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

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Abstract:

While we know that understory vegetation affects the soil microbial biomass and extracellular enzyme activities in subtropical Chinese fir (*Cunninghamia lanceolata*) forests, we are less certain about the degree of its influence. We determined the degree to which the soil abiotic and biotic properties, such as PLFAs and extracellular enzyme activities, were controlled by understory vegetation. We established a paired treatment in a subtropical Chinese fir plantation, which comprised one plot from which the understory vegetation and litter were removed (None) and another from which the litter was removed but the understory vegetation was left intact (Understory). We evaluated how the understory vegetation influenced the soil abiotic properties; the bacterial, fungal, and actinobacterial PLFAs, and the activities of five hydrolases and two oxidative enzymes. The dissolved organic carbon (DOC), particulate organic carbon, soil organic carbon, ammonia nitrogen (NH_4^+-N), and total nitrogen contents and soil moisture were 18%, 25%, 12%, 34%, 8%, and 4% lower in the None treatments than in the Understory treatments, respectively ($P < 0.05$). Soil bacterial, fungal, and total PLFAs, and the potential activities of β-1,4-glucosidase (βG), β-1,4-N-acetylglucosaminidase, phenol oxidase, and peroxidase were as much as 24% lower in None treatments than in the Understory treatments ($P < 0.05$). The specific activities of C-acquiring enzymes were as much as 41% higher ($P < 0.05$), and the ratio of C- to N-acquiring enzymes was also higher, in the None treatments than in the Understory treatments. This suggests that in the absence of understory vegetation microbes invested more in C acquisition than N acquisition because the carbon (C) inputs were less labile. The negative relationship between DOC and AP shows that DOC is consumed when P-acquiring enzymes are produced. The positive correlation between NH_4^+-N and βG suggested the increased availability of N promoted the decomposition of C. More extracellular enzymes that degrade soil organic matter are produced when there

30   is understory vegetation, which leads to losses of soil C. On the other hand, the soil C sink is maintained by increased

31   inputs of C. We can therefore conclude that understory vegetation contributes to C sequestration in Chinese fir forests

32   and suggest that understory should be 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

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 understory vegetation is removed from forest ecosystems, soil processes are influenced, such that above-ground

39   plant diversity and biomass decrease (Lamb et al., 2011; Fu et al., 2015) and the characteristics of the below-ground

40   rhizodeposits change (Li et al., 2013). The understory vegetation absorbs water and nutrients from soil (Wang et al.,

41   2014), and also releases carbohydrates back to the soil as discarded root cap and border cells; mucilage and exudates

42   from roots (McNear Jr, 2013), and cellulose, hemicelluloses, and lignin from leaf litter (Loeppmann et al., 2016a, b).

43   The net effect of understory vegetation on soil nutrients is determined by the balance between its nutrient demand and

44   capacity to release carbohydrates to soil via the decomposition of understory-derived litter and rhizodeposits.

45   Soil extracellular enzymes produced by microorganisms or plant roots catalyze the cycling of soil C, nitrogen (N),

46   and phosphorus (P) (Burns et al., 2013; Nannipieri et al., 2018). Because they respond rapidly to soil environmental

47   changes, soil enzyme activities are often used as indicators of soil quality (Trasar-Cepedaet al., 2008; Burns et al., 2013).

48   Individual enzyme activities can reflect the substrate availability, the nutrient requirements of microorganisms and

49   plants, and the strategies used by microbes and plants to maintain the nutrient balance when the soil environment

50   changes (Burns et al., 2013; Nannipieri et al., 2018). Because it is difficult to know whether changes in the enzymatic

51   activities reflect changes in the soil microbial biomass or differences in the actual activities (Trasar-Cepeda et al., 2008),

52   we need to study the specific enzyme activities, i.e., the activity normalized to the total PLFA contents (Zhang et al.,

53   2015; Zhang et al., 2017). The enzyme ratio is used to examine the relative allocation of energy versus nutrient

54   acquisition, since it intersects the metabolic theory of microbial ecology and the theory of ecological stoichiometry

55   (Stone et al., 2014; Loeppmann et al., 2016a; Xu et al., 2017). By studying how the enzyme activities and ratios change

56   when the understory vegetation is removed, we hope to improve our understanding of how the storage of C in soil is

57   influenced by the understory vegetation, and how microbial nutrient acquisition is affected by microbial biomass and

58   soil nutrients.

59   Studies have shown that understory vegetation-induced changes in soil properties are closely related to climate,

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

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

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

2. Material and Methods

2.1 Experimental treatments

Our study site was in the Shixi forest plantation in Taihe County, Jiangxi Province, China (115°03′29.9″ E, 26°44′29.1″ N). The area has a subtropical monsoon climate with a mean annual temperature of 18.8 ℃ and a mean

90   annual precipitation of 1340 mm. According to the USDA-NRCS soil taxonomy (Soil Survey Staff, 1996), the soil in

91   this area is dominated by Udults, which forms from red sandstone and sandy conglomerate and has moist and dry

92   Munsell values of 7.5 YR 5/6 and 7.5 YR 6/6, respectively.

93   The study site is a second-generation Chinese fir plantation that was planted in 1998. The average tree height and

94   diameter at breast height (measured at 1.3 m above ground level) were about 18 m and 17 cm, respectively. The

95   understory vegetation, including shrubs and herbs, is dominated by Old World forked fern (*Dicranopteris dichotoma*

96   *Berth*), gambir (*Uncaria*), oriental blueberry (*Vaccinium bracteatum*), nutgall tree (*Rhus chinensis*), Chinese witch hazel

97   (*Loropetalum chinense*), short shank robe oak (*Quercus glandulifera BI.*), root of mayflower glorybower (*Clerodendron*

98   *cyrtophyllum Turcz*), and azalea (*Rhododendron*).

99   Three plots, measuring 30 × 30 m and separated by a buffer of a least 10 m to avoid any between-plot influence,

100   were established in the plantation in January 2013. One paired treatment with three replicates was established within

101   each of the three plots. Each plot was divided into 4 subplots (15 × 15 m each) and contained 2 treatments, namely

102   None, from which both the understory vegetation and litter were removed, and Understory, from which the litter was

103   removed but the understory vegetation was left. The two subplots in a plot with the same treatment were distributed

104   across each plot to avoid the effects of slope (Fig. 1) and their results were averaged. The litter and understory were

105   managed monthly. The amount of litter and understory vegetation at the study site amounted to about 1020 and 6236 kg

106   ha^{-1} year^{-1}, respectively, under natural conditions.

107   2.2 Soil sampling and analysis

108   Bulk soil samples were collected in the wet (April and November) and dry (July) seasons in 2015. Five soil cores

109   with an inner diameter of 5 cm were collected randomly from between the surface and a depth of 10 cm from each

110   subplot and then mixed as one composite sample. All fresh soil samples were sieved to 2 mm and stored at 4 ℃ until

111   analysis.

112   Soil physical and chemical properties were determined as outlined by Bao (2008). Soil temperature (ST) was

113   determined at a depth of 10 cm with a soil thermometer (TP101) during sampling. The soil moisture content (SMC) was

114   measured by drying aliquots of soil at 105 ℃ to constant weight. Soil pH was measured at a soil to water ratio of 1:2.5

115   by a pH digital meter. The contents of nitrate N (NO_3^--N) and ammonia N (NH_4^+-N) were measured with a continuous

116   flow analyzer (Bran Luebbe, AA3) after extraction with a 2 M KCl solution (soil: solution ratio of 1: 10). Dissolved

117   organic carbon (DOC) contents were measured with a TOC analyzer (Elementar, Liquid *II*) after extraction with

118   ultra-pure water (soil: solution ratio of 1: 5) (Jones and Willett. 2006). Particulate organic carbon (POC) was determined

119   as outlined by Garten et al. (1999). The contents of soil organic C (SOC) and total nitrogen (TN) were measured with an

4

120   elemental analyzer (Vario Max CN).

121   Soil phospholipid fatty acids (PLFAs) were extracted following the procedure outlined by Bossio and Scow (1998),

122   and were determined with a gas chromatograph (Agilent 6890N). Soil total PLFAs were represented by various PLFA

123   biomarkers; gram positive bacteria (G$^+$) were represented by i14:0, i15:0, a15:0, i16:0, i17:0, and a17:0, and gram

124   negative bacteria (G$^-$) were represented by 16:1ω7c, cy17:0, 16:1ω9c, and cy19:0. The total bacterial PLFAs were

125   represented by biomarkers of G$^+$ and G$^-$. The total fungi PLFAs were represented by arbuscular mycorrhizal fungi

126   (AMF) biomarkers 16:1ω5, as well as 18:1ω9c, 18:2ω6c, and 18:3ω6c, and the actinobacterial PLFAs were represented

127   by 10Me16:0, 10Me17:0, and 10Me18:0 (Bradley et al., 2007; Denef et al., 2009).

128   Soil enzyme activities were measured following the methods of Saiya-Cork et al. (2002). The specific substrates

129   and functions of the enzymes assayed are listed in Table A1. Five hydrolase activities (α-1,4-glucosidase (αG),

130   β-1,4-glucosidase (βG), β-1,4-N-acetylglucosaminidase(NAG), β-1,4-xylosidase (βX) and acid phosphatase (AP)) were

131   assayed using fluorogenically-labeled substrates. Briefly, a soil suspension was prepared by adding 1 g of fresh soil to

132   125 mL of 50 mM acetate buffer. We added 200 μL of the soil suspension and 50 μL of the substrate solution (200 μM)

133   to 96 microplates, making a total of 8 analytical replicates. Methylumbelliferone (MUB) was used to calibrate the

134   hydrolase activities. The microplates were incubated in the dark at 20 ℃ for up to 4 h. After incubation, 10 μL of 1 M

135   NaOH was added to each well to terminate the enzymatic reaction. When the reactions had ended, the fluorescence was

136   measured using a microplate fluorometer (SynergyH4, BioTek) with excitation and emission filters of 365 and 450 nm,

137   respectively. We calculated the specific enzyme activities by dividing the individual potential hydrolase activities by the

138   total PLFA contents (Zhang et al., 2015; Zhang et al., 2017). The total C-acquiring enzyme activity (C$_{enz}$) was

139   operationally defined as the sum of the αG, βG, and βX activities (Stone et al., 2014) (Table A2).

140   The soil oxidase activities (polyphenol oxidase (PPO) and peroxidase (PER)) were assayed spectrophotometrically.

141   We added 600 μL of the soil suspension and 150 μL of the substrate solution to deep-well plates. We also added 30 μL

142   of 0.3% H$_2$O$_2$ solution before determining PER. After incubation in the dark at 20 ℃ for up to 5 h, the deep-well plates

143   were centrifuged for 3 minutes at 3000 r h^{-1}. We then transferred 250 μL of the supernatant to the microplates and

144   measured the absorbance at 450 nm with a microplate fluorometer (SynergyH4, BioTek) (DeForest, 2009).

145   2.3 Statistical Analysis

146   Data are presented as the means ± standard errors. By applying the one-sample Kolmogorov-Smirnov test within

147   SPSS 17.0, we found that the data satisfied the normal distribution criteria. We assessed the differences between the soil

148   abiotic properties, PLFA contents, and enzyme activities for the understory treatments with a paired-sample *t*-test (SPSS

149   17.0). Where two subplots within the same plot had the same treatment, we averaged the data before analysis. We

150   investigated the relationships between the soil abiotic properties, PLFA contents, and enzyme activities for the two

151   treatments using redundancy analysis (RDA, CANOCO, version 4.5) and Pearson correlation analysis (SPSS 17.0). We

152   tested the significance of the variables with the Monte Carlo Permutation Test before applying RDA. Figures were

153   generated using SigmaPlot (Version 10.0). A significance level of $P < 0.05$ was applied throughout.

3. Results

154

155   3.1 Soil abiotic properties

156   The results suggest that the soil abiotic properties were influenced by the understory vegetation management

157   (Table 1). The contents of DOC, POC, SOC, NH_4^+-N, and TN were 18%, 25%, 12%, 34%, and 8% lower in the None

158   treatments than in the Understory treatments ($P < 0.05$), respectively. The SMC and POC/SOC were also 4% and 15%

159   lower in the None treatments than in the Understory treatments, respectively ($P < 0.05$). There were no significant

160   differences between the contents of NO_3^--N, ST, pH, and the SOC/TN ratios in the None and the Understory treatments

161   ($P > 0.05$).

162   3.2 Soil PLFAs

163   The soil total PLFAs were 27% lower in the None treatments than in the Understory treatments (Fig. 2).

164   Specifically, the bacterial and fungal PLFAs were 26% and 20% lower ($P < 0.05$) in the None treatments than in the

165   Understory treatments, respectively, but there were no significant differences between the G^+, G^-, or actinobacterial

166   PLFAs in the two treatments ($P > 0.05$). The fungi/bacteria ratios did not change because the bacterial and fungal

167   PLFAs were both lower in the None treatments.

168   3.3 Soil enzyme activities

169   The soil enzyme activities varied as the understory vegetation management varied. The potential activities of βG,

170   NAG, PPO, and PER were 13%, 24%, 21%, and 20% lower in the None treatments than in the Understory treatments

171   (Fig. 3a and b) ($P < 0.05$), respectively, but the potential activities of acid phosphatases did not differ significantly ($P >$

172   0.05) between the two treatments. The ratio of $lnC_{enz}/lnNAG$ was 6% higher in the None treatments than in the

173   Understory treatments, but the ratios of $lnC_{enz}/lnAP$ were similar for the different treatments. The trends were

174   enzyme-specific when normalized by the total PLFAs (Fig. 3d and e). The specific activities of the C-acquiring

175   enzymes, i.e., $αG_{PLFAs}$, $βG_{PLFAs}$ and $βX_{PLFAs}$, were 40%, 22%, and 41% higher, respectively, in the None treatments than

176   in the Understory treatments ($P < 0.05$), but the specific activities of N- (NAG_{PLFAs}) and P-acquiring enzymes (AP_{PLFAs})

177   were not significantly different between the two treatments ($P > 0.05$).

178   3.4 Correlations between soil enzyme activities, soil PLFAs, and soil abiotic properties

179   The first (RD1) and second (RD2) ordination axes explained 62.0% and 15.5% of the total variability in the

180   different PLFAs, respectively. Soil temperature, SMC, NO_3^--N, NH_4^+-N, DOC, SOC, and SOC/TN were mainly

181   correlated with RD1 (Fig. 4a). Ammonia nitrogen and DOC were positively correlated with bacterial, actinobacterial,

182   and total PLFAs, and SOC was positively correlated with G^-, bacterial, fungal, and total PLFAs ($P < 0.05$) (Table A3).

183   The first (RD1) and second (RD2) ordination axes explained 50.1% and 19.9% of the total variability in the

184   potential enzyme activities, respectively. The contents of DOC, NO_3^--N, and NH_4^+-N were mainly related to RD2 (Fig.

185   4b). Dissolved organic carbon was positively correlated with αG and negatively correlated with βX and AP, and NH_4^+-N

186   was positively correlated with αG and βG ($P < 0.05$; Table A3). Bacterial and total PLFAs were positively correlated

187   with αG, βG, NAG, PPO, and PER, and fungal PLFAs were positively correlated with αG, βG, and NAG ($P < 0.05$;

188   Table A4).

189   **4. Discussion**

190   Consistent with our hypothesis, the contents of organic C (including DOC, POC, and SOC) and N (including

191   NH_4^+-N and TN) were lower in the plots from which the understory vegetation was removed than in those with intact

192   understory vegetation (Table 1), which suggests that understory vegetation promotes C and N storage in soil. Other

193   researchers reported minimal changes in the soil physical and chemical properties when the understory vegetation was

194   removed (Xiong et al., 2008; Zhao et al., 2011), and the different results may reflect the variable composition of the

195   understory vegetation (Nilsson and Wardle, 2005). In our study, we removed the litter from all treatments. The roots of

196   the Chinese fir trees may take over the space previously occupied by the understory vegetation and may increase their

197   exudation to compensate for the reduced C inputs (Li et al., 2016), and the residues from the roots of understory

198   vegetation may also decompose in the soil (Li et al., 2013). However, the increased quantities of labile C from Chinese

199   fir roots and understory vegetation root residues may not fully compensate for the C loss when the understory

200   vegetation is removed. Additionally, soil C tends to be higher when the plant functional diversity is high (Zhou et al.,

201   2016). When the understory vegetation is removed, the plant diversity decreases, and the soil C content also decreases.

202   Previously, researchers found that soil N contents increased when the amount of N taken up by plants decreased during

203   tree girdling experiments (Kaiser et al., 2010) and in soils without live roots (Loeppmann et al., 2016a). However, we

204   found that the soil N increased when the understory vegetation remained intact, which suggests that the amount of

205   available N released from plant roots and SOM degradation exceeded the amount taken up by plants. The POC/SOC

206   ratios were lower for the understory vegetation removal plots than for the plots with intact understory vegetation (Table

207   1), which suggests that POC declined stronger than SOC when the understory vegetation was removed. Since POC is

208   related to aggregate stability, the soil in Chinese fir plantations will be more productive when the understory vegetation

209   remains intact (Bouajila and Gallali, 2010). As also reported by Wang et al. (2014), the SMC decreased when the

210   understory vegetation was removed (Table 1), which shows that the understory vegetation has benefits for soil moisture.

211   Consistent with our hypothesis, total PLFAs declined when the understory vegetation was removed (Fig. 2). It is

212   known that fungal biomass decreases when understory vegetation was removed (Wu et al., 2011; Liu et al., 2012; Zhao

213   et al., 2013). The PLFAs in AMF were lower in the plots with no understory vegetation (Fig. A1), which reflects the

214   reduced plant diversity. Since certain AMF may only grow when specific plants are present, changes in the plant

215   communities over time will result in changes in their mycorrhizal partners (Hart et al., 2001). Other studies have

216   suggested that decreases in the fungal PLFAs were mainly related to a reduction in mycorrhizal fungi, as mycorrhizal

217   fungi are more dependent on below-ground C allocation by plants than other fungi (Kaiser et al., 2010). Mycorrhizal

218   species in the understory vegetation included *Dicranopteris dichotoma*, *Vaccinium bracteatum*, *Loropetalum chinense*,

219   and *Rhododendron*. Chinese fir (arbuscular mycorrhizal plant) monocultures may support fewer fungi biomass than

220   other plantations where the understory vegetation is left intact. The bacterial biomass also decreased after the understory

221   vegetation was removed, mainly because of the decreases in the soil C and N (Table A3) and plant diversity (Lamb et al.,

222   2011). Actinobacteria promote the decomposition of recalcitrant C compounds and, while Brant et al. (2006) considered

223   that they might increase when the available nutrient contents were low, we did not observe such a tendency (Fig. 2),

224   perhaps because of the high variability in the actinobacterial PLFAs in the soils.

225   Consistent with our hypothesis and the results of Huang et al. (2014), we found that the potential extracellular

226   enzyme activities were lower when there was no understory vegetation (Fig. 3). However, Lin et al. (2012) did not

227   observe any changes in soil enzyme activities when understory vegetation was removed. The soil rhizosphere is a

228   hotspot of microbial activities (Kuzyakov and Blagodatskaya, 2015). Decreases in the quantity and diversity of root

229   exudates in the understory vegetation, and changes in the soil abiotic and biotic properties, may cause direct and

230   indirect changes in soil enzyme activities (Liu et al., 2012; Huang et al., 2014). The potential C hydrolase activity was

231   higher when the understory remained intact, indicating the high soil microbial demand for C. The specific C hydrolase

232   activities normalized by PLFAs were lower when the understory vegetation remained intact than when it was removed,

233   which may reflect opportunistic microorganisms (microorganisms that use enzyme products rather than produce

234   enzymes) that emerged in response to an increase in the labile C input (Allison, 2005), and a subsequent decline in the

235   ability of microorganisms to produce C-acquiring enzymes. The ratio of C- to N-acquiring enzymes increased when the

236   understory vegetation was removed, perhaps because the microbes produced enzymes that acquired C rather than N

237   when the labile C inputs were lower. There are various explanations for the changes observed in the potential enzyme

238   activities, as follows: (1) Mycorrhizal fungi vanish when understory vegetation is removed (Fekete et al., 2011), which

239   means there are fewer microorganisms to produce enzymes, so the total amount of enzymes decreases. (2) When the

240 understory vegetation remains intact, root exudates are continuously released to soil, but when the understory vegetation

241 is removed, below-ground root residues are the main source of C for the understory vegetation. Thus, the inputs of C

242 with different chemical compositions may have influenced the enzyme activities (Li et al., 2013).

243   The activities of αG and βG were positively correlated with the contents of the soil inorganic N fractions (Table

244 A3), which suggests that the decomposition of C decreased because of the reduced availability of N when the

245 understory vegetation was removed. The size of soil C pool reflects the balance between the inputs and outputs of C (De

246 Deyn et al., 2008). When understory vegetation is removed, both the soil C inputs, including root exudates, fine root

247 turnover (Liu et al., 2012), and SOM decomposition rate (Wu et al., 2011; Liu et al., 2012; Zhao et al., 2013), and soil C

248 outputs, such as soil respiration (Wang et al., 2013), decrease. The lower SOC contents in the plots from which the

249 understory vegetation was removed therefore indicate that the removal of understory vegetation had more effect on the

250 outputs of soil C than on the inputs. Polyphenols are mainly decomposed by PPO, so the decrease in PPO activity may

251 result in an increase in the content of polyphenols that have toxic effects on soil microbes and inhibit hydrolase

252 activities (Sinsabaugh, 2010). Oxidative enzymes are responsible for the degradation of poor-quality, chemically

253 complex compounds, such as lignin, aromatic compounds, and phenolic compounds (Sinsabaugh, 2010). Therefore, the

254 lower activities of PPO and PER observed after the understory vegetation was removed may result in an increase in the

255 content of refractory compounds in SOM.

256   Phosphorus is generally the most limiting element in the highly weathered red soils in southern China. Soil P is

257 generally present in an organic form or is immobilized when the contents of Al and Fe are high (Margalef et al., 2017).

258 Of all the enzymes we assayed, the activity of AP was the highest (Fig. 3), which may reflect the fact that P was the

259 limiting nutrient in the red soils. Soil microorganisms may produce more phosphatase to mineralize organic P to meet

260 their demand for P (Allison and Vitousek, 2005). Loeppmann et al. (2016a, b) reported that N-degrading enzymes in the

261 rhizosphere of maize-planted soil increased when the available N decreased because of plant N uptake, which suggests

262 that N demand in the rhizosphere might be regulated by a similar mechanism in the cultivated field; we however, did

263 not find any evidence of such a control in our study. The soil nutrient availability affects rhizosphere priming (Dijkstra

264 et al., 2013). The higher potential NAG activity and higher contents of NH_4^+-N in the treatments with the intact

265 understory vegetation suggest that the energy-rich C compounds released through the roots promoted the production of

266 N-acquiring enzymes that released available N from SOM. The low potential activity of NAG in the treatments from

267 which the understory vegetation was removed was related to the reduction in the fungal biomass, and reflects the fact

268 that chitin, a major structural component of fungal cell walls (Loeppmann et al., 2016b), can be degraded by NAG

269 (Mganga et al., 2015). We did not observe any change in the AP activities when the understory vegetation was removed.

270　Because Chinese firs coexist with fungi and form mycorrhizal associates (Li et al., 2011), and mycorrhizal fungi

271　produce soil acid phosphatase (Rosling et al., 2016), these enzymes were most likely produced by Chinese firs. The

272　negative relationships between the potential activity of AP and DOC suggest that DOC was the substrate for microbes,

273　and that large amounts of DOC were consumed when producing P-acquiring enzymes.

5. Conclusions

275　　　Our results demonstrate that understory vegetation plays an important role in enhancing the soil C and N contents,

276　the soil potential activities of C- and N- hydrolase and oxidase, but does not influence the P-hydrolase activity. The ratio

277　of C- to N-acquiring enzymes increased after the understory vegetation was removed, which implies that, under lower

278　inputs of labile C, microbes invest more in C-acquiring enzymes than N-acquiring enzymes. The positive relationship

279　between the activities of C-degrading enzymes and the soil inorganic N contents suggest that C decomposition was

280　inhibited by the lower available N contents after understory vegetation was removed. It could be expected that with less

281　N is taken up by plants after understory vegetation was removed may increase soil N content, however, soil N inputs

282　decrease with reduced understory vegetation root material inputs, which leads to inorganic N decreases over time. The

283　potential activity of AP was negatively correlated with the content of DOC, which indicates that large amounts of DOC,

284　an energy source, were consumed when producing P-acquiring enzymes. Therefore, understory vegetation can

285　contribute to C sequestration by enhancing C inputs to soil, even though C may be lost from soil with understory

286　vegetation through the degradation of SOM by enzymes. We suggest that, as part of routine forestry management,

287　understory vegetation should not be removed from subtropical Chinese fir plantations.

Acknowledgements

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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 (αG), β-1,4-glucosidase (βG), and β-1,4-xylosidase (βX) 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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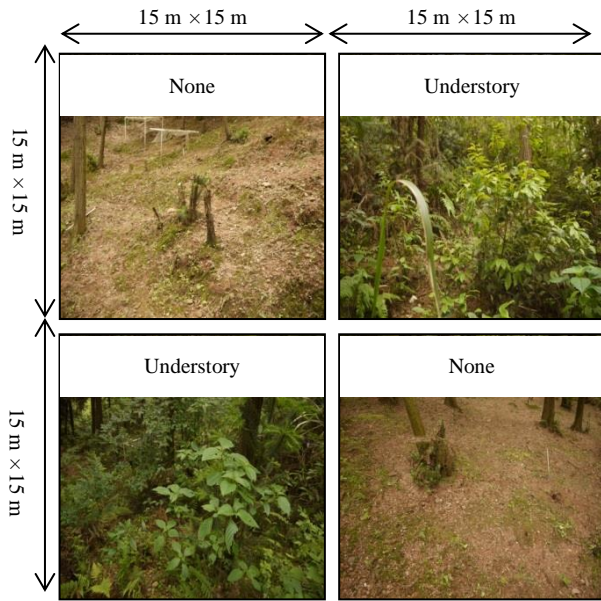
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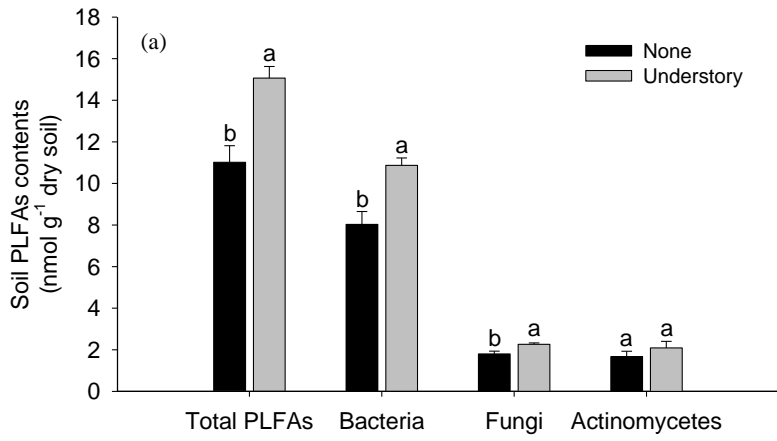
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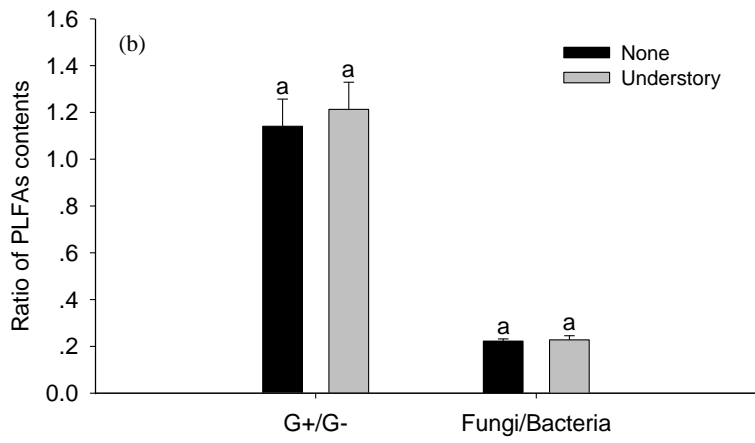
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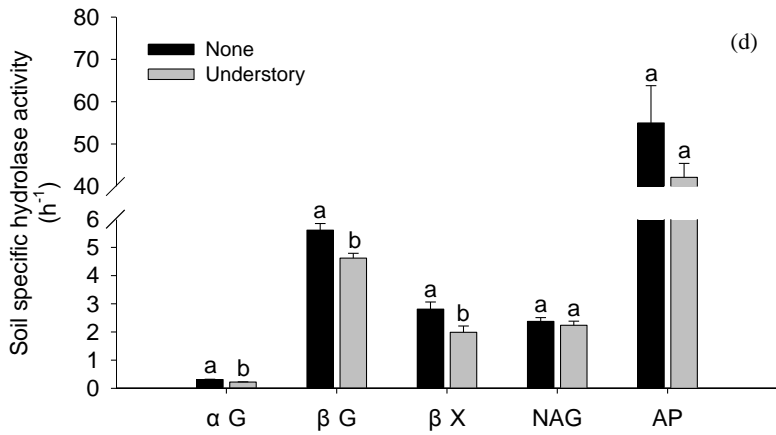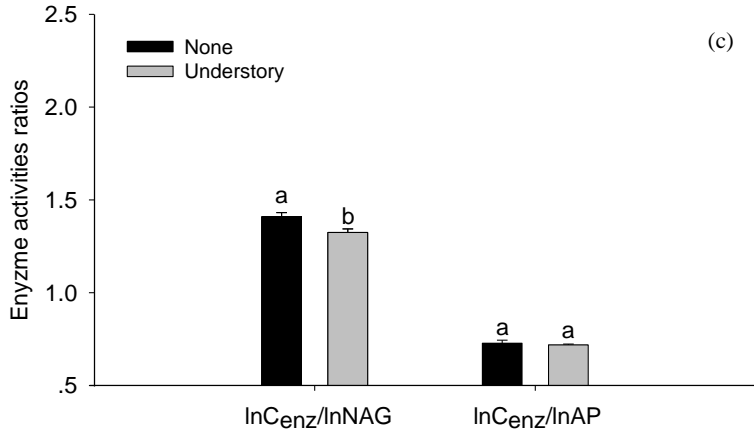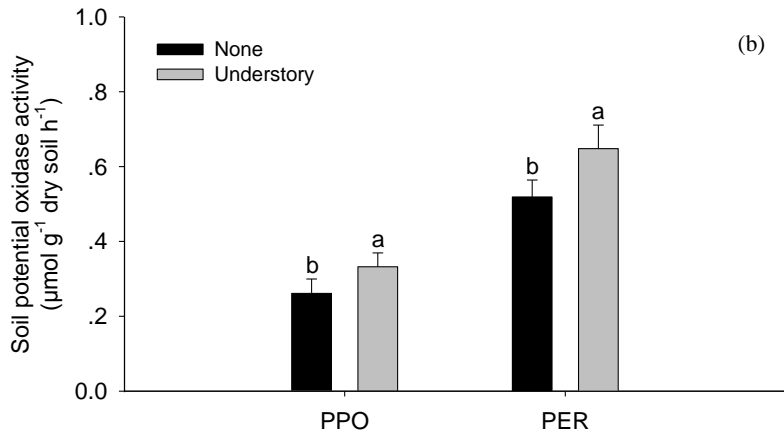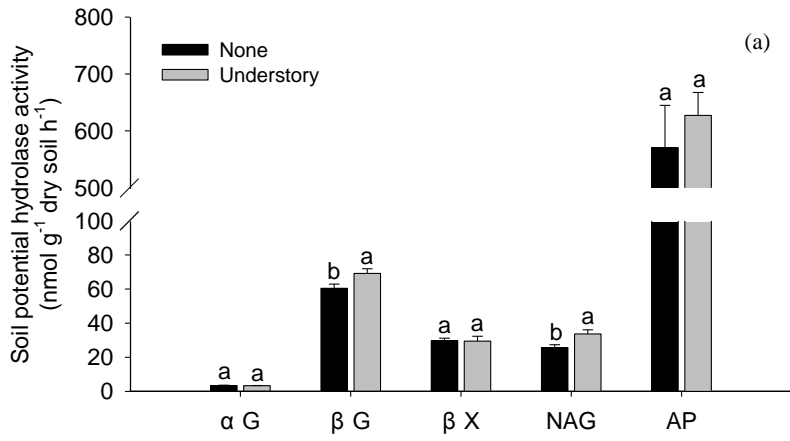
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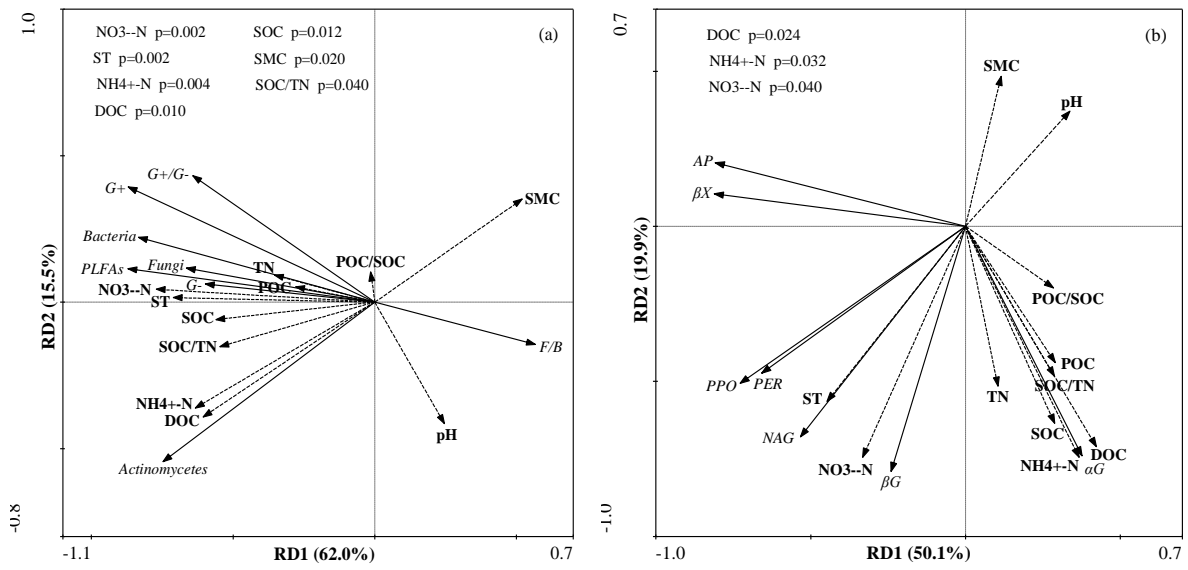
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Table 1 Soil abiotic properties in the different understory vegetation treatments

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±3.4b	3.7±0.3b	17.6±0.8b	4.84±0.6a	14.72±2.5b	1.19±0.04b	20.6±1.0b	14.9±0.4a
Understory	21.0±1.7a	22.92±1.0a	4.87±0.03a	45.4±4.9a	4.9±0.3a	20.0±0.4a	5.50±0.5a	22.25±3.7a	1.30±0.01a	24.2±1.1a	15.4±0.3a

Values in the table are the means ± standard error. *ST* soil temperature, *SMC* soil moisture, *pH* soil pH, NO_3^--*N* soil nitrate nitrogen, NH_4^+-*N* soil ammonia nitrogen, *TN* soil total nitrogen, *DOC* soil dissolved organic carbon, *POC* soil particulate organic carbon, *SOC* soil organic carbon, *POC*/*SOC* ratio of POC to SOC, *SOC*/*TN* ratio of SOC to TN. Different lowercase letters represent significant differences between **None** and **Understory** treatments ($P < 0.05$). Data were means ± standard errors. The same abbreviations are used below.

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Fig. A1

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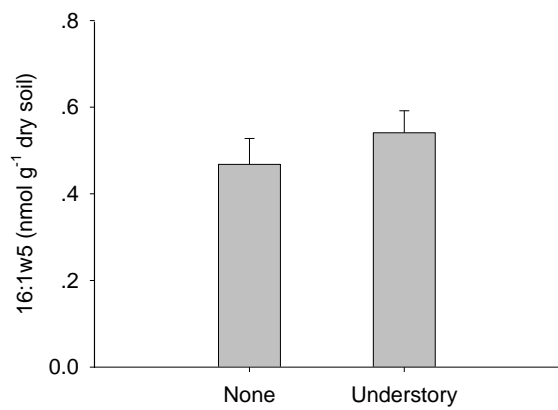
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600   Table A1 Soil enzymes and their corresponding substrates and functions

Enzyme	E. C	Abbreviation	Substrate	Function
Peroxidase	1.11.1.7	PER	L-DOPA	Oxidize lignin and aromatic compounds using H_2O_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	αG	4-MUB-α-D-glucoside	Releases glucose from starch (Stone et al., 2014).
β-1,4-glucosidase	3.2.1.21	βG	4-MUB-β-D-glucoside	Releases glucose from cellulose (Stone et al., 2014).
β-1,4-xylosidase	3.2.1.37	βX	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 (αG), β-1,4-glucosidase (βG), and β-1,4-xylosidase (βX) 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
$αG_{PLFAs}$; $βG_{PLFAs}$; $βX_{PLFAs}$	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
$lnC_{enz}/lnNAG$	Ratio of C- to N- acquiring enzymes	Stone et al., 2014; Loeppmann et al., 2016a; Xu et al., 2017
$lnC_{enz}/lnAP$	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 αG + βG + βX).

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

Abiotic Properties		ST	SMC	pH	NO$_3^-$-N	NH$_4^+$-N	TN	DOC	POC	SOC	POC/SOC	SOC/TN
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
	Actinobacterias	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	-0.17	-0.02	-0.29	0.25
	F/B	-0.50*	0.22	-0.01	-0.30	-0.17	-0.07	-0.15	0.03	-0.18	0.22	-0.24
Enzymes	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*	-0.38	-0.34	-0.38	-0.43
	AP	0.28	0.00	-0.16	0.09	-0.44	-0.21	-0.48*	-0.36	-0.38	-0.32	-0.33
	PPO	0.86**	-0.57*	-0.33	0.72**	0.25	-0.01	0.23	-0.13	0.05	-0.28	0.14
	PER	0.81**	-0.54*	-0.12	0.61**	0.37	-0.01	0.32	-0.03	0.13	-0.18	0.23

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

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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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/SOC (%)	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
	November	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
Understory	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
	November	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

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

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)	Actinobacterias (nmol g^{-1} dry soil)	PLFAs (nmol g^{-1} dry soil)	G$^+$/G$^-$	F/B
None	April	4.25±0.44aB	4.61±0.50aA	8.86±0.94aA	2.07±0.30aA	0.36±0.05aB	2.10±0.22aA	11.56±0.75bA	0.93±0.01aB	0.21±0.01aAB
	July	6.28±0.47aA	3.62±0.08aAB	9.31±0.13bA	1.89±0.03bA	0.69±0.05aA	2.09±0.22aA	13.29±0.30aA	1.59±0.07aA	0.20±0.00aB
	November	2.82±0.34bB	3.11±0.22aB	5.93±0.56bB	1.45±0.07bA	0.35±0.04aB	0.817±0.41aB	8.19±0.52bB	0.90±0.05aB	0.25±0.02aA
Understory	April	3.81±0.46aC	4.32±0.21aA	10.53±0.54aA	2.21±0.08aA	0.43±0.26aB	2.05±0.06aAB	14.62±0.50aAB	0.89±0.05aB	0.26±0.04aA
	July	7.22±0.25aA	4.52±0.29aA	11.76±0.51aA	2.23±0.04aA	0.73±0.43aA	2.99±0.36aA	16.67±0.71aA	1.62±0.04aA	0.19±0.01aA
	November	5.41±0.51aB	4.92±0.28aA	10.32±0.59aA	2.35±0.21aA	0.47±0.45aB	1.23±0.55aB	13.90±0.98aB	1.13±0.15aB	0.23±0.03aA

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679 Table A7 Temporal variation in soil potential enzyme activities

Treatment	Time	αG (nmol g^{-1} dry soil h^{-1})	βG (nmol g^{-1} dry soil h^{-1})	βX (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±0.03bB
	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±0.03bA
	November	2.48±0.12aB	52.8±2.1aB	30.5±1.7aAB	22.8±2.0bA	698.63±70.3aA	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±0.19aAB	75.8±6.1aA	33.3±1.8aA	41.6±2.1aA	699.5±47.8aA	0.48±0.01aA	0.89±0.04aA
	November	2.90±0.12aB	65.7±2.3aA	33.8±2.8aA	32.6±1.6aB	689.32±35.1aA	0.28±0.01aB	0.53±0.04aB

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