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 the 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 is understory vegetation, which leads to

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

1. Introduction

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

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

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 reduced 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 the 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 annual precipitation of 1340 mm. According to the USDA-NRCS soil taxonomy (Soil Survey Staff, 1996), the soil in this area is dominated by Udults, which forms from red sandstone and sandy conglomerate and has moist and dry Munsell values of 7.5 YR 5/6 and 7.5 YR 6/6, respectively.

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

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

2.2 Soil sampling and analysis

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

Soil physical and chemical properties were determined as outlined by Bao (2008). Soil temperature (ST) was determined at a depth of 10 cm with a soil thermometer (TP101) during sampling. The soil moisture content (SMC) was 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 by a pH digital meter. The contents of nitrate N (NO_3^--N) and ammonia N (NH_4^+-N) were measured with a continuous flow analyzer (Bran Luebbe, AA3) after extraction with a 2 M KCl solution (soil: solution ratio of 1: 10). Dissolved organic carbon (DOC) contents were measured with a TOC analyzer (Elementar, Liquid *II*) after extraction with ultra-pure water (soil: solution ratio of 1: 5) (Jones and Willett. 2006). Particulate organic carbon (POC) was determined

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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 16:1ω7c, cy17:0, 16:1ω9c, 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 16:1ω5, as well as 18:1ω9c, 18:2ω6c, and 18:3ω6c, and the actinobacterial PLFAs were represented

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

131 β-1,4-glucosidase (βG), β-1,4-N-acetylglucosaminidase(NAG), β-1,4-xylosidase (βX) 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 μL of the soil suspension and 50 μL of the substrate solution (200 μM)

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 ℃ for up to 4 h. After incubation, 10 μL 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 αG, βG, and βX 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 μL of the soil suspension and 150 μL of the substrate solution to deep-well plates. We also added 30 μL

143 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

144 were centrifuged for 3 minutes at 3000 r h^{-1}. We then transferred 250 μL 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 ± 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.

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 $lnC_{enz}/lnNAG$ was 6% higher in the None treatments than in the

174 Understory treatments, but the ratios of $lnC_{enz}/lnAP$ 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., $αG_{PLFAs}$, $βG_{PLFAs}$ and $βX_{PLFAs}$, 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- (NAG_{PLFAs}) and P-acquiring enzymes (AP_{PLFAs})

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

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

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

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

4. Discussion

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

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

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

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

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

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

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

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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.

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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Figure captions

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

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

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

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

Soil potential hydrolase activities (a), soil potential oxidase activities (b), enzyme activity ratios (c), soil hydrolase activities normalized by total PLFAs (d). 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. The same abbreviations apply to Fig. 4.

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

SMC soil moisture content, 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

Table captions

Table 1 Soil abiotic properties in the different understory vegetation treatments

Supplementary material

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

Table A1 Soil enzymes and their corresponding substrates and functions

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

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

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

Table A5 Temporal variation in soil abiotic properties

Table A6 Temporal variation in soil PLFA contents

Table A7 Temporal variation in soil potential enzyme activities

15 m × 15 m   15 m × 15 m

15 m × 15 m

None   Understory

15 m × 15 m

Understory   None
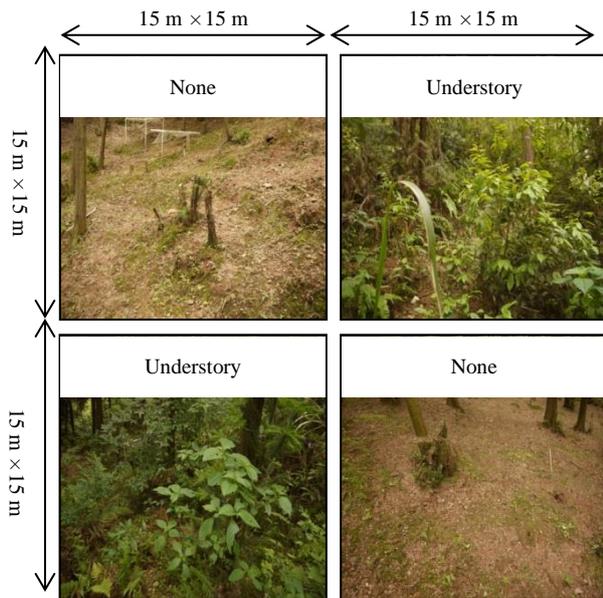
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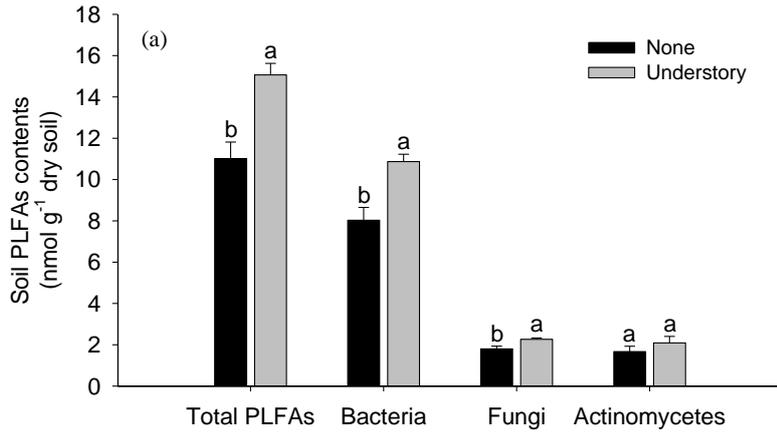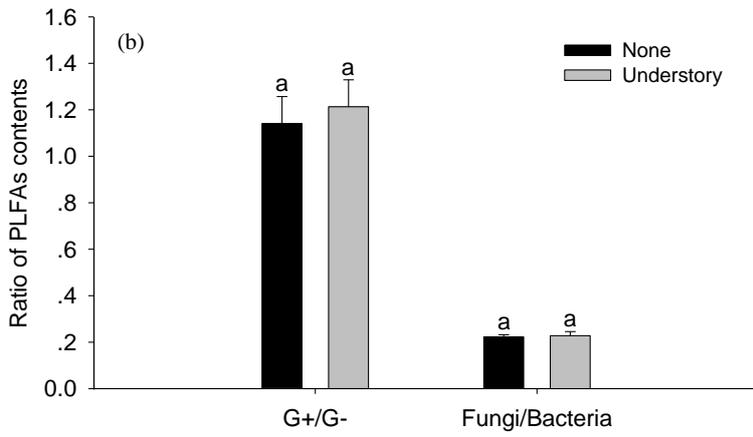
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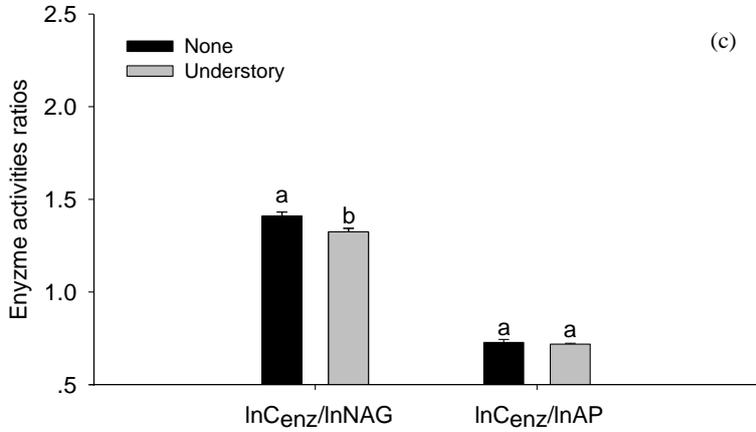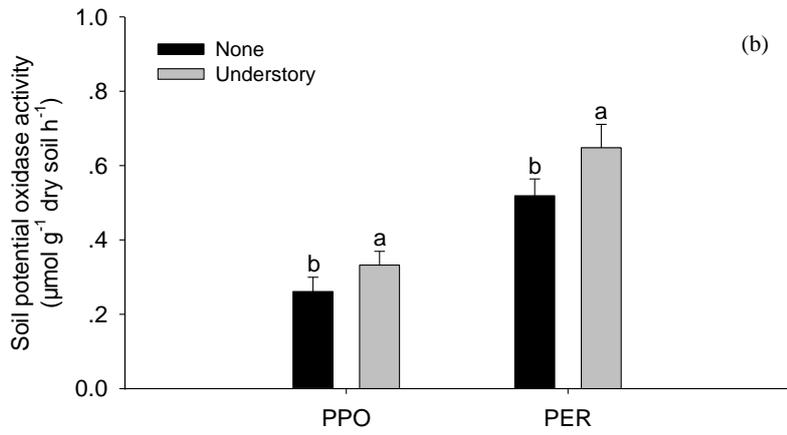517

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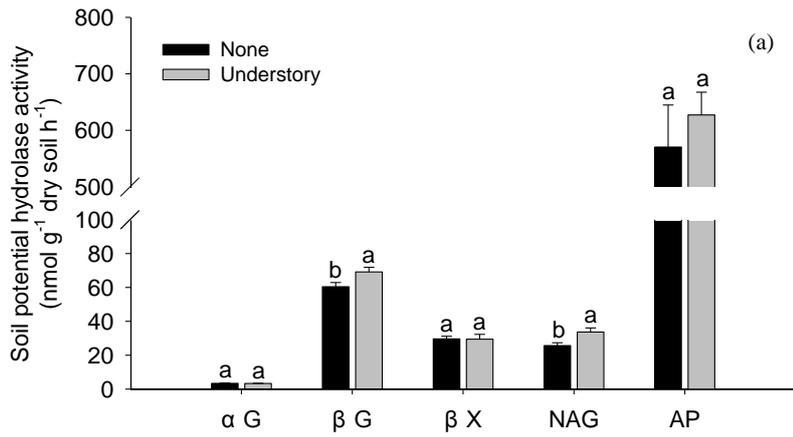524

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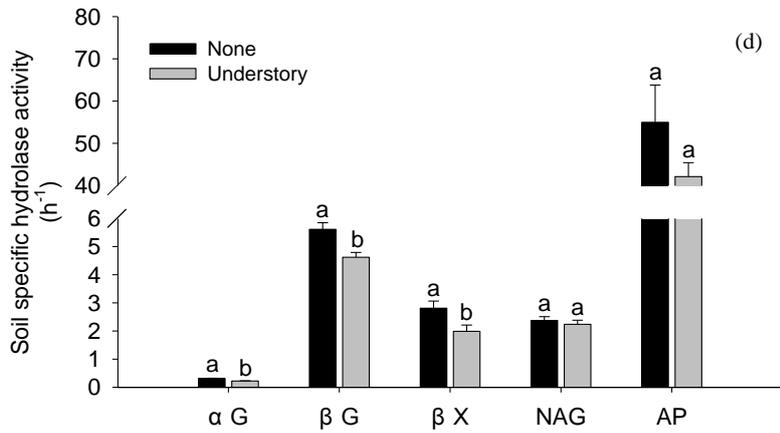
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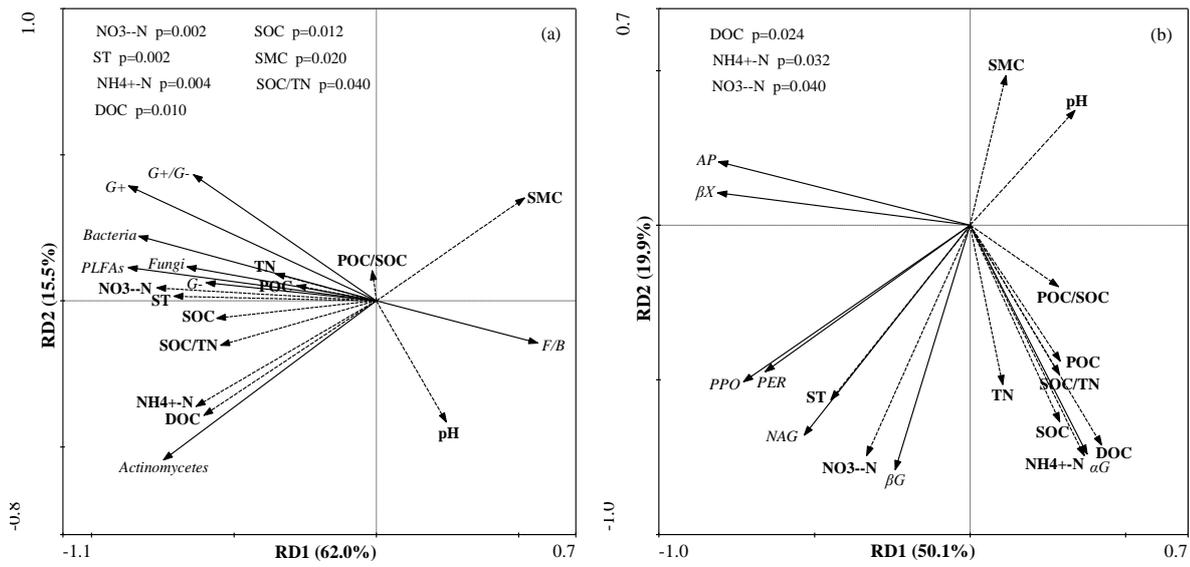
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Table 1 Soil abiotic properties in the different understory vegetation treatments

Treatment	ST (℃)	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.
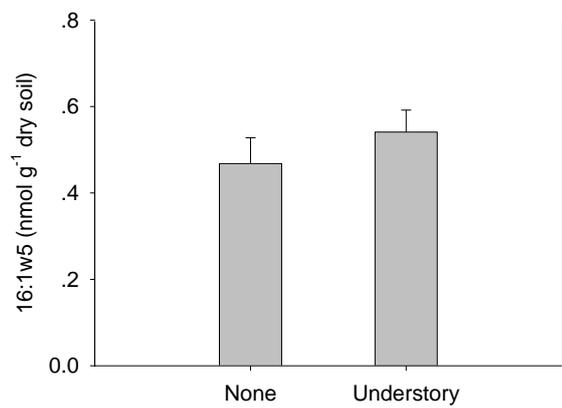
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583   Fig. A1

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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_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.

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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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