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# Understory vegetation plays a key role in sustaining soil microbial biomass and extracellular enzyme activities

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## Abstract:

~~It is desirable to learn more how~~ 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 properties, ~~through an examination of the effects of understory vegetation on soil environmental factors,~~ microbial biomass, and extracellular enzyme activities. One paired treatment, which comprised understory vegetation removal (**None**) and understory vegetation left intact (**Understory**) in the context of litter removal, was established in a subtropical Chinese fir plantation. We mainly evaluated the effects of understory vegetation on soil environmental factors, the biomass of bacteria, fungi and actinomycetes, and the activities of five hydrolases, ~~i.e., α-1,4-glucosidase, β-1,4-glucosidase (βG), β-1,4-N-acetylglucosaminidase(NAG), β-1,4-xylosidase and acid phosphatase (AP), and two oxidase, i.e., phenol oxidase (PPO) and peroxidase (PER).~~ The soil moisture content (SMC), a~~nd the~~ concentrations of soil dissolved organic carbon (DOC), particulate organic carbon (POC), soil organic carbon (SOC), ammonia nitrogen ($NH_4^+$-N), ~~and~~ total nitrogen and the POC/SOC ratio declined by 4% to 34%, and the biomass of soil bacteria and fungi, total PLFA contents, and the activities of βG, NAG, PPO, a~~nd~~ PER were ~~between 13% and~~ 27% lower, when understory vegetation was removed. ~~The highest activity of AP among all the measured enzymes may reflect the P was limited in this area~~, while NAG was positive with the concentration of $NO_3^-$-N, ~~reflected that P- and N- degrading enzyme affected by different mechanism,~~ The positive relationship between DOC and AP implied that microorganisms absorb carbon to meet their needs for phosphorus. The concentrations of $NO_3^-$-N and $NH_4^+$-N were positively correlated with and αG and βG suggested the increased availability of N promoted the decomposition of carbon. Understory vegetation removal inhibited the propagation of microorganisms and restricted

their enzyme activities, by reducing soil energy and above-ground nutrient inputs and altering the soil micro-environment. We therefore propose that, to sustain soil quality in subtropical Chinese fir plantations, understory vegetation should be maintained.

**Keywords**: Chinese fir forest; Enzyme activities; Microbial biomass; 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 C and nutrient cycling in terrestrial ecosystems (Murugan et al., 2014). Understory vegetation removal influence soil process by reducing above-ground plant diversity Lamb et al., (20[illegible]1) and biomass (Fu et al., 2015) and changing under-ground root inputs quality (Li et al., 2013) in ~~the~~ forest ecosystem. While understory vegetation absorbs moisture and nutrients from the soil (Wang et al., 2014), it also releases C and nutrients to soils through root exudates, and ~~the~~ turnover of fine roots and leaf litter (Liu et al., 2012). The net effect of understory vegetation on soil nutrients is therefore the balance between the understory vegetation’s nutrient demand and its capacity to release nutrients to the soil. Soil extracellular enzymes produced by microorganisms or plant roots catalyze soil C, nitrogen (N), and phosphorus (P) cycling (Stone et al., 2014), in line with the nutrient requirements of plants and microorganisms to ensure the nutrient balance is maintained the context of the changes in soil environment (Burns et al., 2013). To study the changes of enzyme activities with understory vegetation removal could reveal how microbial nutrient acquisition is affect by microbial biomass and soil nutrients.

The influences of understory vegetation on soil properties were 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 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 results 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). ~~The brief review therefore shows that there is inconsistency in the~~ information currently available about the responses of soil enzyme activities to understory vegetation, ~~with some studies~~ reporting that soil enzyme activities decreased in the subtropical alpine coniferous forest (Huang et al., 2014), ~~and others reporting that they~~ did not change ~~in the~~ *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). ~~We are not sure~~ how the soil enzyme activities are affected by the understory vegetation removal in Chinese fir plantations.

In this study, we used a long-term field experiment to assess how understory vegetation ~~in the context of without litter~~ influences soil enzyme activities, microbial biomass, and ~~soil environmental factors in~~ Chinese fir plantations. Earlier studies reported that the nutrient contents release from long-term storage pools, such as root exudates, fine root turnover and leaf litter, decreased when understory vegetation was removed (Liu et al., 2012). We therefore hypothesized that soil C and nutrient availability, microbial biomass, and enzyme activities would decline upon removal of the understory vegetation. ~~Furthermore, we expected that our study would highlight~~ the interactions between ~~the microbial biomass, enzyme activities, and soil environmental~~ factors under different ~~forest~~ understory management practices.

## 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, which forms from red sandstone and sandy conglomerate and is classified as Ud

The study site is a second-generation Chinese fir plantation that was planted in 1998. 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 × 30 m plots, with a buffer zone between them exceeding 10 m, were established in the Chinese fir plantation in January 2013. One paired treatment with three replications was established within the three plots. Each plot was divided into four 15 × 15 m subplots and contained two treatments, the same treatment were distributed across each plot to avoid the effects of slope (Fig. 1). The two subplots with the same treatment in one plot were averaged as one analysis replication. The treatments comprised understory vegetation and litter removal (**None**) and understory vegetation left intact but litter removal (**Understory**). 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 hm$^{-2}$ year$^{-1}$, and the amount of understory vegetation in the research site was about 6236 kg hm$^{-2}$ under natural conditions.

## 2.2 Soil sampling and analysis

Soil samples were collected in April, July, and November 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 ℃, and analyzed as early as possible.

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). The soil moisture content (SMC) was measured by drying 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) concentrations 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) concentrations 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) concentrations 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). The total soil microbial biomass was 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, 18:1ω7c, cy19:0), fungi (16:1ω5, 18:1ω9c, 18:2ω6c, 18:2ω9c 18:3ω6c), actinomycetes (10Me16:0, 10Me17:0, 10Me18:0); $G^+$ and $G^-$ bacterial biomass represented total bacterial biomass (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 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. The microplates were incubated in the dark at 20 ℃ for up to 4 h, following which 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 460 nm with a microplate fluorometer. We had eight replicate sample wells for each assay.

### 2.3 Statistical Analysis

The differences of soil environmental factors, microbial biomass and enzyme activities between the understory treatments were assessed by a pa[illegible]ample *t*-test using SPSS 17.0. Data from the two subplots with the same treatment in one plot were averaged and then ~~were~~ analyzed statistically (*n*=3). We investigated the relationships among soil environmental factors and different microbial biomass, and soil enzyme activities 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 environmental factors

Soil C and N concentrations and the SMC were decreased, when understory vegetation was removed (Table 1).

The ~~concentrations~~ of various soil C (including DOC, POC, and SOC) and N (including $NH_4^+$-N and TN) fractions, SMC and POC/SOC ratio were between 4% and 34% lower in the **None** treatment than in the **Understory** treatment ($P < 0.05$). The concentrations 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 microbial biomass

Soil total PLFA contents were 27% lower in the **None** treatment than in the **Understory** treatment (Fig. 2). In specific, bacterial biomass was 26% less in the **None** treatment than in the **Understory** treatment ($P < 0.05$), though the biomass of $G^+$ and $G^-$ did not vary ($P > 0.05$). Soil fungal biomass was 20% lower in the **None** treatment than in the **Understory** treatment ($P < 0.05$). Understory vegetation removal did not change the actinomycetes biomass ($P > 0.05$).

### 3.3 Soil enzyme activities

Some of the soil C- and N- hydrolase and oxidase activities were higher in the treatments with understory vegetation than in the treatment without understory vegetation (Fig. 3). The activities of βG, NAG, PPO, and PER were declined when the understory vegetation was removed, ~~and, for example,~~ were between 13% and 24% lower than those in the **Understory** treatment ($P < 0.05$).  While ~~phosphate hydrolase activities~~ in the **Understory** treatment were the same as in the **None** treatments ($P > 0.05$).

### 3.4 Correlations between soil enzyme activities, soil microbial biomass, and soil environmental factors

The relationships between different microbial biomass and soil environmental factors are shown in Fig. 4 (a). The first (RD1) ordination axis explained 62.0% of the total variability in the PLFA data 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 PLFA data. The ST was positively correlated with $G^+$, actinomycetes, total PLFAs, $G^+/G^-$ and F/B. The SMC was negatively correlated with actinomycetes and $G^+/G^-$. The concentration of $NO_3^-$-N was positively correlated with $G^+$, bacteria, actinomycetes, total PLFAs, and $G^+/G^-$. The concentrations of $NH_4^+$-N and DOC were positively correlated with bacteria, actinomycetes and total PLFAs. The concentration of SOC was positively correlated with $G^-$, bacteria, fungi and total PLFAs. ($P < 0.05$) (Table A2).

The relationships between soil enzyme activities and soil environmental factors 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 enzyme activities, respectively. The concentrations of DOC, $NO_3^-$-N, $NH_4^+$-N were mainly related to RD2 ordination axis. The

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concentration of DOC was positively correlated with αG, and was negatively correlated with βX and AP. The concentration of $NO_3^-$-N was positively correlated with αG, βG, NAG, PPO and PER. The concentration of $NH_4^+$-N was positively correlated with αG and βG ($P < 0.05$; Table A2). Pearson correlation analysis demonstrated that bacteria and total PLFAs were positively correlated with αG, βG, NAG, PPO and PER. The biomass of fungi was positively correlated with αG, βG, NAG. The biomass of actinomycetes was positively correlated with αG, βG and PER. The ratio of $G^+/G^-$ was positively correlated with all the enzymes except αG (Table A3).

## 4. Discussion

Consistent with our hypothesis, the concentrations of ~~soil 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 variety of understory vegetation species in different studies (Nilsson and Wardle, 2005) and the influence of litter. In our study, we removed litter in all treatments to avoid the effects of litter. Studies in the past have shown that a source of soil C and nutrients, such as rhizosphere secretions, fine root turnover (Liu et al., 2012) and the SOM decomposition rate (Wu et al., 2011; Liu et al., 2012; Zhao et al., 2013), decline when the understory vegetation is removed. 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), and understory vegetation root residue also incorporated into soil (Li et al., 2013) after understory vegetation removal. The increased quantities of soil C and N secreted by Chinese fir roots and originated from the root residue of understory vegetation in~~ this study~~ did not fully compensate for the C and N lost when understory vegetation was removed. Additionally, soil nutrients tend to be higher when plant functional diversity is high (Zhou et al., 2016). Therefore, soil C and N concentrations may decrease by removing understory vegetation and reducing plant diversity. The decreased values of the POC/SOC ratio (Table 1) suggest that POC changed more than SOC when understory vegetation was removed. The changes in the POC concentrations indicated that understory vegetation intact improved soil sustainability and productivity in Chinese fir forests, since aggregate stability and POC concentrations were related (Bouajila and Gallali, 2010). In addition, the decrease in the SMC when the understory vegetation was removed (Table 1) reflects the enhanced soil evaporation driven by the increase in soil surface solar radiation (Wang et al., 2014).

Consistent with our hypothesis, the microbial biomass, including total PLFAs, bacterial, and fungal PLFA biomarkers, declined after the understory vegetation was removed in this study (Fig. 2). Previous studies ~~also~~ reported decreases in fungal biomass after understory vegetation removal (Wu et al., 2011; Liu et al., 2012; Zhao et al., 2013), and changes in the structure of the soil microbial community in response to the loss of above-ground plant functional groups (Murugan et al., 2014). ~~In our study,~~ the decline in fungal biomass may reflect the decrease in plant diversity. Some soil fungi, such as AMF, are controlled by plants, and specific AMF may only grow when specific plants are present (Hart et al., 2001). If plant communities change over time, their mycorrhizal partners will also change (Hart et al., 2001). Mycorrhizal species in the study area included understory vegetation, such as *Dicranopteris dichotoma*, *Vaccinium bracteatum*, *Loropetalum chinense*, and *Rhododendron*. Chinese fir monocultures may support fewer fungi biomass than other plantations where the understory vegetation is left intact. Fungal biomass and SOC concentration were positively correlated (Table A2). Therefore when the amounts of C and exuded by the rhizosphere decreased after the understory vegetation was removed, the soil fungal biomass also decreased, since soil fungi dominated decomposition of C in the rhizosphere (Denef et al., 2009). The bacterial biomass also decreased after the understory vegetation was removed, which was mainly the result of reductions in the soil C and N concentrations (Table A2) and plant diversity (Lamb et al., 2011). The F/B ratio did not change because the bacterial and fungal biomass decreased at the same time (Fig. 2). Brant et al., (2006) considered that there might be an increase in the biomass of actinomycetes 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 actinomycetes biomass in the field plots. Our results suggest that bacterial and fungal biomass were better indicators of the changes in understory management practices in the Chinese fir plantation (arbuscular mycorrhizal species) than actinomycetes.

Consistent with our hypothesis, we found a lower extracellular enzyme activity when understory vegetation was removed (Fig. 3), which was agree with the results of Huang et al., (2014), who found soil cellulose activity decline after understory vegetation removal, in spite of Lin et al., (2012) didn't find any changes in soil enzyme activities. The soil rhizosphere has been described as soil microbial hotspots with higher microbial activities than other areas of the soil profile (Kuzyakov and Blagodatskaya, 2015). Decreases in the quantity and diversity of root exudates in the understory vegetation, and changes in the soil environmental factors and soil fauna, may cause direct and indirect changes in soil enzyme activities (Liu et al., 2012; Huang et al., 2014). There are several possible reasons for the decreased enzyme activities observed in our study, as follows. (1) When understory vegetation is removed, less organic matters are released to the soil from the lower amounts of root (Liu et al., 2012), which means there will be less substrates available for enzyme production. (2) Mycorrhizal fungi and rhizosphere microorganisms attached to tree roots 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 concentrations 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 concentrations 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). Of all the enzymes we assayed, the activity of AP was the highest (Fig. 3), perhaps because P was the most limiting nutrient in this acidic Chinese fir forest soil. The mineralization of organic phosphorus might increase as the microorganisms produce more phosphatase to ensure their demand for P when P is limited (Allison and Vitousek, 2005). The results of Loeppmann et al., (2016) 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 ratio of SOC/TN did not change, which indicates that the rhizosphere of the understory vegetation was not N-limited relative to understory vegetation removal. The positive correlation between NAG activity and $NO_3^-$-N concentrations in our study (Table A2) may suggest that more SOM derived from root enhanced NAG activity may in turns promote the mineralization of SOM, thereby increased soil available N concentrations. Chitin, a major structural component of fungal cell wall, 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 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 relationship between the activity of AP and the concentration of DOC indicated that microorganisms absorbed more C to meet the demands for P in the P limited area.

## 5. Conclusions

Our results demonstrate that understory vegetation plays an important role in enhancing soil C- and N- hydrolase and oxidase activities, through increasing soil C and N concentrations, and bacterial and fungal biomass. Understory vegetation, however, does not influence the biomass of actinomycetes or P-hydrolase activity. The activity of AP among all the measured enzymes is the highest may reflect the P was limited in this area, while NAG was positive with the concentration of $NO_3^-$-N, reflected that P- and N- degrading enzyme affected by different mechanism. The positive relationships between the activities of C-degrading enzymes and the concentrations of soil inorganic N implied that the decreased availability of N inhibited the decomposition of C when understory vegetation was removed. The activity of AP is positive with the concentration of DOC indicated that microorganisms absorbed more C to meet the demands for P in the P limited area. 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 of different microbial community compositions

(a) contents of different PLFAs contents, (b) ratio of PLFAs 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 of April, July and November data. N=18, n=3. The same below

Fig. 3 Soil enzyme activities

(a) soil hydrolase activities, (b) soil oxidase activity. *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 soil environmental factors and (a) microbial biomass, and (b) 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 environmental factors

## Supplementary material

Table A1 Soil enzymes and their corresponding substrates and functions

Table A2 Pearson correlation coefficients between soil environmental factors and different microbial biomass and enzyme activities

Table A3 Pearson correlation coefficients between different soil microbial biomass and enzyme activities

Table A4 Soil environmental factors in different months

Table A5 Soil microbial biomass in different months

Table A6 Soil enzyme activities in different months

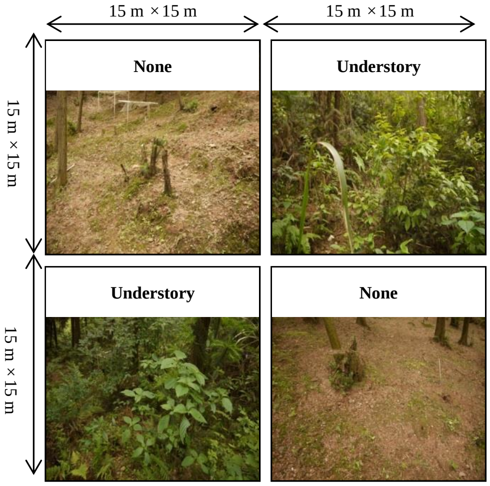
15 m × 15 m
15 m × 15 m
15 m × 15 m
15 m × 15 m
None
Understory
Understory
None

Fig. 1

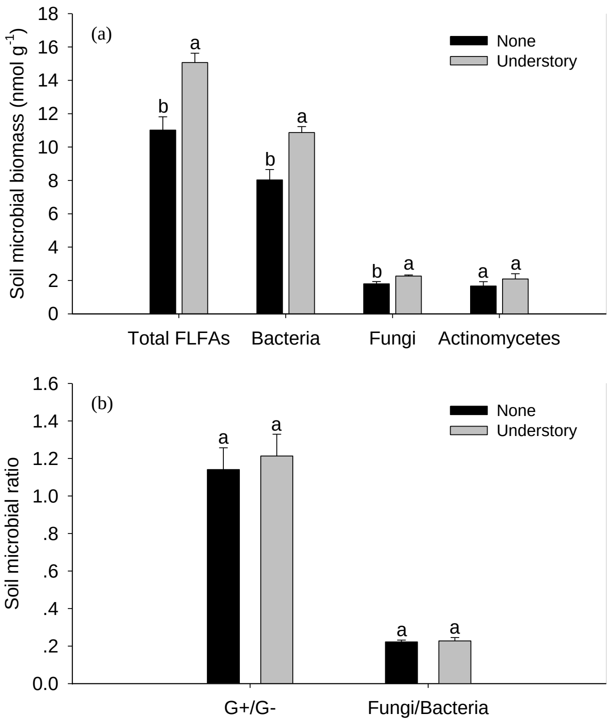
(a)
Soil microbial biomass (nmol g-1)
18
16
14
12
10
8
6
4
2
0
None
Understory
b
a
b
a
b
a
a
a
Total FLFAs
Bacteria
Fungi
Actinomycetes
(b)
Soil microbial ratio
1.6
1.4
1.2
1.0
.8
.6
.4
.2
0.0
None
Understory
a
a
a
a
G+/G-
Fungi/Bacteria

Fig. 2

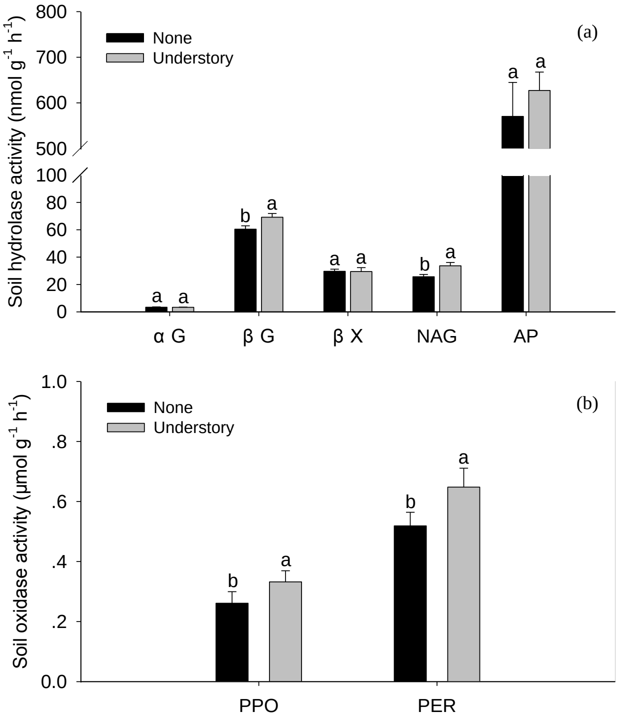
Soil hydrolase activity (nmol g-1 h-1)
800
700
600
500
100
80
60
40
20
0
None
Understory
(a)
a
a
b
a
a
a
b
a
a
a
α G
β G
β X
NAG
AP
Soil oxidase activity (μmol g-1 h-1)
1.0
.8
.6
.4
.2
0.0
None
Understory
(b)
b
a
b
a
PPO
PER

Fig. 3

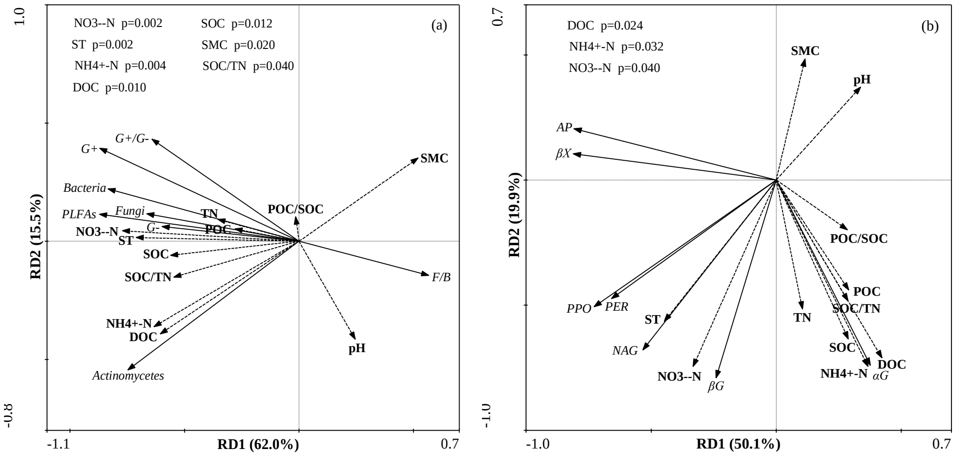
(a)
NO3--N p=0.002
ST p=0.002
NH4+-N p=0.004
DOC p=0.010
SOC p=0.012
SMC p=0.020
SOC/TN p=0.040
1.0
-0.8
RD2 (15.5%)
RD1 (62.0%)
-1.1
0.7
G+/G-
G+
Bacteria
PLFAs
Fungi
G-
TN
POC
POC/SOC
SMC
NO3--N
ST
SOC
SOC/TN
F/B
NH4+-N
DOC
pH
Actinomycetes
(b)
DOC p=0.024
NH4+-N p=0.032
NO3--N p=0.040
0.7
-1.0
RD2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

Fig. 4

Table 1 Soil environmental factors

| 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 of April, July and November data. *N*=18, *n*=3. The same below

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). |

Table A2 Pearson correlation analysis of soil environmental factors and different microbial biomass and enzyme activities

| Environmental factors | | 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 |
| | Actinomycetes | 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 |

Values are *r* value of Pearson correlation analysis. * 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. The same below

Table A3 Pearson correlation analysis of soil different microbial biomass and enzyme activities

| Factors | $G^+$ | $G^-$ | Bacteria | Fungi | Actinomycetes | 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 |

Table A4 Soil environmental factors 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

Table A5 Soil microbial biomass in different months

| Treatment | Time | $G^+$ (nmol $g^{-1}$) | $G^-$ (nmol $g^{-1}$) | Bacteria (nmol $g^{-1}$) | Fungi (nmol $g^{-1}$) | Actinomycetes (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 | 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 | 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 | 0.817±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 | 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 | 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 | 1.23±0.55aB | 13.90±0.98aB | 1.13±0.15aB | 0.23±0.03aA |

Table A6 Soil 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 |