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

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Abstract:

Understory vegetation affects soil microbial biomass and extracellular enzyme activities in a subtropical Chinese fir (*Cunninghamia lanceolata*) forests. The aim of this study was to determine the role of understory vegetation in controlling soil abiotic and biotic properties, such as PLFAs contents, and extracellular enzyme activities. One paired treatment, which comprised understory vegetation removal (**None**) and understory vegetation left intact (**Understor**y) in the context of litter removal, was established in a subtropical Chinese fir plantation. We mainly evaluated the effects of understory vegetation on soil abiotic properties, the PLFA contents of bacteria, fungi and actinobacterias, and the activities of five hydrolases and two oxidative enzymes. The soil moisture content (SMC), contents of soil dissolved organic carbon (DOC), particulate organic carbon (POC), soil organic carbon (SOC), ammonia nitrogen (NH_4^+-N), total nitrogen (TN), and the POC/SOC ratios respectively declined by 4%, 18%, 25%, 12%, 34%, and 12%, and soil bacterial, fungal and total PLFA contents, and the activities of β-1,4-glucosidase (βG), β-1,4-N-acetylglucosaminidase (NAG), phenol oxidase (PPO), as well as peroxidase (PER) were up to 27% lower, when the understory vegetation was removed. The soil ln(αG+βG+βX)/lnAP (βX: β-1,4-xylosidase; AP: acid phosphatase) increased when understory vegetation is removed, which may mean that less labile carbon (C) inputs led microbes to produce more enzymes comes at C cost relative to N cost. The positive relationships between DOC and AP implied that increased DOC contents may be linked to increased root exudation which may increase microbial biomass and therefore to increase P acquisition. The contents of NH_4^+-N were positively correlated with and βG suggested the increased availability of N promoted the decomposition of C. Understory vegetation alter soil microbial biomass, which may influence the decomposition of soil organic matter, by changing soil carbon inputs. We therefore propose that, to sustain soil quality in subtropical Chinese

fir plantations, understory vegetation should be maintained.

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

1. Introduction

The interactions between above-ground vegetation functional groups and soil microbial community structures are thought to be important drivers of carbon (C) and nutrient cycling in terrestrial ecosystems (Murugan et al., 2014). Understory vegetation removal influence soil processes by reducing above-ground plant diversity (Lamb et al., 2011) and biomass (Fu et al., 2015), changing ~~under~~-ground rhizodeposition quality (Li et al., 2013) in forest ecosystems. While understory vegetation absorbs water and nutrients from soil (Wang et al., 2014), it also releases carbohydrates, such as sloughed-off root cap and border cells, mucilage and exudates through root (McNear Jr, 2013), cellulose, hemicelluloses and lignin in the form of leaf litter (Loeppmann et al., 2016a, b), to soils. The net effect of understory vegetation on soil nutrients is decided by the balance between the understory vegetation's nutrient demand and its capacity to release carbohydrates to soil via the decompositions of understory derived litter and rhizodeposition. Soil extracellular enzymes produced by microorganisms or plant roots catalyze soil C, nitrogen (N), and phosphorus (P) cycling (Burns et al., 2013; Nannipieri et al., 2018). Individual enzyme activities reflect the nutrient requirements of microorganisms and the microbial strategies for maintaining the nutrient balance in response to changes in the soil environment (Burns et al., 2013). To study the changes of enzyme activities with understory vegetation removal could reveal how microbial nutrient acquisition is affected by microbial biomass and soil nutrients.

The influences of understory vegetation on soil properties are closely related to climate, soil type, plant species, and how long the manipulations have been applied (Li et al., 2013; Nilsson and Wardle, 2005; Zhang et al., 2014). There is no consensus about how understory vegetation impacts the physical, chemical, and biological properties of forest soils. Various studies have reported that the litter decomposition rate, soil organic matter (SOM) content, and the soil respiration rate decreased when the understory vegetation was removed (Wang et al., 2011; Liu et al., 2012; Wang et al., 2014), while others reported that its removal had little influence on soil properties (Xiong et al., 2008; Zhao et al., 2011). The effects of understory vegetation on soil microbial biomass also varied. Wu et al., (2011) and Zhao et al., (2013) found that fungal biomass and the fungi to bacteria ratio (F/B) decreased in the absence of understory vegetation, while in contrast, Murugan et al., (2014) found that bacterial and saprophytic fungal biomass increased after understory vegetation was removed from eucalyptus plantations. In an alpine shrubland, the soil arbuscular mycorrhizal fungal biomass decreased five months after plant functional groups were removed, but this effect disappeared after seventeen months (Urcelay et al., 2009). There is inconsistent information currently available about the responses of soil enzyme

activities to understory vegetation, reporting that soil enzyme activities decreased in the subtropical alpine coniferous forest (Huang et al., 2014), or did not change under *Pinus sylvestris* var. *mongolica* plantation (Lin et al., 2012), when understory vegetation was removed.

The average net ecosystem productivity of Chinese subtropical forests (362 ± 39 g C m^{-2} yr^{-1}) is approximately 82.6% and 64.9% higher than that of tropical and temperate forests, respectively (Yu et al., 2014). To maintain soil fertility it is important to ensure that C sinks and forest growth are sustained in these forests. Because of its high economic value, Chinese fir (*Cunninghamia lanceolata*) plantations are widespread in 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). To facilitate seed germination, ensure survival of seedlings, avoid the intense competition between understory vegetation and trees for water, nutrients and light, or for fuel, understory vegetation and litter were commonly removed from the forest floor in southern China and elsewhere (Xiong et al., 2008; Wu et al., 2011; Liu et al., 2012). As a shallow-rooted and fast-growing tree species, the Chinese fir competes intensively with understory vegetation for soil nutrients and moisture (He et al., 2015). It is still of high interest how the soil enzyme activities are affected by the understory vegetation removal in Chinese fir plantations.

In this study, we established a long-term field experiment to assess how understory vegetation influences soil abiotic properties, PLFA contents and enzyme activities at Chinese fir plantations. Earlier studies reported that the labile C release from below-ground C input decreased when understory vegetation was removed (Liu et al., 2012). We hypothesized that the removal of understory vegetation decreased rhizodepostion and therefore microbial biomass and activity. The interactions between soil abiotic and biotic properties under different forest understory management practices could gain new insights on forest nutrition.

2. Material and Methods

2.1 Experimental treatments

The study site was located at the Shixi forest plantation in Taihe County, Jiangxi Province, China (115°03′29.9″ E, 26°44′29.1″ N). The plantation experiences a subtropical monsoon climate with a mean annual temperature and precipitation of 18.8 ℃ and 1340 mm, respectively. The main soil type in this area is red soil (Munsell values: moisture, 7.5 YR 5/6 and dry, 7.5 YR 6/6), which forms from red sandstone and sandy conglomerate and is classified as Udults using the USDA-NRCS soil taxonomy (Soil Survey Staff, 1996).

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 andazalea (*Rhododendron*).

Three 30 m × 30 m plots, with a buffer zone between them exceeding 10 m to avoid the influence between each plot, were established in the Chinese fir plantation in January 2013. One paired treatment with three replications was established within each of the three plots. Each plot was divided into four 15 m × 15 m subplots and contained two treatments: understory vegetation and litter removal (**None**) and understory vegetation left intact but litter removal (**Understory**). The two subplots with the same treatment in one plot were distributed across each plot to avoid the effects of slope (Fig. 1) and were averaged as one analysis replication. The litter and understory were managed on a monthly basis. For the **None** treatment, we removed all litter and understory vegetation from the plot. For the **Understory** treatment, we removed the litter from the plot, but left the understory vegetation intact. The amount of litter was about 1020 kg ha^{-1} $year^{-1}$, and the amount of understory vegetation in the research site was about 6236 kg ha^{-1} under natural conditions.

2.2 Soil sampling and analysis

Bulk soil samples were collected in wet season (April and November) and dry season (July) in 2015. Five soil cores with an inner diameter of 5 cm were collected randomly from a depth of 0–10 cm in each subplot and then mixed as one composite sample. All fresh soil samples were sieved through a 2-mm mesh, stored at 4 ℃ prior to 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) when 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. Soil nitrate N (NO_3^--N) and ammonia N (NH_4^+-N) contents were measured with a continuous flow analyzer (Bran Luebbe, AA3) after extraction with 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 as outlined in the method of Garten et al., (1999). Soil organic C (SOC) and total nitrogen (TN) contents were measured with an elemental analyzer (Vario Max CN).

Soil phospholipid fatty acids (PLFAs) were extracted following the procedure outlined by Bossio and Scow (1998), and were determined with a gas chromatograph (Agilent 6890N). Soil total PLFAs were represented by the following

PLFA biomarkers: gram positive bacteria (G^+: i14:0, i15:0, a15:0, i16:0, i17:0, a17:0), gram negative bacteria (G^-: 16:1ω7c, cy17:0, 16:1ω9c, cy19:0), fungi (arbuscular mycorrhizal fungi (AMF, 16:1ω5), 18:1ω9c, 18:2ω6c, 18:3ω6c), actinobacterias (10Me16:0, 10Me17:0, 10Me18:0); G^+ and G^- bacterial PLFA contents represented total bacterial PLFA contents (Bradley et al., 2007; Denef et al., 2009).

Soil enzyme activities were measured following the methods of Saiya-Cork et al., (2002). The specific substrates and functions of the enzymes assayed are listed in Table A1. Five hydrolase activities (α-1,4-glucosidase, β-1,4-glucosidase (βG), β-1,4-N-acetylglucosaminidase(NAG), β-1,4-xylosidase (βX) and acid phosphatase (AP)) were assayed using fluorogenically-labeled substrates. Briefly, a soil suspension was prepared by adding 1 g of fresh soil to 125 mL of 50 m*M* acetate buffer. We added 200 μL of the soil suspension and 50 μL of the substrate solution (200 μ*M*) to 96 microplates in eight analytical replicates. Methylumbelliferone (MUB) was used for calibration of hydrolase activities. The microplates were incubated in the dark at 20 ℃ for up to 4 h. After incubation, 10 μL of 1 M NaOH was added to each well to terminate enzymatic reaction. Following termination of each reaction, the fluorescence was measured using a microplate fluorometer (SynergyH4, BioTek) with excitation and emission filters of 365 nm and 450 nm, respectively.

The soil oxidase activities (polyphenol oxidase (PPO) and peroxidase (PER)) were assayed with spectrophotometrically. We added 600 μL of the soil suspension and 150 μL of the substrate solution to deep-well plates. We also added 30 μL of 0.3% H_2O_2 solution before determining PER. After incubation in the dark at 20 ℃ for up to 5 h, the deep-well plates were centrifuged for 3 minutes at 3000 r h^{-1}. We then moved 250 μL of the supernatant to the microplates and measured the absorbance at 450 nm with a microplate fluorometer (DeForest, 2009). We had eight replicate sample wells for each assay.

2.3 Statistical Analysis

Data we used were the average data of April, July and November. N=18, n=3. All the data satisfy the normal distribution criteria for parameter analysis was tested by one-sample Kolmogorov-Smirnov test using SPSS 17.0. The differences of soil abiotic properties, PLFA contents and enzyme activities between the understory treatments were assessed by a paired-sample *t*-test (SPSS 17.0). Data from the two subplots with the same treatment in one plot were averaged and then analyzed statistically (n=3). We investigated the relationships among soil abiotic properties and PLFA contents and enzyme activities of all soil using redundancy analysis (RDA, CANOCO, version 4.5) and Pearson correlation analysis (SPSS 17.0). Monte Carlo Permutation Test was used to test the significance of the variables before conducted RDA. Figures were generated with SigmaPlot (Version 10.0). The significance level was $P < 0.05$.

3. Results

3.1 Soil abiotic properties

Soil C and N contents and the SMC were decreased, when understory vegetation was removed (Table 1). The contents of various soil organic C (including DOC, POC, and SOC) and N (including NH_4^+-N and TN) fractions, SMC and POC/SOC ratios were respectively 4%, 18%, 25%, 12%, 34% and 12% lower in the **None** treatment than in the **Understory** treatment ($P < 0.05$). The contents of NO_3^--N, ST, pH, and SOC/TN did not differ significantly between the **None** and the **Understory** treatment ($P > 0.05$).

3.2 Soil PLFA contents

Soil total PLFA contents were 27% lower in the **None** treatment than in the **Understory** treatment (Fig. 2). In specific, bacterial PLFA content was 26% less in the **None** treatment than in the **Understory** treatment ($P < 0.05$), though the PLFA contents of G^+ and G^- did not vary ($P > 0.05$). Soil fungal PLFA content was 20% lower in the **None** treatment than in the **Understory** treatment ($P < 0.05$). The ratios of fungi/bacteria did not change because the bacterial and fungal PLFA contents decreased simultaneously when understory vegetation was removed. Understory vegetation removal did not change actinobacterial PLFA contents as well ($P > 0.05$).

3.3 Soil enzyme activities

Understory vegetation significantly affected soil enzyme activities. The potential activities of βG, NAG, PPO, and PER were higher in the treatments with understory vegetation than in the treatment without understory vegetation (Fig. 3a and b) ($P < 0.05$). When the understory vegetation was removed, the potential activities of βG, NAG, PPO, and PER reduced by 13%, 24%, 21% and 20%, respectively ($P < 0.05$), while the potential activity of acid phosphatases were not changed ($P > 0.05$). Soil C/N and C/P potential acquisition activity was indicated by the ratios of ln(αG+βG+βX)/lnNAG and ln(αG+βG+βX)/lnAP (Fig. 3c). The ratios of ln(αG+βG+βX)/lnNAG increased by 6.0%, while the ratios of ln(αG+βG+βX)/lnAP was not changed after understory vegetation was removed.

The trends were enzyme-specific when normalized by total PLFAs (Fig. 3d and e). The specific activities of C hydrolase (αG_{PLFAs}, βG_{PLFAs} and βX_{PLFAs}) significant increased after understory vegetation removal ($P < 0.05$), while the specific activities of N (NAG_{PLFAs}) and P hydrolase (AP_{PLFAs}) were not changed ($P > 0.05$).

3.4 Correlations between soil enzyme activities, soil PLFA contents, and soil abiotic properties

The relationships between different PLFA contents and soil abiotic properties are shown in Fig. 4 (a). The first (RD1) ordination axis explained 62.0% of the total variability in the different PLFA contents and was mainly correlated

with ST, SMC, NO_3^--N, NH_4^+-N, DOC, SOC and SOC/TN, and the second (RD2) ordination axis explained 15.5% of the total variability in the different PLFA contents. The contents of NH_4^+-N and DOC were positively correlated with bacterial, actinobacterial and total PLFAs. The content of SOC was positively correlated with G^-, bacterial, fungal and total PLFAs. ($P < 0.05$) (Table A2).

The relationships between soil potential enzyme activities and soil abiotic properties are shown in Fig. 4 (b). The RD1 and the 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, NH_4^+-N were mainly related to RD2 ordination axis. The content of DOC was positively correlated with αG, and was negatively correlated with βX and AP. The content of NH_4^+-N was positively correlated with αG and βG ($P < 0.05$; Table A2). Pearson correlation analysis demonstrated that bacterial and total PLFAs were positively correlated with αG, βG, NAG, PPO and PER. The PLFA content of fungi was positively correlated with αG, βG, NAG ($P < 0.05$; Table A3).

4. Discussion

Consistent with our hypothesis, the contents of soil organic C (including DOC, POC, and SOC) and N (including NH_4^+-N and TN) were decreased when the understory vegetation was removed (Table 1), which demonstrated that understory vegetation is beneficial to improve the content and availability of soil C and N. Other studies however reported that the responses of soil physical and chemical properties to understory vegetation removal were minimal (Xiong et al., 2008; Zhao et al., 2011). The distinct results might largely depend on the understory vegetation compositions in different studies (Nilsson and Wardle, 2005). In our study, we removed litter in all treatments to avoid the effects of litter. Although Chinese fir roots may occupy the space vacated and may partly compensate for the reduced C inputs by increasing their exudation (Li et al., 2016), understory vegetation root residue also incorporated into soil (Li et al., 2013) after understory vegetation removal. The increased quantities of C secreted by Chinese fir roots and originated from decomposition of the understory vegetation root residues did not fully compensate for the C lost when understory vegetation was removed. Additionally, soil C tends to be higher when plant functional diversity is high (Zhou et al., 2016). Therefore, soil C content may decrease by removing understory vegetation and reducing plant diversity. Previous ~~study~~ have found that the reduction of labile root C input resulted in the increment of soil N contents as a result of reduced plant N uptake (Kaiser et al., 2010; Loeppmann et al., 2016a). However, we found the N contents increased with understory vegetation intact, maybe because more labile C input from root exudates have resulted the accumulation of SOM and promoted the mineralization of organic N simultaneously. The decreased values of the POC/SOC ratios after understory vegetation removal (Table 1) suggest that POC declined more than SOC when

understory vegetation was removed. This was indicated that intact understory vegetation improved soil sustainability and productivity in Chinese fir forests, since aggregate stability and POC contents were related (Bouajila and Gallali, 2010). This also means that when understory vegetation was removed, the decomposition from POC to SOC could occur at higher rates. In addition, the decrease in the SMC by understory vegetation removal (Table 1) reflects that understory vegetation had the ability to hold soil water.

Consistent with our hypothesis, total PLFAs, including bacterial and fungal PLFA biomarkers declined after the understory vegetation was removed in this study (Fig. 2). Previous studies reported decreases in fungal biomass after understory vegetation removal (Wu et al., 2011; Liu et al., 2012; Zhao et al., 2013). The PLFA content of AMF was declined (P = 0.053) after understory vegetation removal (Fig, A1) which may reflect the influence of the reduction of plant diversity. Since specific AMF may only grow when specific plants are present, plant communities' change over time will change their mycorrhizal partners (Hart et al., 2001). Compared with other fungi, mycorrhizal fungi depends highly on belowground C allocation by plants, thus, the reduction of fungal PLFA content was mainly related to the reduction of mycorrhizal fungi (Kaiser et al., 2010). Mycorrhizal species in the study area included understory vegetation, such as *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, which was mainly the result of reductions in the soil C and N contents (Table A2) and plant diversity (Lamb et al., 2011). Brant et al., (2006) considered that there might be an increase in the biomass of actinobacterias to decompose recalcitrant C compounds when nutrient availabilities were low; however, we did not observe this pattern in our research (Fig. 2), perhaps because of the high variability in the actinobacterial PLFA content in the field plots.

Consistent with our hypothesis, we found a lower potential extracellular enzyme activity when understory vegetation was removed (Fig. 3), which was in line with the results of Huang et al., (2014), who found soil potential cellulase activity decline after understory vegetation removal, in spite of Lin et al., (2012) found no changes in soil enzyme activities. 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 increased while the specific C hydrolase activities normalized by PLFAs decreased with understory vegetation intact, which may reflected that more labile C input may led to the emergence of opportunistic microorganisms (the microorganisms that do not produce enzymes but use enzyme products) (Allison, 2005). There are several possible reasons for the changed enzyme activities observed in our study, as follows. (1) The

soil C/N potential acquisition activity increased when understory vegetation is removed, which may mean that less labile C inputs are there led microbes to produce more enzymes comes at C cost relative to N cost (Kaiser et al., 2010). (2) Mycorrhizal fungi vanish when understory vegetation is removed (Fekete et al., 2011), which means there are fewer microorganisms to produce less enzymes. (3) For the understory vegetation remaining and removal treatment, continuous root exudates and discontinuous root residue were incorporated into the soil, respectively (Li et al., 2013). The different chemical composition of SOM sources may have different influence on enzyme activities.

We observed positive relationships between the activities of αG, βG and the contents of soil inorganic N fractions (Table A2), which reflected that the decreased availability of N reduced the decomposition of C when understory vegetation was removed. The size of soil C pool is 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 decreased contents of SOC and TN caused by understory vegetation removal therefore indicate that the removal of understory vegetation had more effect on the outputs than inputs of soil C and N. 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).

In highly weathered red soil in southern China, P is the most limiting element, and most soil P is presented in organic form or is immobilized by high contents of Al and Fe (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 limiting nutrient in red soil. Soil microorganisms may produce more phosphatase to mineralized organic P ~~and release phosphate~~ to meet their demand for P (Allison and Vitousek, 2005). The results of Loeppmann et al., (2016a) suggest that the same mechanism applies to N demand in the rhizosphere, as they found that N-degrading enzymes increased when N was limited in the rhizosphere of maize-planted soil. However, we did not find evidence that N demand is controlled by such a mechanism in this paper. The rhizosphere of the understory vegetation was not N-limited because the ratios of SOC/TN did not change with higher SOM and TN contents relative to understory vegetation removal. In line with Loeppmann et al. (2016b), the potential activity of NAG was lower, when the understory vegetation was removed. The lower potential NAG activity and less NH_4^+-N content after understory vegetation removal reflect that less root exudates might inhibit the decomposition of organic N due to carbon limitation. Chitin, a major structural component of fungal cell wall, and peptidoglycan, a major structural component of bacterial cell wall (Loeppmann et al., 2016b), can be degraded by NAG (Mganga et al., 2015). We also found that there was a significant positive correlation between NAG and ~~fungus~~ biomass

(Table A3). The potential activity of NAG was lower when the understory vegetation was removed than the understory vegetation intact, which might reflect a reduction in fungal biomass. We did not observe any change in AP activities when the understory vegetation was removed, perhaps because Chinese firs, along with their mycorrhizal associates, are the main producers of these enzymes. The negative relationships between the potential activity of AP and the content of DOC indicated that increased DOC contents may be linked to increased root exudation which may increase microbial biomass and therefore to increase P acquisition.

5. Conclusions

Our results demonstrate that understory vegetation plays an important role in enhancing soil potential C- and N-hydrolase and oxidase activities, but does not influence or P-hydrolase activity. The soil C/N potential acquisition activity increased after understory vegetation removal may imply that less labile C inputs are there led microbes to produce more enzymes comes at C cost relative to N cost. The positive relationships between the activities of C-degrading enzymes and the contents of soil inorganic N implied that the decreased availability of N inhibited the decomposition of C when understory vegetation was removed. The potential activity of AP is positive with the content of DOC indicated that increased DOC contents may increase P acquisition by increasing microbial biomass. Therefore, understory vegetation alter soil microbial biomass, which may influence the decomposition of soil organic matter, by changing soil C inputs. From this study, we can conclude that understory vegetation are beneficial for sustaining soil microbial activities in subtropical Chinese fir forests. We suggest that, as part of routine forestry management, understory vegetation should not be removed from, but rather should be maintained in, subtropical Chinese fir plantations.

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Figure captions

Fig. 1 One paired plot design treatments. Understory vegetation was either cut and removed (**None**) or left intact (**Understory**) in the context of removing litter.

Fig. 2 Soil phospholipid fatty acid (PLFAs) contents

(a) Soil PLFA contents, (b) ratio of PLFA contents. *None* **None**, *U* **Understory**, 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 was the average data of April, July and November. N=18, n=3. The same below

Fig. 3 Soil enzyme activities

(a) soil potential hydrolase activities, (b) soil potential oxidase activities, (c) Soil C/N and C/P potential acquisition activity was indicated by the ratios of ln(αG+βG+βX)/lnNAG and ln(αG+βG+βX)/lnAP, (d) soil hydrolase activities normalized by total PLFAs. *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.

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

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

Supplementary material

Fig. A1 Contents of arbuscular mycorrhizal fungi.

Table A1 Soil enzymes and their corresponding substrates and functions

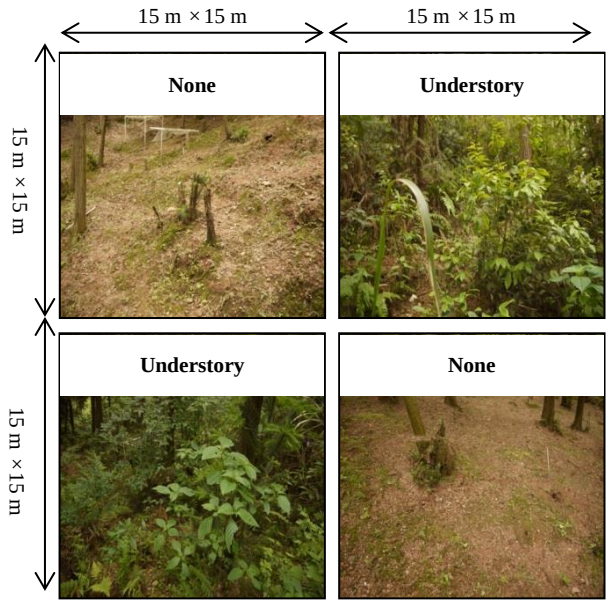
Table A2 Pearson correlation coefficients between soil abiotic properties and different PLFA contents and potential enzyme activities

Table A3 Pearson correlation coefficients between different soil PLFA contents and potential enzyme activities

Table A4 Soil abiotic properties in different months

Table A5 Soil PLFA contents in different months

Table A6 Soil potential enzyme activities in different months


15 m ×15 m
15 m ×15 m
None
Understory
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Understory
None
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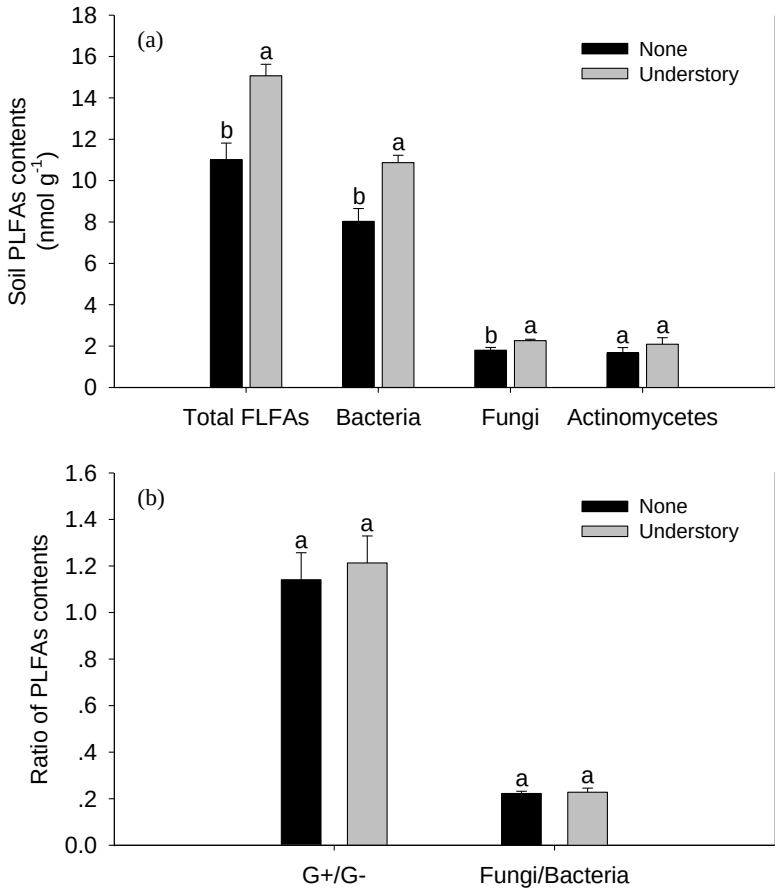
Fig. 1



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507 Fig. 2

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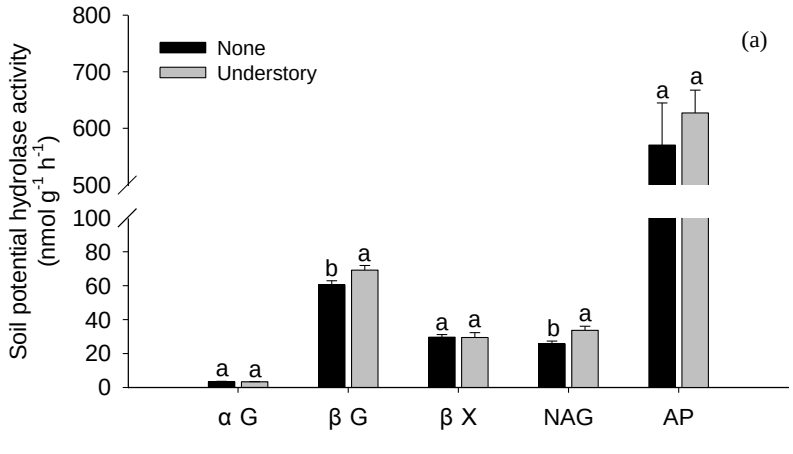

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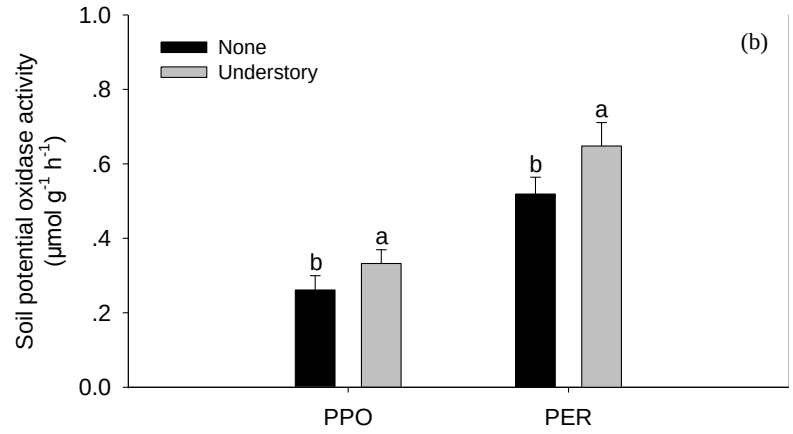

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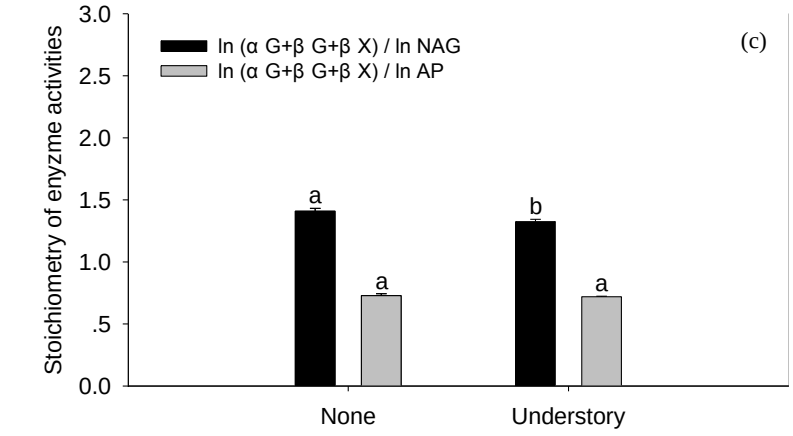

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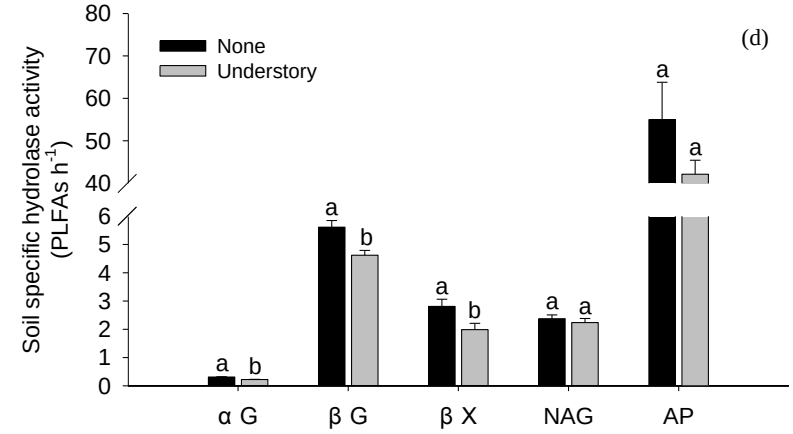

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Fig. 3

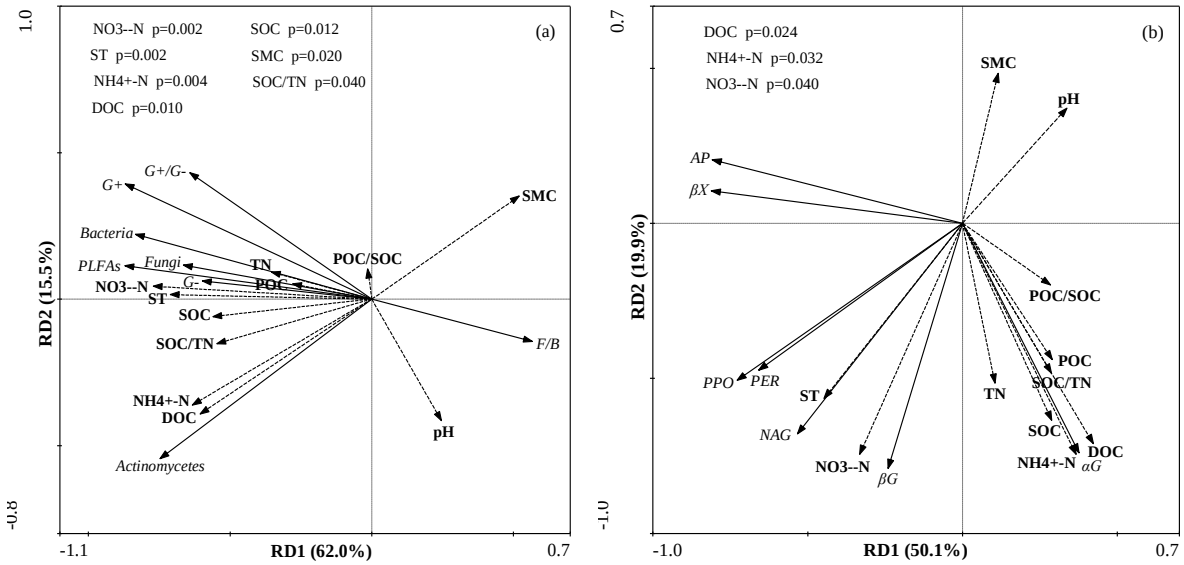
(a)
NO3--N p=0.002
SOC p=0.012
ST p=0.002
SMC p=0.020
NH4+-N p=0.004
SOC/TN p=0.040
DOC p=0.010
G+/G-
G+
SMC
Bacteria
PLFAs
Fungi
TN
POC/SOC
NO3--N
G-
POC
ST
SOC
SOC/TN
F/B
NH4+-N
DOC
pH
Actinomycetes
RD2 (15.5%)
RD1 (62.0%)
1.0
-0.8
-1.1
0.7
(b)
DOC p=0.024
NH4+-N p=0.032
NO3--N p=0.040
SMC
pH
AP
βX
POC/SOC
POC
SOC/TN
PPO
PER
ST
TN
NAG
SOC
DOC
NO3--N
βG
NH4+-N
αG
RD2 (19.9%)
RD1 (50.1%)
0.7
-1.0
-1.0
0.7

Fig. 4

Table 1 Soil abiotic properties

Treatment	ST (℃)	SMC (%)	pH	DOC (mg kg^{-1})	POC (mg kg^{-1})	SOC (g kg^{-1})	NO_3^--N (mg kg^{-1})	NH_4^+-N (mg kg^{-1})	TN (g kg^{-1})	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 mean ± 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 represented significant difference between **None** and **Understory** treatments ($P < 0.05$). Data was the average data of April, July and November. N=18, n=3. The same below

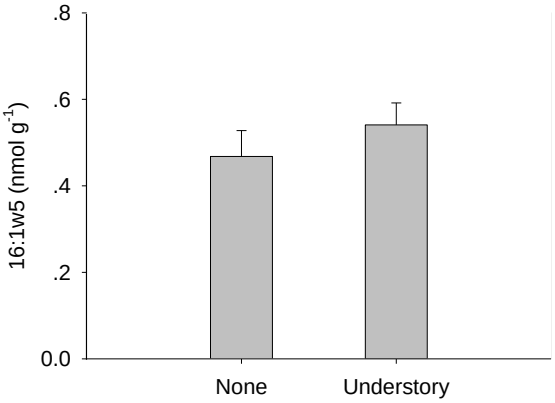

Fig. A1

602 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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618 Table A2 Pearson correlation analysis of soil abiotic properties and different PLFA contents and potential enzyme
619 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
	βX	0.54*	-0.30	-0.40	0.64**	0.32	0.36	0.23	0.25	0.32	0.11	0.15
	NAG	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

620 Values are *r* value of Pearson correlation analysis. * indicates a significant difference at $P < 0.05$; ** indicates a
621 significant difference at $P < 0.01$. G^+ gram positive bacteria, G^- gram negative bacteria, *PLFAs* total PLFAs, G^+/G^-
622 ratio of G^+ to G^-, *F/B* ratio of fungi to bacteria. *αG* α-1,4-glucosidase, *βG* β-1,4-glucosidase, *NAG*
623 β-1,4-N-acetylglucosaminidase, *βX* β-1,4-xylosidase, *AP* acid phosphatase, *PPO* phenol oxidase, *PER* peroxidase. The
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630 Table A3 Pearson correlation analysis of soil different 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 A4 Soil abiotic properties in different months

Treatment	Time	ST (℃)	SWC (%)	pH	NO_3^--N (mg kg^{-1})	NH_4^+-N (mg kg^{-1})	TN (g kg^{-1})	DOC (mg kg^{-1})	POC (g kg^{-1})	SOC (g kg^{-1})	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 represented significant difference between different treatments, and different uppercase letters represented significant difference among different months in the same treatment ($P < 0.05$). The same below

659 Table A5 Soil PLFA contents in different months

Treatment	Time	G^+ (nmol g^{-1})	G^- (nmol g^{-1})	Bacteria (nmol g^{-1})	Fungi (nmol g^{-1})	AMF (nmol g^{-1})	Actinobacterias (nmol g^{-1})	PLFAs (nmol g^{-1})	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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674 Table A6 Soil potential enzyme activities in different months

Treatment	Time	αG (nmol g^{-1} h^{-1})	βG (nmol g^{-1} h^{-1})	βX (nmol g^{-1} h^{-1})	NAG (nmol g^{-1} h^{-1})	AP (nmol g^{-1} h^{-1})	PPO (nmol g^{-1} h^{-1})	PER (nmol g^{-1} 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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