaiser et al., 2010). Mycorrhizal
220 species in the understory vegetation included *Dicranopteris dichotoma*, *Vaccinium bracteatum*, *Loropetalum chinense*,
221 and *Rhododendron*. Chinese fir (arbuscular mycorrhizal plant) monocultures may support fewer fungi biomass than
222 other plantations where the understory vegetation is left intact. The bacterial biomass also decreased after the understory
223 vegetation was removed, mainly because of the decreases in the soil C and N (Table A3) and plant diversity (Lamb et al.,
224 2011). Actinobacteria promote the decomposition of recalcitrant C compounds and, while Brant et al. (2006) considered
225 that they might increase when the available nutrient contents were low, we did not observe such a tendency (Fig. 2),
226 perhaps because of the high variability in the actinobacterial PLFAs in the soils.

227 Consistent with our hypothesis and the results of Huang et al. (2014), we found that the potential extracellular
228 enzyme activities were lower when there was no understory vegetation (Fig. 3). However, Lin et al. (2012) did not
229 observe any changes in soil enzyme activities when understory vegetation was removed. The soil rhizosphere is a
230 hotspot of microbial activities (Kuzyakov and Blagodatskaya, 2015). Decreases in the quantity and diversity of root
231 exudates in the understory vegetation, and changes in the soil abiotic and biotic properties, may cause direct and
232 indirect changes in soil enzyme activities (Liu et al., 2012; Huang et al., 2014). The potential C hydrolase activity was
233 higher when the understory remained intact, indicating the high soil microbial demand for C. The specific C hydrolase
234 activities normalized by PLFAs were lower when the understory vegetation remained intact than when it was removed,
235 which may reflect opportunistic microorganisms (microorganisms that use enzyme products rather than produce
236 enzymes) that emerged in response to an increase in the labile C input (Allison, 2005), and a subsequent decline in the
237 ability of microorganisms to produce C-acquiring enzymes. The ratio of C- to N-acquiring enzymes increased when the
238 understory vegetation was removed, perhaps because the microbes produced enzymes that acquired C rather than N

239 when the labile C inputs were lower. There are various explanations for the changes observed in the potential enzyme
240 activities, as follows: (1) Mycorrhizal fungi vanish when understory vegetation is removed (Fekete et al., 2011), which
241 means there are fewer microorganisms to produce enzymes, so the total amount of enzymes decreases. (2) When the
242 understory vegetation remains intact, root exudates are continuously released to soil, but when the understory vegetation
243 is removed, below-ground root residues are the main source of C for the understory vegetation. Thus, the inputs of C
244 with different chemical compositions may have influenced the enzyme activities (Li et al., 2013).

245 The activities of α G and β G were positively correlated with the contents of the soil inorganic N fractions (Table
246 A3), which suggests that the decomposition of C decreased because of the reduced availability of N when the
247 understory vegetation was removed. The size of soil C pool reflects the balance between the inputs and outputs of C (De
248 Deyn et al., 2008). When understory vegetation is removed, both the soil C inputs, including root exudates, fine root
249 turnover (Liu et al., 2012), and SOM decomposition rate (Wu et al., 2011; Liu et al., 2012; Zhao et al., 2013), and soil C
250 outputs, such as soil respiration (Wang et al., 2013), decrease. The lower SOC contents in the plots from which the
251 understory vegetation was removed therefore indicate that the removal of understory vegetation had more effect on the
252 outputs of soil C than on the inputs. Polyphenols are mainly decomposed by PPO, so the decrease in PPO activity may
253 result in an increase in the content of polyphenols that have toxic effects on soil microbes and inhibit hydrolase
254 activities (Sinsabaugh, 2010). Oxidative enzymes are responsible for the degradation of poor-quality, chemically
255 complex compounds, such as lignin, aromatic compounds, and phenolic compounds (Sinsabaugh, 2010). Therefore, the
256 lower activities of PPO and PER observed after the understory vegetation was removed may result in an increase in the
257 content of refractory compounds in SOM.

258 Phosphorus is generally the most limiting element in the highly weathered red soils in southern China. Soil P is
259 generally present in an organic form or is immobilized when the contents of Al and Fe are high (Margalef et al., 2017).
260 Of all the enzymes we assayed, the activity of AP was the highest (Fig. 3), which may reflect the fact that P was the
261 limiting nutrient in the red soils. Soil microorganisms may produce more phosphatase to mineralize organic P to meet
262 their demand for P (Allison and Vitousek, 2005). Loeppmann et al. (2016a, b) reported that N-degrading enzymes in the
263 rhizosphere of maize-planted soil increased when the available N decreased because of plant N uptake, which suggests
264 that N demand in the rhizosphere might be regulated by a similar mechanism in the cultivated field; we however, did
265 not find any evidence of such a control in our study. The soil nutrient availability affects rhizosphere priming (Dijkstra
266 et al., 2013). The higher potential NAG activity and higher contents of NH_4^+ -N in the treatments with the intact
267 understory vegetation suggest that the energy-rich C compounds released through the roots promoted the production of
268 N-acquiring enzymes that released available N from SOM. The low potential activity of NAG in the treatments from

269 which the understory vegetation was removed was related to the reduction in the fungal biomass, and reflects the fact
270 that chitin, a major structural component of fungal cell walls (Loeppmann et al., 2016b), can be degraded by NAG
271 (Mganga et al., 2015). We did not observe any change in the AP activities when the understory vegetation was removed.
272 Because Chinese firs coexist with fungi and form mycorrhizal associates (Li et al., 2011), and mycorrhizal fungi
273 produce soil acid phosphatase (Rosling et al., 2016), these enzymes were most likely produced by Chinese firs. The
274 negative relationships between the potential activity of AP and DOC suggest that DOC was the substrate for microbes,
275 and that large amounts of DOC were consumed when producing P-acquiring enzymes.

276 5. Conclusions

277 Our results demonstrate that understory vegetation plays an important role in enhancing the soil C and N contents,
278 the soil potential activities of C- and N- hydrolase and oxidase, but does not influence the P-hydrolase activity. The ratio
279 of C- to N-acquiring enzymes increased after the understory vegetation was removed, which implies that, under lower
280 inputs of labile C, microbes invest more in C-acquiring enzymes than N-acquiring enzymes. The positive relationship
281 between the activities of C-degrading enzymes and the soil inorganic N contents suggest that C decomposition was
282 inhibited by the lower available N contents after understory vegetation was removed. The potential activity of AP was
283 negatively correlated with the content of DOC, which indicates that large amounts of DOC, an energy source, were
284 consumed when producing P-acquiring enzymes. Therefore, understory vegetation can contribute to C sequestration by
285 enhancing C inputs to soil, even though C may be lost from soil with understory vegetation through the degradation of
286 SOM by enzymes. We suggest that, as part of routine forestry management, understory vegetation should not be
287 removed from subtropical Chinese fir plantations.

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419 **Figure captions**

420 Fig. 1 Paired-plot design treatments with understory vegetation and litter removal (None), and understory vegetation
421 intact and litter removal (Understory), the same abbreviations are used below

422 Fig. 2 Soil phospholipid fatty acids (PLFAs) in the different understory vegetation treatments

423 Soil PLFA contents (a), ratio of PLFA contents (b). G^+/G^- ratio of gram positive bacteria to gram negative bacteria, F/B
424 ratio of fungi to bacteria. Different lowercases represent significant differences among the **None** and **Understory**
425 treatments ($P < 0.05$). Data are the means \pm standard errors. The same abbreviations apply to Fig. 4.

426 Fig. 3 Soil enzyme activities in the different understory vegetation treatments

427 Soil potential hydrolase activities (a), soil potential oxidase activities (b), enzyme activity ratios (c), soil hydrolase
428 activities normalized by total PLFAs (d). αG α -1,4-glucosidase, βG β -1,4-glucosidase, NAG
429 β -1,4-N-acetylglucosaminidase, βX β -1,4-xylosidase, AP acid phosphatase, PPO phenol oxidase, PER peroxidase. The
430 same abbreviations apply to Fig. 4.

431 Fig. 4 Redundancy analysis of all soil abiotic properties and PLFA contents (a), and potential enzyme activities (b)

432 SMC soil moisture content, pH soil pH, $NO_3^- -N$ soil nitrate nitrogen, $NH_4^+ -N$ soil ammonia nitrogen, TN soil total
433 nitrogen, DOC soil dissolved organic carbon, POC soil particulate organic carbon, SOC soil organic carbon, POC/SOC
434 ratio of POC to SOC, SOC/TN ratio of SOC to TN

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445 **Table captions**

446 Table 1 Soil abiotic properties **in the different understory vegetation treatments**

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473 **Supplementary material**

474 Fig. A1 Contents of arbuscular mycorrhizal fungi in the different understory vegetation treatments

475 Table A1 Soil enzymes and their corresponding substrates and functions

476 Table A2 Enzyme indexes: The potential enzyme activity and the total PLFA contents were used to calculate different
477 enzyme indexes. α -1,4-glucosidase (α G), β -1,4-glucosidase (β G), and β -1,4-xylosidase (β X) represented C-acquiring
478 enzymes, whereas β -1,4-N-acetylglucosaminidase(NAG) represented N-cycling enzymes. Acid phosphatase (AP)
479 represented P-acquiring enzymes

480 Table A3 Pearson correlation coefficients between soil abiotic properties, PLFA contents, and potential enzyme
481 activities

482 Table A4 Pearson correlation coefficients between PLFA contents and potential enzyme activities

483 Table A5 Temporal variation in soil abiotic properties

484 Table A6 Temporal variation in soil PLFA contents

485 Table A7 Temporal variation in soil potential enzyme activities

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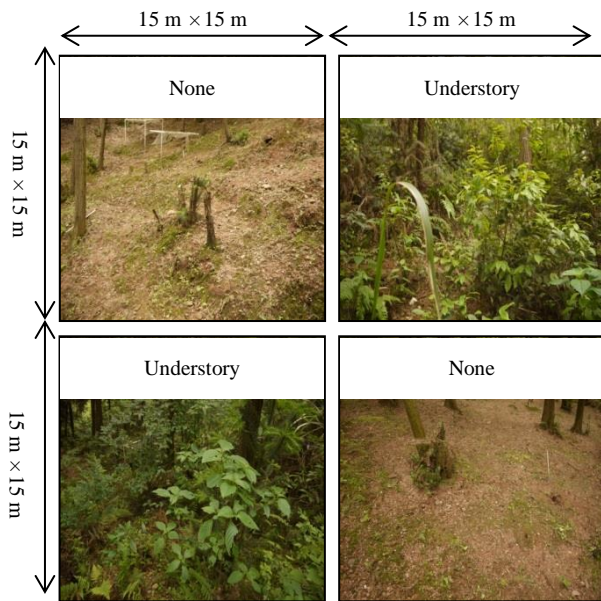
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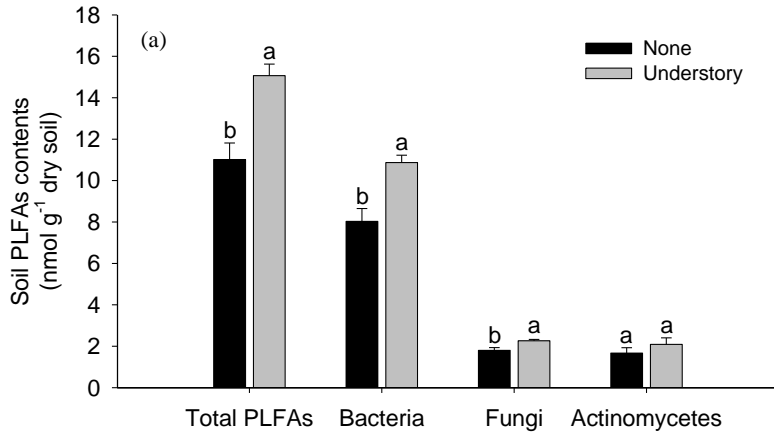
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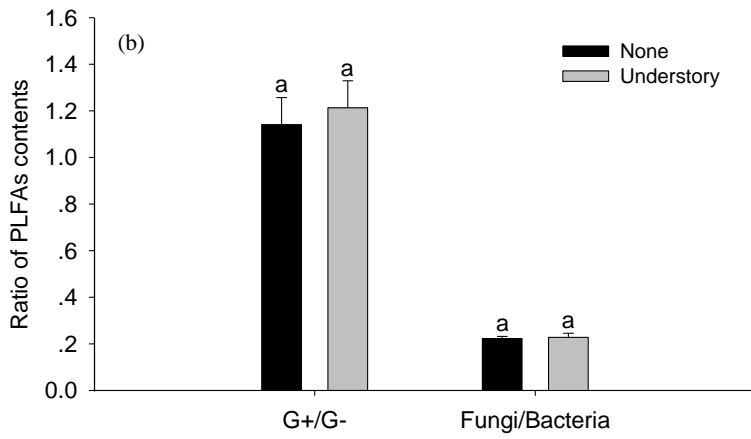
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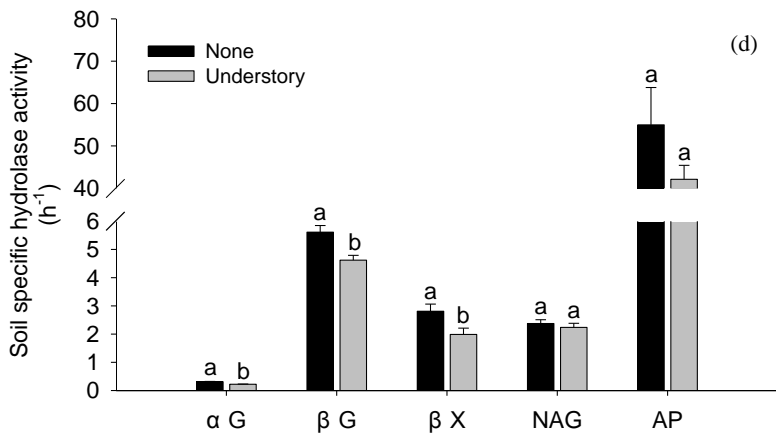
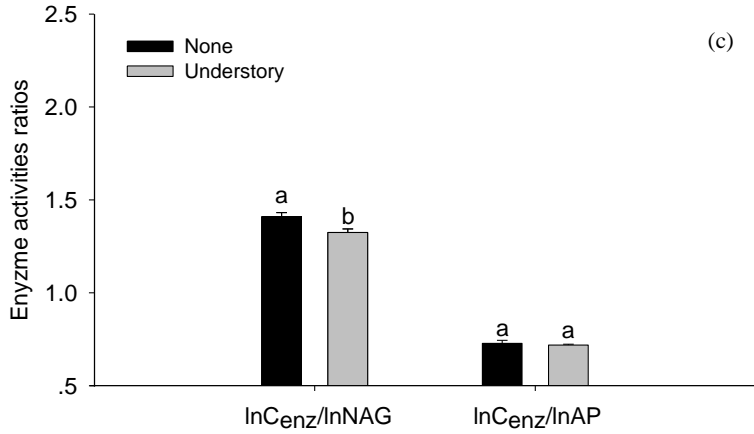
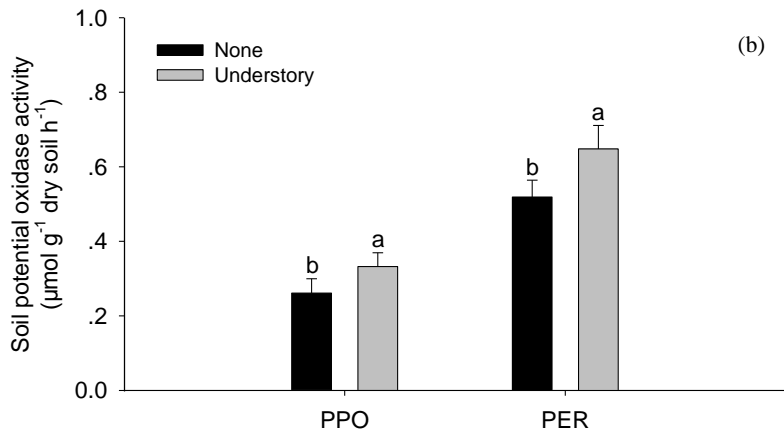
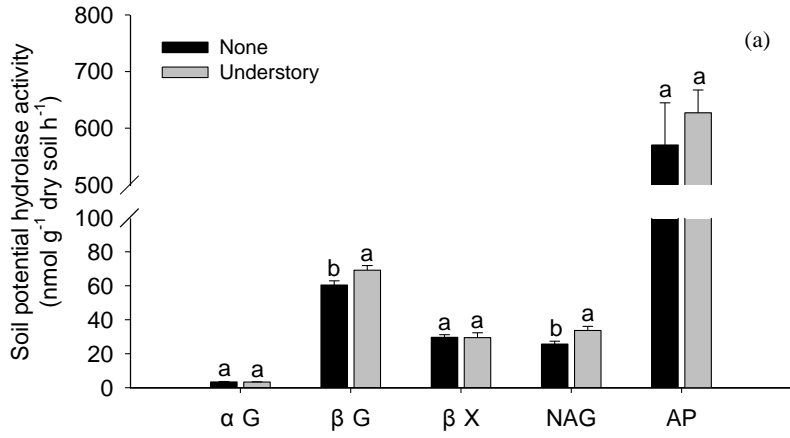
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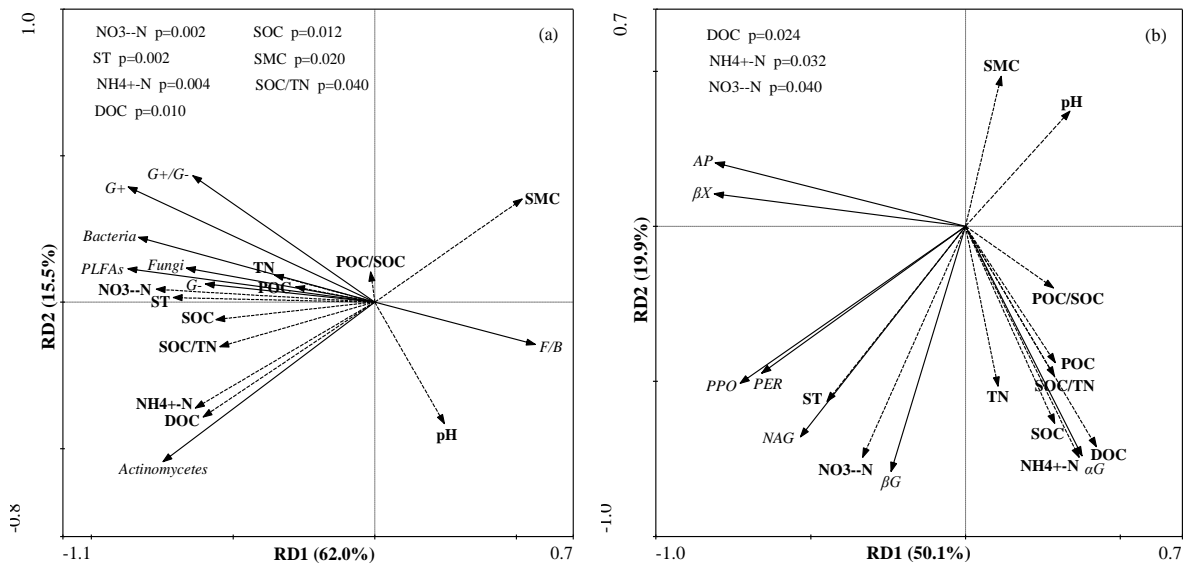
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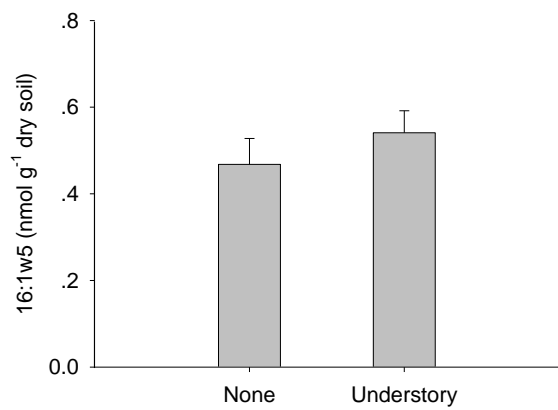
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558 Table 1 Soil abiotic properties in the different understory vegetation treatments

Treatment	ST (°C)	SMC (%)	pH	DOC (mg kg ⁻¹ dry soil)	POC (mg kg ⁻¹ dry soil)	SOC (g kg ⁻¹ dry soil)	NO ₃ ⁻ -N (mg kg ⁻¹ dry soil)	NH ₄ ⁺ -N (mg kg ⁻¹ dry soil)	TN (g kg ⁻¹ dry soil)	POC/SOC (%)	SOC/TN
None	21.1±1. 8a	21.92± 0.9b	4.88±0 .03a	37.3±3.4 b	3.7±0.3b	17.6±0 .8b	4.84±0.6 a	14.72±2. 5b	1.19±0 .04b	20.6±1.0b	14.9±0.4a
Understor y	21.0±1. 7a	22.92± 1.0a	4.87±0 .03a	45.4±4.9 a	4.9±0.3a	20.0±0 .4a	5.50±0.5 a	22.25±3. 7a	1.30±0 .01a	24.2±1.1a	15.4±0.3a

559 Values in the table are the means ± standard error. *ST* soil temperature, *SMC* soil moisture, *pH* soil pH, *NO₃⁻-N* soil
560 nitrate nitrogen, *NH₄⁺-N* soil ammonia nitrogen, *TN* soil total nitrogen, *DOC* soil dissolved organic carbon, *POC* soil
561 particulate organic carbon, *SOC* soil organic carbon, *POC/SOC* ratio of POC to SOC, *SOC/TN* ratio of SOC to TN.
562 Different lowercase letters represent significant differences between **None** and **Understory** treatments ($P < 0.05$). **Data**
563 were means ± standard errors. The same abbreviations are used below.

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583 Fig. A1

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600 Table A1 Soil enzymes and their corresponding substrates and functions

Enzyme	E. C	Abbreviation	Substrate	Function
Peroxidase	1.11.1.7	PER	L-DOPA	Oxidize lignin and aromatic compounds using H ₂ O ₂ or secondary oxidants as an electron acceptor (Sinsabaugh, 2010).
Phenol oxidase	1.10.3.2	PPO	L-DOPA	Oxidize phenolic compounds using oxygen as an electron acceptor (Sinsabaugh, 2010).
α -1,4-glucosidase	3.2.1.20	α G	4-MUB- α -D-glucoside	Releases glucose from starch (Stone et al., 2014).
β -1,4-glucosidase	3.2.1.21	β G	4-MUB- β -D-glucoside	Releases glucose from cellulose (Stone et al., 2014).
β -1,4-xylosidase	3.2.1.37	β X	4-MUB- β -D-xyloside	Releases xylose from hemicellulose (Stone et al., 2014).
β -1,4-N -acetylglucosaminidase	3.2.1.14	NAG	4-MUB-N-acetyl- β -D -glucosaminide	Releases N-acetyl glucosamine from oligosaccharides (Stone et al., 2014).
Acid phosphatase	3.1.3.1	AP	4-MUB-phosphate	Releases phosphate groups (Stone et al., 2014).

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616 Table A2 Enzyme indexes: The potential enzyme activity and the total PLFA contents were used to calculate different
 617 enzyme indexes. α -1,4-glucosidase (α G), β -1,4-glucosidase (β G), and β -1,4-xylosidase (β X) represented C-acquiring
 618 enzymes, whereas β -1,4-N-acetylglucosaminidase(NAG) represented N-cycling enzymes. Acid phosphatase (AP)
 619 represented P-acquiring enzymes.

Enzyme indexes	Description	Reference
$\alpha G_{PLFAs}; \beta G_{PLFAs}; \beta X_{PLFAs}$	Specific enzyme activity of C-acquiring enzymes (enzyme activities to total PLFAs)	Zhang et al., 2015; Zhang et al., 2017
NAG_{PLFAs}	Specific enzyme activity of N-acquiring enzymes (enzyme activities to total PLFAs)	Zhang et al., 2015; Zhang et al., 2017
AP_{PLFAs}	Specific enzyme activity of P-acquiring enzymes (enzyme activities to total PLFAs)	Zhang et al., 2015; Zhang et al., 2017
$\ln C_{enz}/\ln NAG$	Ratio of C- to N- acquiring enzymes	Stone et al., 2014; Loeppmann et al., 2016a; Xu et al., 2017
$\ln C_{enz}/\ln AP$	Ratio of C- to P- acquiring enzymes	Stone et al., 2014; Loeppmann et al., 2016a; Xu et al., 2017

620 C_{enz} : the total C-acquiring enzyme activity (the potential activities of α G + β G + β X).
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624 Table A3 Pearson correlation coefficients between soil abiotic properties, PLFA contents, and potential enzyme
 625 activities

Abiotic Properties		ST	SMC	pH	NO ₃ ⁻ N	NH ₄ ⁺ N	TN	DOC	POC	SOC	POC/SO C	SOC/T N
PLFAs	G ⁺	0.77**	-0.45	-0.38	0.72**	0.28	0.11	0.24	0.06	0.26	-0.13	0.39
	G ⁻	-0.05	0.15	-0.01	0.18	0.38	0.70*	0.27	0.52	0.68*	0.33	0.29
							*		*	*		
	Bacteria	0.44	-0.24	-0.25	0.58*	0.62**	0.53*	0.57*	0.48	0.65*	0.27	0.46
									*	*		
	Fungi	0.11	-0.02	-0.20	0.40	0.43	0.68*	0.39	0.56	0.72*	0.38	0.36
							*		*	*		
Actinobacteria	0.65**	-0.67*	-0.13	0.69**	0.69**	0.22	0.63**	0.08	0.36	-0.14	0.37	
		*										
PLFAs	0.54*	-0.37	-0.26	0.69**	0.63**	0.47*	0.60**	0.41	0.58*	0.20	0.43	
G ⁺ /G ⁻	0.88**	-0.57*	-0.40	0.71**	0.14	-0.17	0.18	-0.1	-0.02	-0.29	0.25	
Enzymes	F/B	-0.50*	0.22	-0.01	-0.30	-0.17	-0.07	-0.15	0.03	-0.18	0.22	-0.24
	αG	0.40	-0.54*	-0.30	0.51*	0.64**	0.30	0.69**	0.23	0.45	0.04	0.44
	βG	0.57*	-0.41	-0.40	0.67**	0.50*	0.38	0.42	0.16	0.37	-0.03	0.22
	NAG	0.54*	-0.30	-0.40	0.64**	0.32	0.36	0.23	0.25	0.32	0.11	0.15
	βX	0.30	-0.06	-0.49*	0.30	-0.46	-0.06	-0.52*	-0.3	-0.34	-0.38	-0.43
	AP	0.28	0.00	-0.16	0.09	-0.44	-0.21	-0.48*	-0.3	-0.38	-0.32	-0.33
	PPO	0.86**	-0.57*	-0.33	0.72**	0.25	-0.01	0.23	-0.1	0.05	-0.28	0.14
PER	0.81**	-0.54*	-0.12	0.61**	0.37	-0.01	0.32	-0.0	0.13	-0.18	0.23	

626 Values are the Pearson *r* value. * indicates a significant difference at $P < 0.05$; ** indicates a significant difference at P
 627 < 0.01 . G⁺ gram positive bacteria, G⁻ gram negative bacteria, PLFAs total PLFAs, G⁺/G⁻ ratio of G⁺ to G⁻, F/B ratio
 628 of fungi to bacteria. αG α-1,4-glucosidase, βG β-1,4-glucosidase, NAG β-1,4-N-acetylglucosaminidase, βX
 629 β-1,4-xylosidase, AP acid phosphatase, PPO phenol oxidase, PER peroxidase. These abbreviations apply to Table A4,
 630 A5, A6 and A7.

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635 Table A4 Pearson correlation coefficients between PLFA contents and potential enzyme activities

Factors	G ⁺	G ⁻	Bacteria	Fungi	Actinobacterias	PLFAs	G ⁺ /G ⁻	F/B
αG	0.29	0.46	0.53*	0.51*	0.61**	0.48*	0.12	-0.17
βG	0.67**	0.57*	0.83**	0.65**	0.70**	0.83**	0.52*	-0.27
βX	0.71**	0.46	0.73**	0.58*	0.47	0.73**	0.60**	-0.28
NAG	0.40	-0.15	0.01	0.02	-0.11	0.02	0.52*	-0.02
AP	0.32	-0.24	0.03	-0.14	-0.15	0.08	0.49*	-0.07
PPO	0.84**	0.09	0.57*	0.28	0.46	0.64**	0.91**	-0.44
PER	0.79**	0.04	0.55*	0.21	0.47*	0.62**	0.86**	-0.46

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650 Table A5 Temporal variation in soil abiotic properties

Treatment	Time	ST (°C)	SWC (%)	pH	NO ₃ ⁻ N (mg kg ⁻¹ dry soil)	NH ₄ ⁺ -N (mg kg ⁻¹ dry soil)	TN (g kg ⁻¹ dry soil)	DOC (mg kg ⁻¹ dry soil)	POC (g kg ⁻¹ dry soil)	SOC (g kg ⁻¹ dry soil)	POC/S OC (%)	SOC/TN
None	April	18.9±0.3aA	22.8±0.5aA	4.88±0.04aA	4.9±0.8aA	23.1±1.8bA	1.29±0.08aA	45.9±3.5bA	4.36±0.63aA	19.7±1.7aA	21.9±1.5aA	15.3±0.8aA
	July	28.1±0.2aA	18.8±0.5aB	4.80±0.04aA	6.5±0.4aA	14.6±0.4bB	1.13±0.06aA	40.5±3.6bA	3.03±0.37aA	16.9±0.7aA	18.1±2.2bA	15.4±0.9aA
	Novemb er	16.4±0.2aC	24.1±1.0bA	4.95±0.04aA	3.1±0.3aB	6.4±0.4aC	1.16±0.03aA	25.6±0.2bA	3.55±0.03bA	16.3±0.3bA	21.8±0.4aA	14.0±0.6aA
Understo ry	April	18.8±0.0aB	22.6±0.6aB	4.89±0.07aA	4.9±0.7aB	29.8±2.1aA	1.29±0.00aA	57.3±4.0aA	5.17±0.43aA	20.3±0.9aA	25.6±1.5aA	15.8±0.7aA
	July	27.6±0.2bA	19.9±0.4aC	4.86±0.07aA	7.1±0.4aA	29.24±0.8aA	1.29±0.03aA	51.4±5.0aA	4.48±0.84aA	19.9±1.2aA	22.1±2.9aA	15.4±0.7aA
	Novemb er	16.5±0.2aC	26.3±0.9aA	4.86±0.04aA	4.5±0.3aB	7.8±0.2aB	1.32±0.01aA	27.5±0.2aA	4.93±0.28aA	19.7±0.3aA	24.9±1.0aA	15.0±0.3aA

651 Different lowercase letters represent significant differences between different treatments, and different uppercase letters
652 represent significant differences among different months in the same treatment ($P < 0.05$). The same abbreviations
653 apply to Table A6 and A7.

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664 Table A6 Temporal variation in soil PLFAs

Treatment	Time	G ⁺ (nmol g ⁻¹ dry soil)	G ⁻ (nmol g ⁻¹ dry soil)	Bacteria (nmol g ⁻¹ dry soil)	Fungi (nmol g ⁻¹ dry soil)	AMF (nmol g ⁻¹ dry soil)	Actinobacte rias (nmol g ⁻¹ dry soil)	PLFAs (nmol g ⁻¹ dry soil)	G ⁺ /G ⁻	F/B
None	April	4.25±0.	4.61±0.	8.86±0.9	2.07±0.3	0.36±0.0	2.10±0.22a	11.56±0.75	0.93±0.01a	0.21±0.
		44aB	50aA	4aA	0aA	5aB	A	bA	B	01aAB
	July	6.28±0.	3.62±0.	9.31±0.1	1.89±0.0	0.69±0.0	2.09±0.22a	13.29±0.30	1.59±0.07a	0.20±0.
		47aA	08aAB	3bA	3bA	5aA	A	aA	A	00aB
	November	2.82±0.	3.11±0.	5.93±0.5	1.45±0.0	0.35±0.0	0.817±0.41	8.19±0.52b	0.90±0.05a	0.25±0.
		34bB	22aB	6bB	7bA	4aB	aB	B	B	02aA
Understory	April	3.81±0.	4.32±0.	10.53±0.	2.21±0.0	0.43±0.2	2.05±0.06a	14.62±0.50	0.89±0.05a	0.26±0.
		46aC	21aA	54aA	8aA	6aB	AB	aAB	B	04aA
	July	7.22±0.	4.52±0.	11.76±0.	2.23±0.0	0.73±0.4	2.99±0.36a	16.67±0.71	1.62±0.04a	0.19±0.
		25aA	29aA	51aA	4aA	3aA	A	aA	A	01aA
	November	5.41±0.	4.92±0.	10.32±0.	2.35±0.2	0.47±0.4	1.23±0.55a	13.90±0.98	1.13±0.15a	0.23±0.
		51aB	28aA	59aA	1aA	5aB	B	aB	B	03aA

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679 Table A7 Temporal variation in soil potential enzyme activities

Treatment	Time	α G (nmol g ⁻¹ dry soil h ⁻¹)	β G (nmol g ⁻¹ dry soil h ⁻¹)	β X (nmol g ⁻¹ dry soil h ⁻¹)	NAG (nmol g ⁻¹ dry soil h ⁻¹)	AP (nmol g ⁻¹ dry soil h ⁻¹)	PPO (nmol g ⁻¹ dry soil h ⁻¹)	PER (nmol g ⁻¹ dry soil h ⁻¹)
None	April	3.93±0.41aA	61.9±4.3aAB	24.8±0.2aB	24.9±3.2aA	300.5±22.9aB	0.18±0.02aB	0.40±0.03b B
	July	3.74±0.09aA	66.7±1.3aA	33.6±2.7aA	29.3±3.1bA	711.9±79.8aA	0.41±0.02aA	0.69±0.03b A
	November	2.48±0.12aB	52.8±2.1aB	30.5±1.7aAB	22.8±2.0bA	698.63±70.3a A	0.20±0.03aB	0.47±0.02aB
Understory	April	3.72±0.15aA	65.9±3.9aA	21.3±5.8aA	26.8±3.1aB	492.4±48.8aB	0.24±0.01aC	0.52±0.03aB
	July	3.35±0.19aA B	75.8±6.1aA	33.3±1.8aA	41.6±2.1aA	699.5±47.8aA	0.48±0.01aA	0.89±0.04a A
	November	2.90±0.12aB	65.7±2.3aA	33.8±2.8aA	32.6±1.6aB	689.32±35.1a A	0.28±0.01aB	0.53±0.04aB

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